Methods to Assess, Control, and Manage Risks for Two Invasive Mollusks in Fish Hatcheries

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ABSTRACT

Fish hatcheries are vulnerable to infestations of invasive mollusks such as the New Zealand mudsnail *Potamopyrgus antipodarum* (NZMS) and quagga mussels (*Dreissena rostriformis bugensis*) because aquaculture facilities often are characterized by constant flows and temperatures and enhanced nutrients. Aquaculture facilities are often located near recreational areas, open water resources, and production and distribution plans include fish transport, which are pathways that invasive mollusks utilize. Infestations of invasive mollusks have limited the transport and stocking of fish into other locations because of potential risks of being liable for new introductions. In extreme cases, hatcheries have been closed. In this thesis, methods to assess, control, and manage the risks associated with invasive mollusk infestations were evaluated. Studies included laboratory testing of the efficacy, appropriate applications, and safety of Virkon® Aquatic as a disinfectant to kill NZMS and quagga mussels. In other studies, the potential for use of a mixed cell raceway to effectively depurate fish infested with NZMS was evaluated to consider the hydraulics, particle removal rates, and velocities. A comparison of the removal of particles in a mixed cell system with a traditional Burrow's pond rearing system illustrated the enhanced removal efficiency of the mixed cell system. The results of these studies and information in peer reviewed literature, reports, and theses were incorporated to provide a risk analysis and decision document for hatchery managers to reduce the potential risk of invasive mollusk infestations. The risk and decision analysis focuses on NZMS pathways and control measures to assess probability and consequences of infestation, monitoring and disinfection methods, and management and prevention measures that can be used with high certainty to limit the risk of infestation.

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Dedication

This thesis is in loving memory of my mother, Mary Ann Stockton, the inspirer and motivator for my master's degree.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Table of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1: Evaluation of the toxicity of Virkon® Aquatic against two invasive mollusks

Abstract

In the western United States, populations of New Zealand mudsnails (NZMS) (*Potamopyrgus antipodarum*) and quagga mussels (*Dreissena rostriformis bugensis*) have become established in many fish hatchery facilities. Virkon® Aquatic is a broad-spectrum disinfectant used in many hatchery facilities as a disinfectant on footwear, nets, and equipment for protection from bacteria, viruses and one fungus. This chemical has the potential to be used more extensively, but the appropriate dosage, duration of exposure, and safety limits must be determined. We conducted experiments to determine the lethal concentration and duration of exposure for achieving 100% mortality of NZMS and quagga mussel adult and veligers. We also estimated the safety limits of Virkon® Aquatic to steelhead trout (*Oncorhynchus mykiss*). Utilizing static conditions at a range of temperatures, various concentrations of Virkon® Aquatic were poured into test systems with each species. After exposure, test organisms were rinsed and put into a recovery container for up to 72 h. We found all life stages of NZMS were killed after a 20 min bath exposure to 20 g/L Virkon® Aquatic. All quagga mussel veligers exposed to a 5 g/L Virkon® Aquatic bath solution were dead after 10 min, and adult quagga mussels were killed after a 10 min bath exposure to 20 g/L Virkon® Aquatic. No observed effects concentration was 10 mg/L for approximately 1 g steelhead. Lethal concentrations varied with size and exposure time. Disinfectant residues on equipment or if bath systems were spilled into raceways, the risks to fish would be very low. Procedures using this disinfectant would be safe and efficacious.

Introduction

Global climate change, intensive agricultural and aquacultural practices, and increasing human populations have altered ecosystems (Virousek et al. 1997; Pimentel et al. 2005). These disturbances in the ecosystems and the increased chances for foreign organisms to be introduced have increased the rate of invasion (Vitousek et al. 1997; Ricciardi and MacIsaac 2000; Hulme 2009). Invading organisms often have an r-selected life history, are very

prolific or parthenogenic, and have high growth rates (Moyle and Light 1996). The most successful invading organisms have the ability to be transported easily and survive transport (Kolar and Lodge 2001). The invaders that we care about are the ones that cause the abundant ecological and economic damage (Mack et al. 2000; Pimentel et al. 2000; 2005). Aquatic mollusks, such as New Zealand mudsnails *Potamopyrgus antipodarum*, zebra mussels *Dreissena polymorpha*, and quagga mussels *Dreissena rostriformis bugensis* survive transport, are easily transported, and can cause ecological and economic damage. Aquaculture facilities, fish hatcheries, in particular, are affected by aquatic mollusks (Naylor et al. 2001; Aquatic Nuisance Species Task Force and National Invasive Species Council 2007).

The New Zealand mudsnail, (NZMS) is an aquatic mollusk of particular interest to the Western United States. The first known reported identification of NZMS in North America was in southern Idaho, at The Nature Conservancy's Thousand Springs Preserve in 1987 (Bowler 1991). Dybdahl and Drown (2011) evaluated the genetic relatedness of populations from New Zealand, Australia, and Europe to populations in the United States. They found a common genotype (US1) in Washington, Oregon, California, Nevada, Arizona, Utah, Colorado, Wyoming, and Montana from Australia and New Zealand. Aquaculture facilities in California, Colorado, Idaho, Montana, Arizona, and Utah have reported infestations of NZMS. Another population with a different genotype, US2 (Dybdahl and Drown 2011), was found in the Great Lakes area in 1991; first Lake Ontario and later in Lake Superior and Lake Erie (Levri et al. 2008). US2 is the same genotype as found in Europe populations (Dybdahl and Drown 2011). Many vectors and vehicles including, fish, birds and mammals, aquarium traders, ship ballast water, recreationalists, agency personnel, and other water users can distribute NZMS to new locations (Bowler 1991; Alanso and Castro-Diez 2008; Bruce and Moffitt 2010).

NZMS are easily transported because they are very small, ranging in size from 80 µm to 6 mm and live in a variety of habitats, such as estuaries, lakes and rivers (Winterbourn 1970; Alanso and Castro-Diez 2008; Bersine et al. 2008). As a freshwater prosobranch, NZMS have an operculum that closes tightly resisting desiccation and chemical treatment, allowing them to survive transit to new areas (Richards et al. 2004). NZMS are prolific as one NZMS

female will populate an area, producing up to 50 neonates in her brood pouch, and reproducing one to six times throughout the year (Møller et al. 2004; Alanso and Castro-Diez 2008). In some conditions, NZMS will colonize an area and reach densities of over 500,000 snails per square meter (Hall et al. 2006). Gravel, sand, and mud are typical substrates NZMS colonize; they are also found on aquatic vegetation (Richards et al. 2001; Alanso and Castro-Diez 2008). The snails can out-compete native snails and macroinvertebrates (Kerans et al. 2005; Riley et al. 2008), and in some circumstances have consumed up to 75% of the primary productivity (Hall et al. 2003, 2006). Some fish eat NZMS (Bersine et al. 2008; Bruce and Moffitt 2010). NZMS can survive the digestive tract of salmonids (Haynes et al. 1985; Bruce et al. 2008). Parasites are known to infect NZMS in their native range, but no evidence of infection has been documented in the invaded range (Gérard et al. 2003).

Another organism of risk to the Western United States is the quagga mussel. Originally from Ukraine, quagga mussels were first found in the United States in 1987 in the Great Lakes area (May and Marsden 1992). In 2007, the quagga mussel was identified as inhabiting Lake Mead (Kennedy 2007). Researchers in Colorado, Utah, Arizona, Nevada, and California began to find quagga mussels in their water bodies (Fuller 2009). Fish hatcheries in Colorado, Arizona, and Nevada are infested with quagga mussels. The major pathway of quagga mussel introduction is by recreational boaters (Johnson et al. 2001; Leung et al. 2006). Quagga mussels are a freshwater bivalve that grows to a maximum size of 4 cm (Benson et al. 2011). They can live up to 5 years, spawning twice per year, producing millions of gametes (Ram et al. 1996). Larvae are planktonic having many named life stages, but are classified generally as veligers. Embryos that are 80-100 microns are trochophores, 92-112 microns are straight-hinged veligers, and the most typical veligers found are 112-347 microns (USACE 2002). The last stage when the veligers start to settle is the pediveliger stage, 231-462 microns (USACE 2002). Quagga mussels will settle on anything hard; however, they have been found on the soft muddy bottoms of reservoirs (Roe and MacIsaac 1997). They attach to surfaces tightly with their byssal threads, but they can detach and move to another area (Peyer et al. 2009).

The quagga mussel along with its congeneric, the zebra mussel *Dreissena polymorpha*, can affect ecosystems and damage infrastructure of cooling pipes, water intake pipes, head

gates, and hatchery facilities (Pimentel et al. 2000, 2005; Peyer et al 2009). Quagga mussels are very efficient filter feeders, filtering up to one liter of water per day, which leaves little food resources for native aquatic species (Roe and MacIsaac 1997; Strayer 1999; Barbiero et al. 2006; Bunnell et al. 2009). Mussels react to environmental stimuli and close their shells and siphons, avoiding short duration chemical treatments and surviving out of water for days depending on temperature and humidity (Johnson et al. 2001; Britton 2007). More research on quagga mussels is needed to identify specific habitat characteristics and ranges that are different from zebra mussels.

Since these invasive mollusk species continue to have a high probability of successful transport to new locations, effective and safe methods of disinfection in various water conditions and temperatures are needed. Chemical disinfectants are widely used in animal husbandry to reduce risks from infective agents (Johnson et al. 2003; Gehan et al. 2009; Burridge 2010). Virkon® Aquatic (reformulated from Virkon® S in 2007) is one of very few US Environmental Protection Agency-registered disinfectants that are labeled specifically for use in aquaculture facilities (Mainous et al. 2010). Reformulation of Virkon® S, involved the removal of the indicator dye and fragrance, but all other constituents remained the same in Virkon® Aquatic. It is labeled for use on aquatic bacterial, fungal, and viral pathogens (DuPont 2010). This chemical is composed of an oxidizing agent, two organic acids, a buffer, and a surfactant (Western Chemical 2008). Active ingredients of Virkon® Aquatic are potassium monopersulphate and sodium chloride, 21.9% (Mitchell and Cole 2008). The mode of action is by oxidizing proteins and other components of cell protoplasm, resulting in inhibition of enzyme systems and loss of cell-wall integrity (Curry et al. 2005). The recommended concentration for disinfecting most surfaces for a majority of the organisms is a 10 g/L concentration with an exposure time of at least 5 min (Western Chemical 2008).

The efficacy of Virkon® Aquatic on NZMS or quagga mussels has not been determined. Connor et al. (2008) reported that Virkon® Aquatic would have injurious effects on Sydney rock oysters *Saccostrea glomerata*. They determined that 5 mg/L of Virkon® Aquatic caused 100% abnormal embryo development. The only snails that have been tested are the red-rim melania, *Melanoides tuberculata,* and the faucet snail, *Bithynia tentaculata,* which had different reactions. The red-rim melania had 100% mortality at Virkon® concentrations

greater than 5 g/L (Mitchell et al. 2007). However, when *B. tentaculata* was exposed to Virkon® concentrations greater than 5 g/L, there was low mortality (Mitchell and Cole 2008).

Considered environmentally safe, Virkon® Aquatic is recommended for use in and around aquatic environments (Mainous et al. 2010). Virkon® Aquatic is safe at very low concentrations in the water. Studies conducted by Dr. Ronald Roberts determined that Koi could easily tolerate 5 mg/L Virkon® Aquatic solution in a flow through system, keeping background bacterial levels low (Holmes 2006). Other studies show that all life stages of shrimp can withstand 1.2 mg/L Virkon® Aquatic solutions (DuPont 2006). An unpublished study conducted by Dr. Ron Hardy in collaboration with Dr. Ronald Roberts indicated that rainbow trout would survive exposure to 8 mg/L of Virkon® Aquatic in freshwater (Ron Hardy, Hagerman Fish Culture Experiment Station, Hagerman, Idaho, personal communication). No other data on safety limits for fish were determined.

The objectives of this study were to determine if Virkon® Aquatic will effectively kill NZMS and quagga mussels and to determine the safety limits of Virkon® Aquatic for improved biosecurity protocols in fish hatcheries.

Methods

Experimental Organisms

New Zealand mudsnails- New Zealand mudsnails were collected from springs at Hagerman National Fish Hatchery (HNFH) on 14 June through 11 August 2009, packaged at the hatchery in moist towels, placed into plastic bags, and shipped in coolers to the University of Idaho fisheries wet laboratory. Upon arrival, the snails were washed through a 2.0 mm and 0.85 mm sieve to remove the mud. They were transferred into 2 L containers containing dechlorinated, aged well water equilibrated to 15°C. Snail containers were placed into an aquarium. The water in each container was changed every other day, and temperature in the test room was maintained at 15°C throughout trials. Flow through water baths were setup to conduct testing at 8 and 22°C. Temperatures were monitored and recorded at 15 min intervals with a HOBO data logger (Onset Computer Corporation, Bourne, MA). A natural

photoperiod for the latitude of HNFH was maintained in the test room. Snails were retained in the laboratory for no more than three weeks.

Quagga mussels- All mussels for tests were collected from raceways at Willow Beach National Fish Hatchery (WBNFH), Willow Beach, AZ on 13 October through 22 October 2009. Veligers were collected with a 30 µm mesh plankton tow net hung from the grating system of the head box in the second in-line raceway for at least an hour. The filtrate was then filtered through a 500 µm nylon mesh screen into a 500 mL Nalgene sample bottle. The contents of each bottle was allowed to settle at room temperature (18°C) for at least 3 h. Adult sized quagga mussel adults were removed from the walls of the hatchery head boxes of the raceways and were placed into a 15.24 x 15.24 x 7.62 cm plastic container. The mussels used in testing ranged in size from 5 mm to 20 mm. In the lab, the mussels were rinsed two or three times with aerated well water at 18°C. Debris and cracked or dead mussels were removed.

Steelhead trout-Fish were obtained as eggs from Dworshak National Fish Hatchery brood stock and raised at the University of Idaho, College of Natural Resources fisheries wet laboratory March - December 2009. Feeding fry were transferred into two 0.76 m diameter circular tanks on 27 June 2009. Tank temperatures were maintained at 8 or 15°C and trout were fed 1 or 2% body weight daily with #1 BioVita feed (Skretting-BioOregon, Longview, WA). Temperatures were recorded and monitored at 15 minute intervals with a HOBO data logger.

Test Substance

Virkon® Aquatic (lot # 2258523) (Western Chemical, Ferndale, WA) was used for all testing. Powdered Virkon® Aquatic was measured (0.01 g) and mixed in volumetric flasks with deionized water at 20°C for trials at the University of Idaho, or well water at 18°C for trials at WBNFH. All solutions were placed at test temperatures for at least an hour acclimation and activation of the test solution. The concentrations were verified with Virkon® Aquatic test strips (Western Chemical, Ferndale, WA).

Experimental Design

New Zealand mudsnail- All trials were conducted in 150 mL acid washed glass beakers. A test beaker contained 10 healthy NZMS of >2 mm for each concentration and time tested. Test solutions were concentrations of 10 and 20 g/L Virkon® Aquatic. To begin a trial, 100 mL of temperature equilibrated test solution at 8, 15, or 22°C was poured into each beaker. Deionized water was poured into test beakers to serve as controls. Each time and concentration combination was replicated three times per trial.

NZMS were removed from the test system at 5, 10, 15, or 20 min. The snails were removed by pouring the test system into a small stainless steel sieve to recover all snails, and snails were rinsed three times with aged well water. The test liquid was retained in small plastic cups for tests of water chemistry conducted immediately, at 24 and 48 h post testing. The NZMS were then placed into small plastic cups with aged water; mortality was assessed immediately, then again at 24 and 48 h post testing with the aid of a dissecting microscope. Individual snails were touched with a probe to elicit movement or tactile response. The number of neonates was counted at each time interval and assessed for mortality. Each trial was repeated at least three times for a total of 9 beakers tested at each concentration.

Quagga mussel veliger- A serological pipette was used to transfer 1 mL of settled sample water containing veligers from the bottom of the sample bottle into a 150 mL glass beaker. Then 9 mL of stock Virkon® Aquatic concentrations were added to make a final concentration of 0.5, 1, 2.5, 5, or 20 g/L and aerated well water was used for control concentrations. Exposure times were 5, 10, 15, or 20 min. About 3 mL of test solution from the bottom of the beaker was pipetted into a 30 mm petri dish. A dissecting microscope was used to locate at least 10 veligers for each test. The veligers were placed onto the Sedgewick rafter microscope slide at 40 to 100 times magnification to confirm mortality. No movement of cilia and visible organs, and darkening or crystallization of the veliger constituted mortality. Veligers were then placed in aerated well water for 24 h. After 24 h, if no movement or color change was observed in dead tested veligers and control treatment veligers were still alive the recovery period did not continue.

Quagga mussel adult- Ten randomly sized adult quagga mussels were placed into 10 mL of aerated well water within a 150 mL beaker. A volume of 90 mL of aerated well water or the stock 20,000 mg/L Virkon® Aquatic was added to each beaker. The adult quagga mussels were exposed for 10, 15, 20, or 30 min. Mussels from each combination of time and disinfectant concentration were removed, rinsed, and distributed into each of three 30 mm petri dishes. Aerated well water was added to each petri dish to cover the quagga mussels. Quagga mussel mortality was reported after 72 h. Mortality was determined if mussels shells were agape or did not respond to touch.

Steelhead trout- To test the toxicity of Virkon® Aquatic to steelhead, we placed steelhead from the stock tanks into test system containers containing water for a given test concentration and test replicate. Tests were conducted at 8 and 15°C with fish with an average size of 0.89 g and 4.88 g or 1.06 g and 12.89 g respectively (Table 1.1). The fish were maintained overnight in test system containers and testing began the next morning. Test solutions of Virkon® Aquatic were made at higher concentrations so the dilution rate into the test system was one part solution and four parts water.

Static tests at 8°C were conducted with two replicates of 3 fish each (X=0.9 g) in acid washed 2 L beakers with 800 mL of water (Table 1.1). Beakers were placed into flow through water baths to maintain temperature of 8°C. Testing began by pouring 200 mL of 8°C test solution into each beaker. Final test concentrations were 0, 5, 10, 40, and 80 mg/L of Virkon® Aquatic. Testing ended after a 48 h of exposure time.

For static tests conducted at 15°C, two replicates of 5 steelhead each (X=1.1 g) in acid washed 2 L beakers with 800 mL of water (Table 1.1). Beakers were placed into flow through water baths to maintain temperature of 15°C. Testing began by pouring 200 mL of 15°C test solution into each beaker. Final test concentrations were 0, 5.6, 10, 17.8, and 31.6 mg/L of Virkon® Aquatic. Exposure times for every concentration were 1, 2, 3, or 5 h; some exposure times were shortened because moribund fish were placed into recovery before mortality.

Static conditions were maintained for testing at 8°C, with three replicates of eight steelhead trout (X=4.9 g) each in 15.15 L plastic tanks containing 1.6 L of water (Table 1.1). Testing began by pouring 400 mL of 8°C test solution into each tank. Final test concentrations were 0, 19.95, 31.62, 39.81, 63.10, and 79.43 mg/L of Virkon® Aquatic. Exposure time for every concentration was 3.5 h; some exposure times were shortened because moribund fish were placed into recovery.

Static conditions were maintained for testing at 15°C, with a maximum of three replicates of eight steelhead trout (X=12.9 g) each in 15.15 L plastic tanks containing 6.4 L of water (Table 1.1). Testing began by pouring 1.6 L of 15°C test solution into each tank. Final test concentrations were 0, 19.95, 31.62, 39.81, 63.10, 79.43, and 200 mg/L of Virkon® Aquatic. Exposure time for every concentration was 3.5 h; some exposure times were shortened because moribund fish were placed into recovery.

For all temperature and fish sizes, observations of each test system were made at every 15 min for the first hour and then once an hour until fish were put into recovery. Test systems were observed three times throughout the next day. Observations ceased when all fish were dead or recovery time ended. Mortalities were removed and measured for length (0.1 cm) and weight (0.01g). The surviving steelhead were removed after 48 h, rinsed three times with fresh water, and placed into beakers containing fresh water. The fish were allowed to recover 48 h before the test system was ended. A lethal dose (6ml/L) of 250 mg/L tricaine methanesulfonate (MS-222) was used to kill all fish before lengths and weights were measured.

Water Chemistry

For each trial with NZMS one replicate with and without NZMS was analyzed for dissolved oxygen (DO) (mg/L), pH, temperature, and conductivity (mS/cm) with a YSI 556 MPS multiprobe (YSI, Inc., Yellow Springs, Ohio).

For trials with steelhead trout, we recorded dissolved oxygen (mg/L), pH, and temperature with the YSI multiprobe and ammonia levels (LaMotte test kit, Chestertown, Maryland) during testing and recovery stages.

Statistical Analysis

Survival analyses were conducted using SAS 9.2 (SAS Institute Inc. 2002-2008). We explored variable interactions in the NZMS experiments using PROC LIFETEST with number of dead NZMS as the failure variable in the model as it relates to time. A Wilcoxon rank test was used to determine variable significance for replicate, concentration, and temperature. The nonparametric estimates of the survivor function were computed for NZMS survival. The survivor function is: $S(t) = \Pr(T > t)$; where T is the lifetime of a randomly selected experimental unit and t is the failure time. The hazard rate is defined as the probability per time unit, which a test organism that has survived to the beginning of the respective interval will fail in that interval. Specifically, it is computed as the number of failures per time units in the respective interval, divided by the average number of surviving test organisms at the mid-point of the interval. Hazard rates were calculated using PROC LIFETEST with the life method. The life-table estimates are computed by counting the numbers of censored and uncensored observations that fall into each of the time intervals, where conditional probabilities of an event are calculated. Paired t-test of the means in water chemistry parameters were used to determine significant differences, $\alpha \leq 0.05$.

Lethal concentrations and time to mortality were evaluated for age 0 steelhead trout for seperate sizes from 0.89 to 12.9 g at two temperatures. The no observed effects concentrations (NOEC) were determined for each size class and temperature with the criteria of no mortality (USEPA 2002).

Results

New Zealand Mudsnail

Concentration and test temperature were significant covariates affecting survival of NZMS (Table 1.2). Survival of snails exposed to the highest concentrations, 20 g/L, had significantly higher mortality over snails tested at 10 g/L ($P = 0.04$; Table 1.2). Temperature and replicate covariates were not significant between the two concentrations. To determine which temperatures were significant, the data was analyzed by concentration. We found that there was a significant difference in temperature within the controls, 0 g/L (Table 1.2), but not in the 10 and 20 g/L concentrations. In Figure 1.1, it is apparent that survival at 22°C in

the control is different from survival at 8 and 15°C. Figure 1.1 also shows the variation between the different concentrations at the different temperatures. The hazard rates increased as concentration, temperature, and time increased (Table 1.3). At all three temperatures, 100% mortality occurred in 20 min exposure at 20 g/L. The presence of neonates in the 20 g/L concentration at 20 min exposure times at 15 and 22°C indicate that at least one snail was not immediately killed by the exposure of Virkon® Aquatic (Table 1.4) and released a neonate.

No significant differences were detected in dissolved oxygen, pH, or conductivity measured in test solutions with and without snails. Dissolved oxygen ranged 6 and 9 mg/L with a mean of 8.25 mg/L for all test beakers (Table 1.5). The pH averaged 7.93 for the controls. Test systems with concentrations of Virkon® Aquatic had low pH, 2.25 and 2.01 for 10 and 20 g/L Virkon® Aquatic, respectively (Table 1.5). Conductivity increased with increasing concentration; average conductivity for control water was 196 mS/cm, 5663 for 10 g/L, and 10320 for 20 g/L Virkon® Aquatic (Table 1.5).

Quagga Mussel

Concentrations of Virkon® Aquatic of 2.5 g/L were lethal to veligers after 15 to 20 min, Table 1.6. However, a concentration of 5 g/L is more effective, killing 100% of veligers in 10 min (Table 1.6). The test system had no effect on the veligers, as the controls were alive for the time tested. Low concentrations, ≤0.5 g/L, of Virkon® Aquatic had very little effect on the veligers. This study determined that the adult quagga mussels were susceptible to Virkon® Aquatic. Solutions of 20 g/L at all exposure times had 100% mortality as shown in Table 1.7. The controls were alive for 72 h and all exposed quagga mussels were dead at 72 h. Further testing on quagga mussels needs to be conducted before toxicity limits are determined.

Steelhead Trout

High concentrations, greater than 10.0 mg/L, of Virkon® Aquatic were needed to cause mortality in steelhead trout. However, steelhead trout exposed to concentration of greater than 10 mg/L Virkon® Aquatic showed some signs of respiratory distress and engorged gills. Fish were quiet and on the bottom of the beaker or tank; as time progressed, the fish gills

were flared and red. Some of the fish were observed coughing, shaking their heads, or gulping for air. Dead fish were found with their mouth wide open. On some fish, excessive production of mucus was observed. The DO, pH, and ammonia levels were within tolerable ranges to have no effect on fish.

In the first test at 8°C with 0.89 g trout, mortality was observed first in the highest concentrations, 40 and 80 mg/L, (Table 1.8) where all trout were dead at 0.75 and 0.5 h, respectively. Trout tested at 10 mg/L were dead after 24 h. A trend was established of increasing mortality with increasing concentration and exposure time.

The 1.06 g trout that were exposed to concentrations of Virkon® Aquatic between 17.8 and 31.8 mg/L at 15°C for 1-5 h exhibited mortality, Table 1.9 and Figure 1.2; lower concentrations showed no mortality. The trout that were exposed to 31.8 mg/L generally died in a very short time, less than 0.75 h; however, one fish did survive approximately 24.5 h after being exposed to 31.8 mg/L for 0.75 h. Mortality of 1.06 g trout at 15°C was variable at 17.8 mg/L, as shown in Figure 1.2, which did not show a trend of increasing mortality with increased exposure time.

In the trial with 4.88 g trout at 8°C, there was a trend that higher concentrations increased mortality, Table 1.10. At 39.81 mg/L, mortality was 54.2%; most of the mortality occurred before exposure time ended (3.5 h), Figure 1.3. Following the trend, the 4.88 g trout at 8°C that were exposed to 63.1 mg/L all died before exposure time ended, also shown in Figure 1.3. All 4.88 g trout that were exposed to 79.43 mg/L died after being exposed for 0.75 h.

However, the 12.89 g trout that were exposed to the same concentrations at 15°C showed no mortality, Table 1.11, until being exposed to a 200 mg/L concentration. At 200 mg/L, 12.89 g fish were all dead within 0.25 h of exposure.

The no observed effects concentrations are listed in Table 1.12 for each size category per temperature tested. As fish weight increased, the NOEC increased. For the 0.89 g trout at 8°C, the NOEC was 5 mg/L; however, the NOEC for 1.06 g trout at 15°C was 10 mg/L. The linear relationship continues as 4.88 g trout at 8°C had a NOEC of 31.6 mg/L. The highest

NOEC found was with 12.89 g trout at 15°C, which was 79.4 mg/L. Low doses of Virkon® Aquatic were tolerated by steelhead with a limited exposure time.

Discussion

New Zealand Mudsnails

There were no significant differences between mortality rates at 10 and 20 g/L at all temperatures tested, 8, 15, and 22°C. The hazard rates illustrated that as concentration increased, NZMS mortality also increased. However, to achieve 100% mortality of all NZMS, a 20 g/L concentration of Virkon® Aquatic is recommended (Figure 1.1). Temperature was not a significant covariate in determining mortality of NZMS. The presence of neonates in the 20 g/L, 20 min recovery cup demonstrated that not all NZMS are dead within the exposure time. However, the NZMS are weak enough to die within 48 h. The presence of neonates in the recovery cups of the 20 g/L, 20 min exposure time, illustrates the importance of evaluating neonate survival and presence in testing, and is of concern in disinfection protocols. We think that the production of the neonates in the 20 g/L baths at 15 and 22°C were largely due to large NZMS, >4mm or not being exposed to the solution. Possible mechanisms to mitigate survival are to increase exposure time, remove large snails, as they are the most resistant to treatment, or do not allow dead snails to enter the waterway. The only snails tested with Virkon® previously did not have an operculum, which were the red-rim melania, *Melanoides tuberculata,* and the faucet snail, *Bithynia tentaculata*. Mitchell et al. (2007) reported 100% mortality of the red-rim melania when exposed 1 h to >5 g/L Virkon® Aquatic. When 10 *B. tentaculata* were exposed to 10 g/L Virkon® at approximately 20°C for 1 h, *B. tentaculata* showed no mortality (Mitchell and Cole 2008). Our results determined that higher concentrations of Virkon® Aquatic than previously tested were needed to achieve 100% mortality of NZMS especially when using short exposure times. At least a 20 min exposure time with a 20 g/L Virkon® Aquatic solution is recommended for use on gear to obtain 100% mortality of NZMS.

Quagga Mussels

Quagga mussel veligers were killed by concentrations ≥5 g/L with a short exposure time of 10 min. The variability in the percent mortality in veligers exposed to 1 g/L can be

attributed to veliger life stage. Larger veligers were found to be alive after 20 min exposure times whereas the smaller veligers were affected by Virkon® Aquatic. Adult quagga mussels were also killed after 30 min at 5 g/L concentration. A 20 g/L concentration was tested the most on adult quagga mussels (Table 1.6), which resulted in 100% mortality for all times tested; the minimum exposure time tested was 10 min. It is recommended that a 5 g/L concentration with an exposure time of 10 min be used to kill quagga mussel veligers and a 20 g/L concentration for 10 min be used to kill adult quagga mussels.

The results of the quagga mussel veliger toxicity support O' Connor et al. (2008) report, which showed that 5 mg/L of Virkon® Aquatic caused 100% abnormal embryo development in Sydney rock oysters. More experimentation with lower concentrations and times on all life stages of quagga mussels are recommended to develop a better model of survival, providing more certainty regarding use of lower concentrations for short time exposures. This study was only conducted at one temperature, so a broad range of temperature testing would also be applicable. The protocols used in this study are recommended for use in further studies. Zebra mussels also would be susceptible though more testing needs to be conducted.

Steelhead Trout

We observed considerable variation in the mortality of fish exposed to Virkon® Aquatic. Generally, as size of fish increased, higher concentrations of Virkon® Aquatic were needed to obtain mortality. The highest temperature, 15°C, also required a higher concentration of Virkon® Aquatic to attain mortality in steelhead. Some of the variation can be attributed to the allometric relationship of fish surface to length in the test organism (Newman and Heagler 1991). Larger fish had increased mucus production and a larger surface area for the active ingredients of Virkon® Aquatic to bind with, thus deactivating the chemical compared to the smaller fish. Virkon® Aquatic was deactivated by organic material breaking down into environmental salts (Western Chemical 2008). Fish metabolism is faster at higher temperatures, thus allowing reactions to occur, such as increased mucus production and perhaps epithelial cell production, reducing the chemical effect (Barton et al. 2002).

Virkon® Aquatic is recommended for use in and around aquatic environments. Studies determined that Koi could easily tolerate 5 mg/L Virkon® Aquatic solution in a flow through

system and that rainbow trout would survive exposure to 8 mg/L of Virkon® Aquatic in freshwater (Holmes 2006; Ron Hardy, personal communication). The sizes of fish tested were not reported in the above studies. Our studies results match the safety limits of rainbow trout, 8 mg/L; our NOEC was 10 mg/L in approximately 1 g fish at 8 and 15°C. Safety increases as the fish increases in size. During this study, we also noticed that the dilution ratio was also a factor in fish mortality. Studies described in this paper had volume-to-volume ratio of 1 part Virkon® Aquatic to 4 parts water. If dilution ratios were less, mortality did not occur, data not reported. The other oversight that occurred during the study was that the chemical was not homogeneous enough to measure quantities less than 5 g. There was too much variability observed in the composition of the concentrations to obtain consistent concentrations and results.

The results of this study can be applicable to biosecurity measures in fish hatchery facilities. If a container of Virkon® Aquatic solution is dumped accidentally into a raceway within a fish hatchery, then fish will be able to survive limited exposure to Virkon® Aquatic. For example, if 189 L of 10 g/L Virkon® Aquatic solution were spilled into a typical raceway containing 102,206 L, then the resulting mixed concentration would be 18.5 mg/L. Fish would only be affected at the source of the spill. Residence times for most raceways are less than an hour and dilution occurs as more water is supplied to the raceway. If sampling or protective gear is disinfected with Virkon® Aquatic, then it does not need to be washed before using in a large raceway or pond of fish. Rubber boots immersed into a disinfectant bath will hold about 0.04 L of disinfectant that could potentially be contaminating water (Schmidt et al. 2009). A 20 g/L footbath of Virkon® Aquatic could potentially result in a 7.8 µg/L concentration when worn immediately in a raceway. Fish with an average weight of 0.89 g can survive concentrations of 10 mg/L; therefore, gear does not need to be rinsed. This is especially true if the gear is used in a flow through system.

The active ingredient in Virkon® Aquatic is potassium permonosulfate and sodium chloride (Western Chemical 2008; Mitchell and Cole 2008). Potassium and sodium gradients control the neuron response in humans (Starr and Taggert 1998). The neuron response was first identified in *Aplysia californica* (sea slug or sea hare) (Russell and Brown 1972). Previous research conducted in the Moffitt lab (unpublished data) shows that NZMS gape

when exposed to low concentrations of potassium permanganate. There are also studies exposing NZMS to sodium chloride solutions resulting in the NZMS tightly closing their operculum (Oplinger et al. 2009). No mortality in NZMS was associated with exposure to low concentrations of potassium permanganate or sodium chloride. The likely mechanism of inhibition is through the potassium gradient of the natural inervation of closure of the operculum. The potassium in the Virkon® Aquatic solution does not allow the NZMS operculum to close, which lets the other components of Virkon® Aquatic to affect the NZMS. The low pH of Virkon® Aquatic, approximately 2, or the oxidizing system, could kill the NZMS with an open operculum.

D. polymorpha has a low tolerance for elevated potassium concentrations by destroying the integrity of the mussels' gill epithelium, which leads to asphyxiation (Fisher et al. 1991). However, exposure to 10 mg/L potassium permanganate at 17°C for 24 h produced less than 50% mortality of zebra mussel juveniles (Waller et al. 1996). Waller et al. (1996) also reported that when veligers were exposed to 10 g/L sodium chloride solutions for at least 12 h only 76.8% mortality was achieved. The combination of the components in Virkon® Aquatic was adequate to kill quagga mussels; perhaps the same mechanisms are occurring that might be occurring in NZMS. Histology was not conducted on the quagga mussels to determine which tissues were affected to cause mortality.

A study conducted by Avikainen et al. (1993) on disinfecting cucumber, lettuce, and cauliflower seedlings from fungi found that Virkon® efficacy increased as temperature increased. They reasoned that the low activity of Virkon® could be attributed to lower solubility of the chemical in cold water. In this study, we did not find a temperature effect. We made the chemical at approximately 20°C to ensure that the chemical was completely mixed and transparent before the organisms were exposed.

Virkon® Aquatic is an effective disinfectant that can be used to eradicate NZMS and quagga mussels, and safety limits show that it is safe in low concentrations around fish. In low concentrations, we have supported existing literature that shows Virkon® Aquatic is safe for use in and around aquatic environments. Tadpoles, *Bufo bufo* and *Rana temporaria*, were exposed to high concentrations, 4 g/L, and had high survival (Schmidt et al. 2009). Virkon®

Aquatic may have the potential to be used to kill a multitude of aquatic invasive species. Virkon® S, 10 g/L effectively killed after 20 s the zoosporangia *Batrachochytrium dendrobatidis*, the causative fungus of chytridiomycosis (Johnson et al. 2003). However, effects of 4 g/L Virkon® S on zooplankton survival was low, approximately 30% (Schmidt et al. 2009). Further testing of the efficacy of Virkon® Aquatic on other aquatic invasive species is recommended to enable broad-spectrum use. We determined the lethal dose for Virkon® Aquatic to reduce the uncertainty for managers when using Virkon® Aquatic. Managers could recommend this product for use within their biosecurity protocols to reduce their risk on NZMS. Ideally, water users need to use one method of disinfection to remove all aquatic invasive species from gear.

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Table 1.1. Summary of numbers, mean length and weight with standard error (SE) for steelhead trout tested with concentrations of Virkon® Aquatic.

Temp °C	Length mm (SE)	Weight g (SE)	Concentrations (mg/L)	Replicates
8	46.8 (3.2)	0.89 (0.24)	0, 5, 10, 40, and 80	2
15	50.2 (4.7)	1.06 (0.33)	0, 5.6, 10, 17.8, and 31.6	2
8	80.5 (6.0)	4.88 (1.19)	0, 19.95, 31.62, 39.81, 63.10 and 79.43	3
15	99.3 (16.3)	12.89 (3.55)	0, 19.95, 31.62, 39.81, 63.10, 79.43 and 200	3

Table 1.2. Results from the univariate chi square Wilcoxon test for the NZMS toxicity. $P<0.05$ was indicative of significant differences.

	Univariate chi squares for the Wilcoxon test				
Data	Variable	Test statistic	Standard error	Chi-square	*P*-value
0, 10, 20 g/L	Concentration	-595.30	25.40	549.1	<0.01
8, 15, 22°C	Temperature	-1369.00	180.90	57.28	<0.01
1, 2, 3	Replicate	-23.72	26.16	0.82	0.36
10, 20 g/L	Concentration	-27.93	13.50	4.28	0.04
8, 15, 22°C	Temperature	<0.01	151.20	<0.01	1
1, 2, 3	Replicate	<0.01	22.06	<0.01	1
0 g/L	Temp (8, 15, 22°C)	-1672.8	85.15	385.90	<0.01
10 g/L	Temp (8, 15, 22°C)	<0.01	106.40	<0.01	1
20 g/L	Temp (8, 15, 22°C)	<0.01	107.30	<0.01	1

Table 1.3. NZMS toxicity hazard rates resulting from the lifetest using the life method. Hazard statistics shows how increased time affects the risk in mortality for adult NZMS estimated from the data set. As the hazard value increases to 1, the risk of mortality increases. The full model includes all the data tested, including concentrations and temperatures. A is the hazard statistic by concentration, and B is by temperature. Periods indicate insufficient data to calculate the standard error.

A	Full model		Control		10 g/L		20 g/L	
Interval (min)	Hazard	Hazard standard error	Hazard	Hazard standard error	Hazard	Hazard standard error	Hazard	Hazard standard error
0 to 5	0	.	0	.	0	.	0	.
5 to 10	0.036	0.002	0.012	0.002	0.044	0.003	0.054	0.003
10 to 15	0.061	0.002	0.024	0.003	0.077	0.005	0.083	0.005
15 to 20	0.109	0.004	0.044	0.004	0.143	0.008	0.143	0.008
20 to 25	0.314	0.008	0.160	0.013	0.400	.	0.400	.

B	8°C temp		15°C temp		22°C temp	
Interval (min)	Hazard	Hazard standard error	Hazard	Hazard standard error	Hazard	Hazard standard error
0 to 5	0	.	0	.	0	.
5 to 10	0.038	0.003	0.022	0.002	0.051	0.003
10 to 15	0.053	0.004	0.051	0.004	0.080	0.005
15 to 20	0.089	0.006	0.117	0.007	0.119	0.007
20 to 25	0.311	0.014	0.240	0.014	0.400	.

Table 1.4. The number of live NZMS neonates in recovery cups after 48 h. Replicates are combined to determine effectiveness of Virkon® Aquatic on NZMS. The presence of live neonates indicates that the exposed NZMS were not killed immediately; the snails released their brood pouches and then died.

		Presence of live neonates		
Concentration (g/L)	Time (min)	8°C	15°C	22°C
0	5	19	114	269
0	10	37	115	271
0	15	45	107	195
0	20	49	113	317
10	5	4	9	109
10	10	10	35	66
10	15	1	16	58
10	20	5	5	35
20	5	7	9	60
20	10	0	2	37
20	15	1	2	12
20	20	0	2	6

Table 1.5. Summary of dissolved oxygen (DO), pH and conductivity, readings for mean and range from trials conducted on NZMS toxicity to Virkon® Aquatic , 10 and 20 g/L for all temperatures tested, 8, 15, and 22°C; N=11.

		DO (mg/L)	pH	Conductivity (mS/cm)
Control	Mean	9.03	7.93	195.85
	Range	6.63-10.5	7.07-9.97	156-253
10 g/L	Mean	8.35	2.25	5,663.23
	Range	6.71-9.93	1.97-3.26	1,997-6,552
20 g/L	Mean	6.78	2.01	10,319.94
	Range	2.21-9.27	1.76-2.45	1,128-11,637

Table 1.6. Mortality of quagga mussel veliger testing to various concentrations of Virkon® Aquatic, including the exposure times.

Concentration (g/L)	Time (min)	Number dead	Number alive	Percent mortality
0	5	0	60	0.0
	10	0	20	0.0
	15	0	20	0.0
	20	0	30	0.0
0.5	5	0	10	0.0
	20	0	10	0.0
1.0	5	2	26	7.1
	10	22	12	64.7
	15	4	10	28.6
	20	0	10	0.0
2.5	5	3	7	30.0
	10	31	1	96.9
	15	20	0	100.0
	20	20	0	100.0
5.0	5	110	7	94.0
	10	80	0	100.0
20	5	30	0	100.0

Table 1.7. Number of live and dead, and percent mortality for adult quagga mussels; approximately 10 randomly sized mussels per replicate, 11 replicates conducted.

Concentration (mg/L)	Time (min)	Number dead	Number alive	Percent mortality
0	30	0	134	0
20,000	10	112	0	100
	15	113	0	100
	20	118	0	100
	30	124	0	100

Table 1.8. Summary of tests of toxicity of steelhead trout by test concentration of Virkon® Aquatic, including time to death, percent mortality, length measured in millimeters (mm) with the standard error (SE), and the weight in grams (g) with the standard error for 0.89 g trout exposed to Virkon® Aquatic concentrations for 48 h at 8°C, N=6.

Concentration (mg/L)	Time to death (h)	Percent mortality	Length mm (SE)	Weight g (SE)
0	0	0	46.33 (4.80)	0.78 (0.30)
5	0	0	46.17 (2.79)	0.78 (0.20)
10	24	100	47.83 (0.55)	1.06 (1.33)
40	0.75	100	47.33 (3.44)	0.90 (0.22)
80	0.5	100	46.5 (3.33)	0.93 (0.30)

Table 1.9. Steelhead toxicity summary including time to death, percent mortality, length measured in millimeters (mm) with the standard error (SE), and the weight in grams (g) with the standard error for 1.06 g fish exposed to Virkon® Aquatic concentration at 15°C. [a]Figure 1.2. Steelhead toxicity of 1.06 g trout at 15°C 17.8 mg/L provides detailed time to mortality. [b] 20% mortality at 24.5 h in recovery.

Concentration (mg/L)	Exposure time (h)	N	Percent mortality	Length mm (SE)	Weight g (SE)
0	1	10	0	51.80 (3.910	1.132 (0.30)
0	2	10	0	51.20 (5.51)	1.10 (0.38)
0	3	10	0	49.60 (5.04)	1.03 (0.34)
0	5	10	0	48.8 (5.05)	0.91 (0.26)
5.6	1	10	0	51.9 (4.63)	1.15 (0.37)
5.6	2	10	0	50 (3.53)	1.00 (0.20)
5.6	3	10	0	50.2 (4.69)	1.05 (0.35)
5.6	5	10	0	49.9 (3.28)	1.03 (0.22)
10	1	10	0	51.8 (4.83)	1.15 (0.32)
10	2	10	0	50.3 (3.86)	1.08 (0.28)
10	3	10	0	50.67 (4.37)	1.09 (0.30)
10	5	10	0	48 (3.28)	0.86 (0.22)
17.8	1	16	25[a]	50.19 (5.65)	1.08 (0.39)
17.8	1.5	5	100[a]	49.6 (7.67)	1.14 (0.61)
17.8	2	5	100[a]	47 (4.85)	0.97 (0.42)
17.8	3	5	80[a]	53.6 (4.51)	1.23 (0.38)
31.8	0.5	5	100	49.7 (4.41)	1.17 (0.32)
31.8	0.75	5	80[b]	50.2 (7.26)	1.25 (0.54)

Table 1.10. Steelhead toxicity summary including time to death, percent mortality, length measured in millimeters (mm) with the standard error (SE), and the weight in grams (g) with the standard error for 4.88 g trout exposed to Virkon® Aquatic concentrations at 8°C, N=24. [a]Figure 1.3. Steelhead toxicity of 4.88 g trout at 8°C provides detailed time to mortality.

Concentration (mg/L)	Exposure time (h)	Percent mortality	Length mm (SE)	Weight g (SE)
0	3.5	0	80.54 (5.32)	4.43 (0.84)
19.95	3.5	0	81.04 (5.69)	4.56 (0.98)
31.62	3.5	0	78.92 (5.36)	4.32 (0.82)
39.81	3.5	54.2[a]	82.58 (7.06)	5.44 (1.52)
63.1	3.5	100[a]	78.58 (6.45)	4.95 (1.22)
79.43	0.75	100	81.17 (5.77)	5.59 (1.19)

Table 1.11. Steelhead toxicity summary including time to death, percent mortality, length measured in millimeters (mm) with the standard error (SE), and the weight in grams (g) with the standard error for 12.89 g trout exposed to Virkon® Aquatic concentrations at 15°C.

Concentration (mg/L)	Exposure time (h)	N	Percent mortality	Length mm (SE)	Weight g (SE)
0	3.5	8	0	105.75 (11.95)	10.37 (3.98)
19.95	3.5	16	0	107.38 (14.55)	11.50 (5.33)
31.62	3.5	16	0	97.81 (2.84)	8.24 (2.84)
39.81	3.5	24	0	101.75 (12.21)	9.24 (3.65)
63.1	3.5	24	0	96.13 (11.14)	7.99 (3.00)
79.43	3.5	16	0	100.19 (13.39)	8.79 (3.66)
200	0.25	24	100	85.75 (24.16)	8.18 (1.70)

Table 1.12. The no observed effects concentration with the tested exposure time to Virkon® Aquatic for the different sizes of steelhead tested at either 8 or 15°C.

Average weight (g)	Temp. (°C)	No observed effects conc. (mg/L)	Exposure time (hr)
0.9	8	5.0	5
1.1	15	10.0	5
4.9	8	31.6	3.5
12.9	15	79.4	3.5

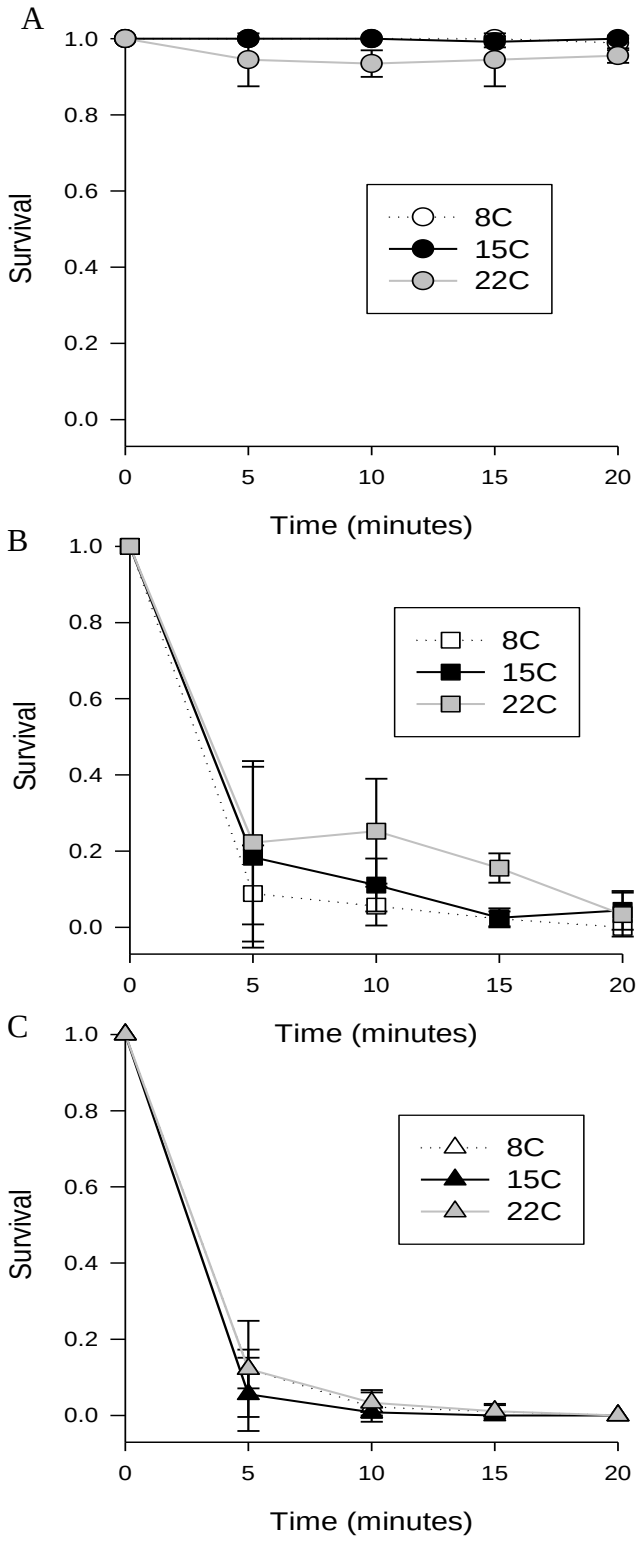

Figure 1.1. Survival of NZMS at three temperatures, 8, 15, and 22°C exposed to control treatment (A) and Virkon® Aquatic at 10 g/L (B) and 20 g/L (C).

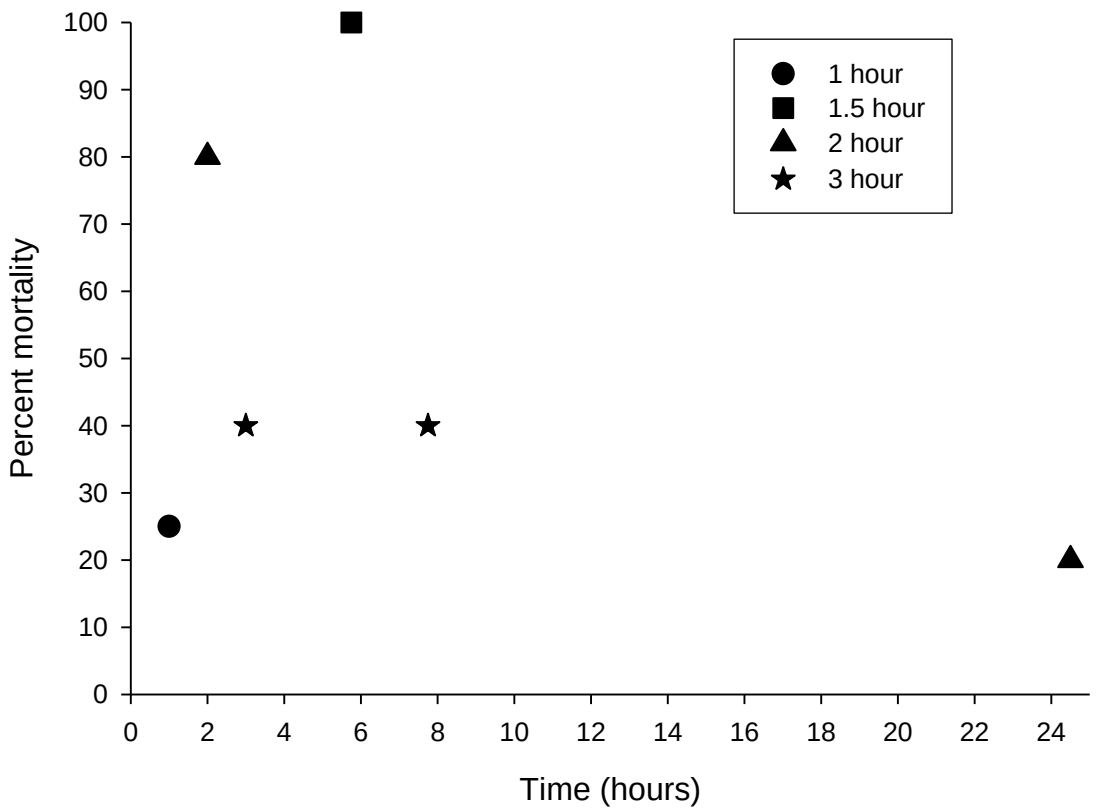

Figure 1.2. Time to death for 1.06 g steelhead fish at 15°C exposed to 17.8 mg/L Virkon® Aquatic in static conditions for each exposure time. Time to deaths greater than exposure time indicates that moribund fish died in recovery.

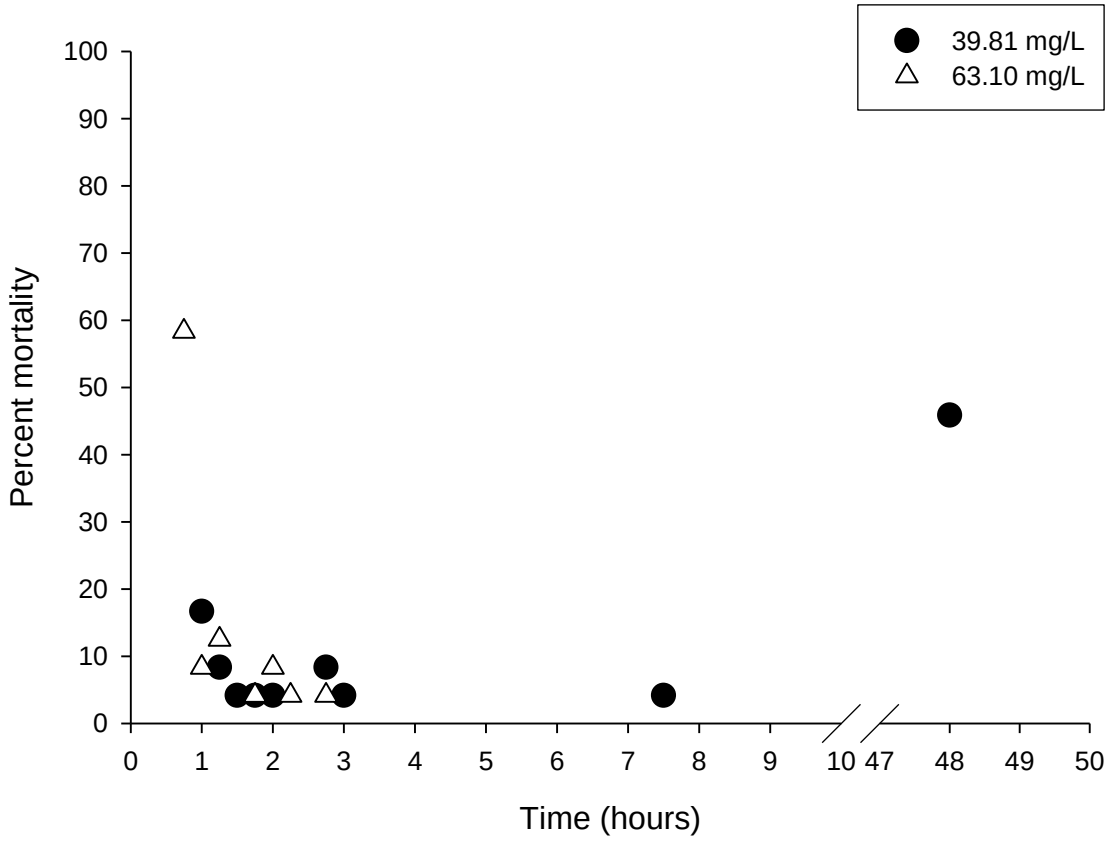

Figure 1.3. Time to death for the 4.88 g steelhead fish at 8°C (N=24) exposed to 39.81 mg/L and 63.10 mg/L Virkon® Aquatic in static conditions for 3.5 h in static conditions. Time to deaths greater than exposure time indicates that moribund fish died in recovery. The fish that was exposed to 39.81 mg/L died between 36 and 48 hours in recovery.

Chapter 2: Efficacy of Virkon® Aquatic in disinfecting three types of wading gear infested with New Zealand mudsnails

Abstract

New Zealand mudsnails (NZMS) *Potamopyrgus antipodarum* are an invasive mollusk that can easily be transported to new areas by attaching to wading gear. These small operculate snails have high reproductive rates, are parthenogenic, and can rapidly establish populations in suitable habitats. In laboratory trials, we evaluated the differences in colonization rates and the efficacy of application methods of disinfection on felt, neoprene, and rubber soled boots infested with NZMS using Virkon® Aquatic. In addition, we determined the potential harm to gear from these methods and the oxidative activity of disinfectant solutions contaminated with two types of organic material. Bath application was a more reliable method for disinfecting waders over spray applications. A bath exposure of 15, 20, and 30 min in 20 g/L Virkon® Aquatic successfully killed all NZMS (adults, juveniles, and neonates). Felt soled boots were the most vulnerable to infestation, followed closely by neoprene and then rubber soled wading boots, but all boots became infested when in contact with NZMS. Boots and waders were not damaged easily by the use of Virkon® Aquatic. Organic material deactivated Virkon® Aquatic in 4-24 h. Our results provide strong evidence that bath disinfection procedures are preferable to spray applications to assure biosecurity in field and hatchery settings.

Introduction

Many non-native species have been introduced into North America through various vehicles such as boats, wading gear, or through vectors from stocking of fish (Mills et al. 1993; Ricciardi and Rasmussen 1998). Transport of the New Zealand mudsnails, *Potamopyrgus antipodarum* (NZMS) in western North America and in other regions in the world is associated with human activities, including recreational fishing and aquaculture operations (Bowler 1991; Dwyer et al. 2003; Richards et al. 2004; Hosea and Finlayson 2005; Loo et al. 2007; Alanso and Castro-Diez 2008; Bruce and Moffitt 2010). A method of ensuring that there are no organisms present is to use a chemical disinfectant on potentially

contaminated gear. Chemical disinfectants are needed that are effective, safe, and practical for field and hatchery applications (Dwyer et al. 2003; Hosea and Finlayson 2005). Tested chemical disinfectants that have been effective at killing a high percentage of NZMS during a 15 min exposure time are household ammonia, benzethonium chloride, Bleach®, copper sulfate, Formula 409® All Purpose Cleaner, Formula 409® Degreaser and Disinfectant, hyamine, hydrogen peroxide, Pine-Sol®, potassium permanganate, Sparquat 256®, and Virkon® Aquatic (Hosea and Finlayson 2005; Schisler et al. 2008; Oplinger and Wagner 2009; Stockton, this thesis). However, some of these disinfectants are not environmentally safe, harsh on fishing gear, or not labeled for use.

Hosea and Finlayson (2005) studied the effect of the most effective disinfectants that killed NZMS. These disinfectants were copper sulfate, benzethonium chloride, Formula 409® Degreaser and Disinfectant, Pine-Sol®, and Bleach®, and they tested the exposure effects on wading gear. They observed that the tested chemical disinfectants except copper sulfate caused some damage to wading gear. Copper sulfate, however, is not highly recommended because of the associated aquatic toxicity (Oplinger and Wagner 2009). Schisler et al. (2008) determined that Formula 409® was not as effective at killing NZMS as Hosea and Finlyason described; however, Schisler et al. used Fomula 409® All Purpose Cleaner instead of the Degreaser and Disinfectant. Formula 409® All Purpose Cleaner was effective at killing NZMS at a 100% concentration instead of a 50% concentration as recommended by Hosea and Finlayson (2005). Oplinger and Wagner (2009) also tested Formula 409® Degreaser and Disinfectant at 100% and determined that a 15 min exposure time was 100% effective in killing NZMS. Formula 409® caused cracks on rubber waders and removed the water repellency of the waders after seven exposures (Hosea and Finlyason 2005), but is also classified as a quaternary ammonia compound, which are not labeled for use in aquatic systems (Schisler et al. 2008).

Concentrations of Virkon® Aquatic have been shown to be effective on NZMS when target snails are immersed in bath exposures of 20 g/L for 20 min (Stockton, this thesis). Virkon® Aquatic (reformulated from Virkon® S in 2007) is one of very few US Environmental Protection Agency-registered disinfectants that are labeled specifically for use in aquaculture facilities (Mainous et al. 2010). Reformulation of Virkon® S, involved the

removal of the indicator dye and perfume, but all other constituents remained the same in Virkon® Aquatic. It is labeled for use on aquatic bacterial, fungal, and viral pathogens (DuPont 2010) and this chemical is composed of an oxidizing agent (triple salt of potassium monopersulphate), two organic acids (sulfamic acid and malic acid), a buffer (sodium hexamethaphosphate), and a surfactant (sodium alkyl benzene sulphonate) (Western Chemical 2008). Active ingredients of Virkon® are potassium monopersulphate and sodium chloride, 21.9% (Mitchell and Cole 2008). The mode of action is by oxidizing proteins and other components of cell protoplasm, resulting in inhibition of enzyme systems and loss of cell-wall integrity (Curry et al. 2005). The recommended concentration for disinfecting most surfaces for a majority of the organisms is a 10 g/L concentration with an exposure time of at least 5 min (Western Chemical 2008).

The efficacy of Virkon® S and Virkon® Aquatic have been evaluated in several studies; finding that they are effective disinfectants for use around aquatic systems. A 10 g/L concentration of Virkon® S is 100% effective in killing bacteria, *Edwardsiella* spp. in 1 min exposure (Mainous et al. 2010), *Batrachochytrium dendrobatidis* with a 20 s exposure (Johnson et al. 2003) and *Flavobacterium psychrophilum* with a 10 min exposure (Madsen et al. 2005). Kilroy et al. (2007) determined that *Didymosphenia geminate* was effectively killed by 10 g/L Virkon® S in 30 s. Schmidt et al. (2009) showed that zooplankton could be reduced with a 4 g/L Virkon® S concentration with a 1 week exposure. O'Conner et al. (2008) determined that Virkon® S and Virkon® Aquatic concentration of 0.05 g/L after 48 h would cause 100% mortality in oyster veligers. Snails when exposed to 5 g/L Virkon® S for 24 h had variable mortality (Mitchell et al. 2007; Mitchell and Cole 2008). Vertebrates may be more tolerant to this chemical, as Schmidt et al. (2009) found tadpoles survive limited exposures to 4 g/L Virkon even after a week exposure. Unpublished studies on fish pathogens are reported by the manufacturer, DuPont™, reporting that a 10 g/L concentration of Virkon® is effective; some of these include infectious salmon anemia virus, infectious pancreatic necrosis birnavirus, Rhabdvirus, *Aeromonas* spp., *Pseudomonas* spp., *Renibacterium salmoninarum, Vibrio anguillarum,* and *Yersinia ruckeri* (DuPont 2011). Virkon® S was used in the Antarctic for successfully disinfecting boots of visitors to reduce the risk of translocation of microbial pathogens (Curry et al. 2005).

Virkon® Aquatic is deactivated over time and is reported to be stable for up to 7 days, but the effectiveness is influenced by organic material and UV light (Syndel 2008). The active ingredients of Virkon® Aquatic are potassium permonosulfate and sodium chloride. These compounds undergo catalytic decomposition by iron or other transition metal salts to form potassium sulfate and oxygen (Syndel 2011a). The other ingredients are composed of inorganic components, which have decomposition mechanisms that are abiotic, resulting in inorganic salts (Syndel 2011b). The two organic acids, malic and sulphamic acid, are both classified as biodegradable (Syndel 2011b). To dispose of Virkon® Aquatic, DuPont™ recommends land application or disposal down a foul sewer drain using water to dilute (Bradan Limited 2010). Severs and Lamontagne (2002) reported that Virkon® Aquatic is safe on gear, with limited use on metals; however, little is known about the details regarding its effect on wading gear or the effectiveness of this chemical used in spray applications. There is little documentation about the specifics of deactivation of Virkon® Aquatic by organic material.

Many federal and state agencies, including fish hatcheries, recommend the use of disinfectants to disinfect contaminated gear. Biosecurity protocols in fish hatcheries also recommend the use of disinfectants to clean gear that is used throughout the facility to limit the spread of diseases and non-native species. NZMS can be introduced on contaminated gear; their small size and pointy hard shell enable NZMS to be transported on wading gear that has cracks, crevices, layers of materials, holes, or laces (Dwyer et al. 2003; Richards et al. 2004; Hosea and Finlayson 2005). NZMS can be found in the felt of wading soles, traction of rubber soles, layers between the tongue on the boots, laces, gravel guards and many other places on waders (Dwyer et al. 2003). States, such as Alaska, Vermont, Maryland, Oregon, Montana, and Idaho are considering banning the use of felt soles in their waterways because of transport of invasive species, especially *Didymosphenia geminate*. Maryland initiated their ban March 2011; Vermont's ban started April 2011 and Alaska's ban will start January 2012, pending no other legislative actions (Center for Aquatic Nuisance Species 2008).

The objectives of this study were to test three types of wading boots: those with felt, neoprene, or rubber soles to determine 1) the likely colonization of NZMS, 2) the

effectiveness of disinfecting with spray or bath application of Virkon® Aquatic , and 3) the effect of the disinfection procedures on the reliability of the gear to function as a barrier to water. We also determined the effectiveness of solutions of Virkon® Aquatic using three scenarios of contamination with organics.

Methods

Test Substance

Virkon® Aquatic (lot # 2258523 or 2258515) (Western Chemical, Ferndale, WA) was used for testing. Powdered Virkon® Aquatic was measured (0.01 g) and mixed with distilled water at 20°C in volumetric flasks or large plastic totes (11.4 or 32 L) depending on the amount of solution needed; concentrations were weight to volume proportions. At least an hour was allowed for all solutions to acclimate to test temperature and to fully activate. Solubility and concentration were verified with Virkon® Aquatic test strips (Western Chemical, Ferndale, WA) before use to ensure chemical was mixed and active.

Experimental Animals

New Zealand mudsnails were collected from springs at Hagerman National Fish Hatchery (HNFH) on 20 January through 13 July 2010, packaged at the hatchery in moist towels, placed into plastic bags, and shipped in coolers to the University of Idaho fisheries wet laboratory. Upon arrival, the snails were washed through a 2.0 mm and 0.85 mm sieve to separate snails from the mud. There were other aquatic invertebrates such as other snails, Trichoptera, Oligochaeta, and Chironomidae mixed in with the NZMS. They were transferred into 2 L containers with dechlorinated, aged well water equilibrated to 15°C. Snail containers were placed into an aquarium to assure security, and the water in each container was changed every other day. Temperature in the test room was maintained at 15°C throughout trials monitored and recorded every 15 min with a HOBO data logger (Onset Computer Corporation, Bourne, MA). A natural photoperiod for the latitude of HNFH was maintained in the test room. NZMS were retained in the laboratory for no more than three weeks.

Experimental Design

Spray versus bath- Tests to compare the mortality of NZMS in a bath versus spray application were conducted using two concentrations of disinfectant in small containers at 15°C in triplicate. Test solutions were 10 g/L and 20 g/L of Virkon® Aquatic. Approximately ten NZMS >2 mm were each placed into a 30 mm diameter glass petri dish or a 150 mL acid washed glass beaker test container for each test replicate trial. For the bath application test, 100 mL of a test solution was poured into the glass beaker. A spray application test was begun by spraying 2 sprays (~2 mL) from a 1 L squirt bottle with test solution onto the petri dish. To serve as controls, we treated snails in beakers and petri dishes with deionized water. Each trial was conducted three times for 9 test applications tested at each concentration (Table 2.1).

The test was ended when NZMS were removed from each container after 15, 20, or 30 min for bath application and after 20, 30, or 40 min for spray application by pouring the test system into small sieve to recover all NZMS and rinsing the snails three times with clean, aged laboratory water. Test system liquid was saved in small plastic cups for water chemistry analysis immediately, 24, and 48 h post testing. Snails were then placed into small plastic cups (250 mL) with aged water, and mortality was assessed immediately, 24, and 48 h post testing. Snails in each cup were inspected with the aid of a dissecting microscope to observe snail movement, and individual snails that were not active were probed to elicit movement or tactile response. Neonates released from test snails were counted at each time interval and assessed for mortality.

Disinfection of three types of naturally infested wading boots- To evaluate efficacy of disinfecting three types of wading boot surfaces in bath applications, we tested a variety of boots obtained from field workers. Felt, neoprene, and rubber soled wading boots, 18 of each type (54 boots total) were tested to determine if bath applications of Virkon® Aquatic would kill exposed NZMS, or if the NZMS would crawl out of the bath. Boots were cut at the knee; washed and numbered with permanent marker prior to the experiments with 20 g/L Virkon® Aquatic to ensure that there was no contamination of the NZMS container.

After each application, wading gear was inspected to determine if Virkon® Aquatic caused any damage during the experiment. Prior to any experiments with Virkon® Aquatic, all waders were pre-inspected and type of damage and location was recorded, such as cracks, holes, stains, tears, and discoloration. After the experiments, the boots were inspected; the damage was ranked as none if there were no cracks, discoloration, loss of flexibility, tearing, or stitching failure, and the gear could still repel water; mild if the wading gear had slight discoloration, small cracks, or tearing, and the gear still repelled water; or severe if the wading gear showed signs of high discoloration, flexibility loss, large cracks and tears, stitching failure, and the boots did not repel water or leaked significantly. All boots were reused in the experiments until no longer needed.

To allow for colonization, the test NZMS were transferred into a 114 L tote (55 x 37 x 43 cm) two days prior to testing. Snails were maintained in dechlorinated, aged well water equilibrated to 15°C. Three boots with the same sole type were placed into each tote containing live NZMS at the same time. Prior to the waders being placed into the NZMS tote, the system was disturbed using a plastic spoon to scrape and swirl the NZMS off the sides of the tote. The boots were colonized naturally by NZMS for 20 to 60 min. Wading boots were weighted down with plastic bags containing bricks or gravel to ensure that the sole of the boots were touching the bottom of the NZMS tote.

The NZMS colonized boots were transferred to a disinfection bath with tap water (control) or 20 g/L Virkon® Aquatic. The boots were exposed for 15, 20, or 30 min. Tests were ended when the boots were removed from the disinfection bath, and inspected visually. Boots were placed into a rinse system containing three separate baths with tap water. Waders were rinsed thoroughly and visual inspected to ensure all snails were removed. Each rinse bath was then sieved and any NZMS that fell off in the rinse system were placed in a recovery cup. The disinfection baths were also sieved to collect NZMS that were from the wading boots. All snails recovered were rinsed in three separate baths and placed into recovery cups with aged laboratory water. Disinfection bath snails were kept separate from the snails of the rinse baths. The disinfection bath was briefly inspected to ensure all snails were removed, and was then reused for the remaining exposure times for the day. The final

number of NZMS tested per exposure time per wading boot type was at least 100 NZMS (Table 2.1).

NZMS placed into the recovery cups were assessed to determine mortality immediately, 24, and 48 h post exposure. Snails in each cup were inspected with the aid of a dissecting microscope to observe snail movement, and individual snails that were not active were probed to elicit movement or tactile response. Any neonates released from test snails were counted at each time interval and assessed for mortality.

Colonization likelihood of NZMS on the three different boot sole types- A controlled 3x3 factorial designed study was conducted to determine the colonization likelihood of NZMS on the three different boot sole types. Two densities of NZMS were evaluated: low (50 g/114 L tote) or high (100 g/114 L).

Three boots, each with the different sole types, neoprene, felt, and rubber, were placed into the 114 L tote containing live NZMS at the same time. Boots were placed in a different order within the NZMS tote each time to account for placement variation; 6 combinations were conducted. The boots were allowed to be naturally colonized for 30 min. The boots were then removed and placed into a 20 g/L Virkon® Aquatic disinfecting bath for 20 min. Rinse and recovery protocols were followed. NZMS adults, juveniles and neonates, and aquatic insects were observed and counted. Test organisms were then destroyed; no recovery was assessed. Testing was replicated three times at each density of NZMS in the tote (Table 2.1).

Effect of organic contaminants on oxidizing activity- To determine the effect of organic contaminants on the effectiveness of the agent, we tested the decay of solution activity with introduction of NZMS, peat moss, or stream mud. Commercially available peat moss was purchased and stream mud was collected from nearby Paradise Creek, Moscow, Idaho. To determine the proportion of organic content, representative samples were dried at 105°C to a constant weight and then the loss-on-ignition method was used to determine the amount of organic material (ASTM 2007). Samples were combusted in a muffle furnace at 440°C for about 4 h until a constant weight of ash. Samples were then cooled in a desiccator and

weighed. Organic material content was calculated as the difference between the initial dry weight and final sample ash weight divided by the initial sample dry weight to determine the percentage organics (Schumacher 2002).

The available oxidizing agent (active Virkon® Aquatic) was determined using the chlorine (residual) iodometric method I (Method 4500-ClB). The iodometric method, a titration that used the principle that chlorine will liberate free iodine from potassium iodide solutions at pH 8 or less, where the liberated iodine is titrated with a standard solution of sodium thiosulfate with starch as the indicator (Clesceri et al. 1996). A 15 g sample of the tested 20 g/L or a 30 g sample of the tested 10 g/L Virkon® Aquatic solution was added to a beaker, then approximately 1 g potassium iodide, KI (lot # 143188, Sigma Aldrich, Inc.) and 10 ml of 200 mL/L sulfuric acid, H_2SO_4, was added and mixed. The sample was immediately titrated with 0.1 N sodium thiosulfate, $Na_2S_2O_3$, (lot # 100705, Fisher Scientific) until the yellow color was almost gone. Then 2 to 4 ml of starch indicator solution 1% w/v (lot # 0155-03, Fisher Scientific) was added to make a blue color and titration continued until the solution remained clear for at least 30 s. The percent available active oxidizer (%AO) was calculated with the following equation provided by Thomas P. Tufano (Laboratory Scientist, DuPont Chemical Solutions Enterprise, personal communications):

$$\%AO = \frac{V_1 * N_{thio} * 100 * 152.17\frac{g}{mole}KHSO_5 * 16\frac{g}{mole}O_2}{1000mL * W_s(g) * 2e^- * 152.17\frac{g}{mole}KHSO_5}; \qquad \text{Equation 1}$$

where V_1=volume of sodium thiosulfate mL;

N_{thio}= normality of sodium thiosulfate;

W_s= weight of Virkon® Aquatic sample tested.

In the study to determine the effect of NZMS as an organic contaminant, 100 mL of a 20 g/L Virkon® Aquatic solution was poured into 150 mL glass beakers. There were two glass beakers replicates per day with and without 10 NZMS. Four beakers were tested with the iodometric method each day for up to seven days.

In the study with the peat moss and stream mud, 10 g of dried peat moss or stream mud was added to 10 and 20 g/L Virkon® Aquatic solutions in 1 L glass beakers with stir bars on a stir plate. Samples were stirred so that all organic material was mixed; 15 or 30 g samples from the 20 and 10 g/L solutions were measured into three glass beakers from each test system. The consistently stirred test solutions containing organics were analyzed with the iodometric method at 0.5, 2, 4, 24, and 48 h. A 10 and 20 g/L Virkon® Aquatic solution with no organic material was also tested; times that samples were measured for iodometric analysis were 0, 4, 24, 48, 72, 120, and 168 h. Test were repeated three times over three weeks (Table 2.1).

Deterioration of waders with repeated disinfecting- A small experiment was conducted to determine if the test solution would deteriorate nylon breathable or neoprene waders. Two pairs of new nylon breathable and one pair of neoprene waders (CADDIS Systems, La Pine, OR) were exposed to a Virkon® Aquatic solution to determine if the disinfectant would cause waders to deteriorate. Test solution was a 20 g/L concentration of Virkon® Aquatic in a 30.2 L plastic tote. Waders were soaked in the tote at 15°C, weighted down to ensure all material below the front chest pocket was disinfected. Waders were soaked for 20 min and then examined for holes, discoloration, tears, failure of water proofing, or any other signs of wear. One pair of nylon breathable waders was rinsed with tap water; the other pair of nylon breathable and neoprene waders were not rinsed. The waders were then hung up to dry for at least an hour. Waders were worn into a large tank full of water for 5 min to determine if there were leaks. New Virkon® Aquatic solutions were made weekly. Testing continued until waders exhibited damage.

Water Chemistry

Temperature, pH, and conductivity (mS/cm) were recorded on one replicate container from only bath application trial involving NZMS with a YSI 556 MPS multiprobe (YSI, Inc., Yellow Springs, Ohio). Water chemistry was not conducted on concentrations used in testing the colonization likelihood of NZMS on the three different boot sole types.

Statistical Analysis

Disinfection of three types of naturally infested wading boots- One-way analysis of variance (ANOVA) tests were used in SAS 9.2 (SAS Institute Inc. 2002-2008) using PROC GLM to evaluate the unbalanced model of total number of NZMS colonizing each sole type as related to sole type. Numbers of colonizing NZMS in each replicate were combined to compare the total number colonizing each boot type. An ANOVA was also conducted to evaluate the total number of NZMS colonizing each sole type per replicate as related to sole type. All data were analyzed at the $\alpha = 0.05$ significance level.

Colonization likelihood of NZMS on the three different boot sole types- We used a one way ANOVA to evaluate the main effects of density (high or low) and total colonization (combined high and low densities) as it contributed to the number of adult NZMS (adultNZMS), juvenile and neonate NZMS (other NZMS), aquatic insects (no AI) and total invertebrates (total invert) colonizing each boot type (sole). A Tukey's HSD was used to determine significant differences among the different types of wader soles for each category of colonizing adult NZMS, juvenile and neonate NZMS, aquatic insects, and total invertebrates. Data were analyzed at the $\alpha = 0.05$ significance level and normal quantile plots were used to check assumptions of normality and distribution of errors.

Effect of organic contaminants on oxidizing activity- Repeated measures analysis of variance with PROC GLM in SAS was used to determine the correlation between organic type and concentration (treatment) and the percent active oxidizer over time. The four treatments that were compared over the three trials were the stream mud in 10 g/L, stream mud in 20 g/L, peat moss in 10 g/L, and peat moss in 20 g/L. The time intervals were 0.5, 2, 4, 24, and 48 h. A polynomial transformation was used to account for the uneven time intervals.

Results

Spray Versus Bath

A bath of 20 g/L for 30 min was effective in killing all stages of NZMS on boots (Table 2.2); other times of exposure had some surviving snails or neonates. There were no neonates

found alive in the 20 g/L solution of Virkon® Aquatic. Live neonates in recovery cups were observed with adult NZMS in control and 10 g/L bath applications. Spray applications were not effective in killing NZMS. Neonates were released and survived at all concentration and exposure times studied in spray testing.

The pH, temperature, and conductivity measurements of the test solutions were consistent and varied little throughout the three trials. The temperature of all solutions was 15°C before and after testing. pH was an average of 7.16, 2.48, and 2.35 for control, 10, and 20 g/L Virkon® Aquatic. Conductivity increased with increasing concentrations; control water's conductivity was 268 mS/cm, 10 g/L was 8,123 mS/cm, and 2 g/L was 14,500 mS/cm. Exposure to NZMS did not change the results of the temperature, pH, or conductivity (Table 2.3).

Disinfection of Three Types of Naturally Infested Wading Boots

The 20 g/L concentration was 100% effective at each time interval in all studies conducted on rubber, felt, and neoprene soled wading boots (Table 2.4). No neonates were found in bath exposures at 20 g/L Virkon® Aquatic, indicating that no NZMS were alive when put into recovery. The number of NZMS that attached to the felt soled boots after three experiments were 108, 116, 150, 174, 233, and 247 snails (Table 2.4). NZMS that attached to the rubber soled boots after four experiments were much lower 48, 56, 113, 207, 233, and 532 (Table 2.4) than the amount attached to felt or neoprene soled boots. Neoprene soled boots had the following number of NZMS attach after three experiments: 152, 216, 235, 267, 730, and 967 snails (Table 2.4). Some NZMS mortality was observed in control solutions with no Virkon® Aquatic. All NZMS exposed to 20 g/L Virkon® Aquatic were killed at all exposure times and no neonates were found indicating that the bath application is effective in killing NZMS passively attached to all types of wading soles.

The ANOVA determined that there was a significant difference between the total numbers of NZMS colonizing each boot type (Table 2.5). Least square mean numbers of NZMS that were attached to each sole type are listed; the exposure times, concentrations, and replicates were combined, since none of these factors effected the colonization of NZMS on to wading boots. When the number of replicates was accounted for because of the different

amount of replicates conducted, the ANOVA still calculated significant differences between the wading sole types (Table 2.5).

There were no differences in pH, temperature, and conductivity of the test solutions throughout trials. Felt, rubber, and neoprene soled wading boots did not show any damage caused by 20 g/L Virkon® Aquatic. Pre-observation and post-observation tests on damages revealed that the holes, cracks, stains, discoloration, and tears did not increase in severity throughout the study. There was also no waterproofing failure in tested wading boots.

Colonization Likelihood of NZMS on the Three Different Boot Sole Types

NZMS of all sizes, including neonates, and aquatic insects, specifically caddis flies colonized the boot soles. The ANOVA showed that there was a significant difference between the wading sole types for the total number of invertebrates colonizing the wading boot, $F = 45.32$, $P < 0.0001$. Tukey's HSD showed that the total numbers colonizing felt soled wading boots was significantly different from rubber and neoprene soled wading boots. The rubber and neoprene soled wading boots were not significantly different from each other (Table 2.6). When only the numbers of adult NZMS colonizing the different sole types were analyzed, the ANOVA showed that there was a significant difference between the sole types, $F = 12.09$, $P < 0.0001$ (Table 2.6). Tukey's HSD showed that felt and neoprene soled wading boots were significantly different from rubber soled wading boots. The ANOVA showed that there was a significant difference between the sole types in the number of juvenile and neonates colonizing, $F = 65.11$, $P < 0.0001$. Juvenile and neonates colonized each wading sole type in significantly different amounts, with neoprene soled boots having the lowest amount of juvenile and neonate NZMS attached as determined by Tukey's HSD. Lastly, when the number of aquatic insects colonizing each wading sole type was analyzed there was a significant difference within the group, $F = 26.56$, $P < 0.0001$. The Tukey's HSD determined that the numbers of aquatic insects colonizing felt soled boots were significantly different from the numbers colonizing neoprene and rubber soled boots (Table 2.6).

When density of the aquatic invertebrates was analyzed, we found that the high-density contributed to the significant differences more than the low-density. The high-density ANOVA shows that there was a significant difference between the wading sole types for the

total number of invertebrates colonizing the wading boot, F = 78.38, *P* <0.0001. Tukey's HSD for the high-density showed that the total number of aquatic invertebrates colonizing felt soles was significantly different from the number colonizing rubber and neoprene soled wading boots. The low-density ANOVA shows that there is not a significant difference between the wading sole types for the total number of invertebrates colonizing the wading boot, F = 1.5, *P*= 0.2258 (Table 2.6). When only the numbers of adult NZMS colonizing the different sole types from the high-density were analyzed, the ANOVA determined that there was a significant difference between the sole types, F = 15.95, *P* <0.0001 (Table 2.6). Tukey's HSD showed that all wading sole types were significantly different from each other in the number of adult NZMS colonizing each sole type. The low-density analysis of number of adult NZMS colonizing each wading type did show a significant difference within the sole types, F =17.52, *P* <0.0001; Tukey's HSD determined that the number of adult NZMS colonizing felt and rubber soled boots were significantly different from the number colonizing neoprene soled boots. High-density juvenile and neonates colonized each wading sole type in significantly different amounts, F = 27.49, *P* <0.0001, with felt soled boots having the highest amount of juvenile and neonate NZMS attached. The low-density analysis showed that the number of juveniles and neonates colonizing each boot type also had a significant difference between the sole types, F = 41.28, *P* <0.0001. Tukey's HSD determined that the number of juvenile and neonate NZMS colonizing felt and neoprene soled boots were significantly different from the number on rubber soled boots. Lastly, when the numbers of aquatic insects colonizing each wading sole type at high-density (F = 30.25, *P* <0.0001) and low-density (F = 10.54, *P* <0.0001) were analyzed there were significant difference within the groups. The Tukey's HSD determined that in both densities, the numbers of aquatic insects colonizing felt soled boots were significantly different from the number of colonizing neoprene and rubber soled wading boots (Table 2.6).

Effect of Organic Contaminants on Oxidizing Activity

The 20 g/L solutions with and without NZMS had some variability, but stayed above the level of inactivity. If the percent AO was above 9.0, then the Virkon® Aquatic solution was considered active (Jeffery Odle, DuPont Animal Health Solutions, Wilmington, DE, personal communications). The organic loading of the NZMS was not enough to deactivate Virkon®

Aquatic (Figure 2.1). When Virkon® Aquatic was undisturbed compared to being constantly stirred; the undisturbed solution had higher percent AO (Figure 2.1). A 10 g/L Virkon® Aquatic solution had more percent AO than a 20 g/L solution over time, Figure 2.1. Organic material deactivated Virkon® Aquatic solutions after 4 to 24 h of exposure (Table 2.7 and Figure 2.2). The amount of organic material in peat moss was 92.92% and paradise creek mud was 3.87%. Peat moss deactivated the 10 and 20 g/L solutions very quickly, Figure 2.2. The Paradise Creek stream mud deactivated the 10 g/L Virkon® Aquatic solution much more than the 20 g/L solution; the difference is apparent at 48 h. Standard error for this concentration is very low (Table 2.7). The repeated measures ANOVA showed that there was a time and treatment effect, indicating the time and treatment were significant variables. The Wilk's Lambda statistic for the hypothesis of no time effect was significant, F = 56.22 and *P*= 0.0002; and for the hypothesis of no time by treatment effect, the Wilk's Lambda statistic was also significant, F =3.39 and *P*= 0.0175. The data fit the model, satisfying the criteria for the Sphericity tests. The repeated measures ANOVA indicated that the linear part of the polynomial model was significant, but the quadratic, cubic, or quartic trends were not significant. Figure 2.2 shows the least square means of percent available oxidizer for each treatment over time.

Deterioration of Waders with Repeated Disinfecting

The nylon breathable pair of waders that was not rinsed after every exposure leaked behind both knees after the 29th exposure to Virkon® Aquatic. The waterproofing sealant seemed to disintegrate from around the knees. After 30 exposures to 20 g/L Virkon® Aquatic, the crotch of the nylon breathable pair of waders that was rinsed after every exposure started to leak. Testing continued with these waders until the legs leaked. The legs leaked in the seam of the calf on both legs after 43 exposures to 20 g/L Virkon® Aquatic and being thoroughly rinsed. Virkon® Aquatic appears to disintegrate the glue around the seams. The crotch of the neoprene waders started to leak after 65 exposures to Virkon® Aquatic. Testing continued with the neoprene waders until the legs leaked water. After 77 exposures to 20 g/L Virkon® Aquatic solution, both booties leaked around the seams. There was no discoloration, tears, or holes in any of the waders. The only damage that occurred to the waders was the seam or waterproofing glue disintegrating.

Discussion

A disinfection bath application of Virkon® Aquatic for 30 min was the only method tested in all trials that killed all NZMS and neonates. Spray applications tested in petri dishes provided only 99% mortality after 30 to 40 min and live neonates were present in the recovery system. Spray is not a recommended application technique for most chemical disinfection protocols tested on NZMS (Hosea and Finlayson 2005; Schisler et al. 2008) because spray applications do not cover all surfaces with the full amount or concentration of disinfectant for the required time. Moreover, spray application is inconsistent with different individuals.

We validated that the procedures used in small lab test systems were also effective on boots infested with NZMS. No crawling snails were observed while the boots were in the disinfectant bath. We observed the NZMS falling off the boots when the boots were placed into Virkon® Aquatic solutions. Very few NZMS were found in the rinse tubs and those found were all dead. All NZMS were dead at all exposure times in the bath disinfectant treatment, even with a15 min exposure time. The effective shorter exposure time in this study could be attributed to Virkon® Aquatic being more effective on attached NZMS that do not have their operculum closed. Agitation of the boot while in solution could help remove the NZMS that are stuck in any cracks and crevices.

The number of NZMS volitionally infesting boots was highest for neoprene and felt soled wading boots, regardless of the density of the snails. Rubber soled boots were still colonized, but with less intensity. The same is true for the number of juvenile and neonate NZMS and aquatic insects colonizing felt, neoprene, and rubber soled wading boots. Different amounts of aquatic invertebrates on each wading sole type could also be attributed to neoprene and felt materials being easier to stay attached compared to the rubber material.

Virkon® Aquatic solutions were deactivated by organic material after 4 to 24 h of exposure. The stream mud deactivated 10 g/L Virkon® Aquatic at a faster rate than the peat moss. Stream mud was a heterogeneous mixture of metals and organic material and through mixing would not make the mixture homogenous. Since Virkon® Aquatic is broken down by metal salts (Syndel 2011b), there could have been a different composition of organic material

and metal salts in the mud. Another explanation for the different decomposition rates between 10 and 20 g/L solutions was that a 20 g/L concentration had a higher amount of active ingredients to prolong the reaction compared to the 10 g/L concentration. Many chemical processes occurred, which aided in the breakdown of Virkon® Aquatic. A higher ratio of organics to solution could speed up the process of deactivation and breakdown of Virkon® Aquatic. The peat moss, which had a high percent organic material, deactivated Virkon® Aquatic faster than the stream mud.

Wading boots and waders did not show any deterioration or discoloration when exposed to Virkon® Aquatic. Other chemicals such as bleach are very harsh on boots and waders (Hosea and Finlayson 2005). These studies showed that Virkon® Aquatic was not as harsh on waders and boots as other chemicals. Hosea and Finlayson (2005) had deterioration of boots and waders after 7 exposures to chemicals such as Bleach®, Pine-sol®, Formula 409®, and benzethonium chloride. Virkon® Aquatic disintegrated the glue of the seams after approximately 30 exposures to a 20 g/L Virkon® Aquatic solution with an exposure time of 20 min. Neoprene seam glue lasted longer, failing after 67 exposures. Glue disintegrated faster in areas where Virkon® Aquatic was able to sit and dry, such as in the crotches of the waders. Further testing needs to be conducted on different manufacturer's seam glue to determine the best compound to withstand the high acidity of Virkon® Aquatic. No control was used to compare the waders, but the normal use of waders, without being exposed to any chemicals, would last longer than 67 trips or times in water.

Agencies can use Virkon® Aquatic as a gear disinfectant in effective biosecurity protocols to ensure that NZMS are not transported to new areas. Banning different gear types has been one solution of controlling the spread of aquatic invasive species, specifically didymo and whirling disease (Spaulding and Elwell 2007; Gates et al. 2008; Bothwell et al. 2009). Wading gear is also a pathway that NZMS utilize to spread to new areas (Dwyer et al. 2003; Richards et al. 2004; Hosea and Finlayson 2005). However, this research shows that banning felt soled wading boots does not eliminate the risk of NZMS being introduced. NZMS could still be attached to the neoprene or rubber soled wading boots. Physical decontamination methods are recommended protocols, but freezing, boiling, or drying may not be the best protocols for agency personnel (Dwyer et al. 2003; Richards et al. 2004). In

such cases chemical decontamination methods are prescribed (Hosea and Finlayson 2005; Schisler et al. 2008). Precaution must be exercised when using chemical disinfectants to ensure efficacy and safety (Oplinger and Wagner 2009). The chemicals that have been tested on NZMS are grapefruit seed extract, isopropanol, household ammonia, Bleach®, copper sulfate, hydrogen peroxide, potassium permanganate, Virkon® Aquatic, Pine-Sol®, benzethonium chloride, Formula 409® All Purpose Cleaner, Formula 409 ®Degreaser and Disinfectant, Hyamine, sodium chloride and Sparquat 256® (Hosea and Finlayson 2005; Schisler et al. 2008; Oplinger and Wagner 2009). Of these, the only effective chemical that is also safe in the environment is hydrogen peroxide; the others are not effective, not labeled for use in or around water, very toxic, or are considered hazardous waste. However, Virkon® Aquatic is effective with a short duration time using a bath application on gear, is deactivated quickly by organic material, and is minimally corrosive on gear. Virkon® Aquatic can be used in many places, and be a key component of any management or biosecurity plan to disinfect contaminated gear. Pre-rinsing to remove soil, excess organic material, and large organisms is an essential step before using any disinfectant. The use of Virkon® Aquatic ensures effective disinfection of wading gear. As with most chemical disinfectants, rinsing thoroughly with water is recommended after chemical exposure. Anyone using or working in infested waters should clean and disinfect their equipment to lower the risk of spreading invasive species.

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Table 2.1. Experimental design of spray versus bath, disinfection of three types of naturally infested wading boots, colonization likelihood of NZMS on the three different boot sole types, and effect of organic contaminants. Virkon® Aquatic concentrations that are used in each study are listed, the number of replicates containing approximately 10 NZMS per trial conducted on different days.

Experiment	Concentrations	Replicates	Trials
Spray versus bath	10 & 20 g/L	3	3
Naturally infested boots	20 g/L	3	Repeated until number adult NZMS >100
Colonization likelihood	20 g/L	3	3
Effect of organic contaminants	10 & 20 g/L	3	3

Table 2.2. Summary of mortality and number of live neonates released for different applications, spray versus bath, testing adult NZMS with different exposure times to 10 and 20 g/L Virkon® Aquatic.

Application	Concentration (g/L)	Time (min)	Number dead	Total tested	Mortality (SE)	Number of neonates
		15	2	90	0.02 (0.04)	19
		20	0	90	0 (0)	19
	Control	30	1	90	0.01(0.03)	22
		15	81	90	0.9 (0.15)	5
		20	85	92	0.92(0.09)	7
	10	30	89	92	0.97 (0.03)	5
		15	89	90	0.99 (0.03)	0
		20	89	90	0.99 (0.03)	0
Bath	20	30	90	90	1 (0)	0
		20	0	90	0 (0)	22
		30	0	89	0 (0)	26
	Control	40	0	90	0 (0)	23
		20	67	91	0.74 (0.17)	27
		30	84	91	0.92 (0.09)	9
	10	40	84	90	0.93 (0.07)	3
		20	86	91	0.95 (0.07)	6
		30	89	90	0.99 (0.03)	2
Spray	20	40	89	90	0.99 (0.03)	1

Table 2.3. Summary of water chemistry results for spray versus bath application methods; time is referring to results before exposure to NZMS or after exposure to NZMS.

Concentration	Time	Temperature (˚C)	pH	Conductivity (mS/cm)
Control	Before	15.29	7.17	270.33
Control	After	14.39	7.15	264.67
10 g/L	Before	15.50	2.46	8,154.00
10 g/L	After	14.81	2.49	8,092.33
20 g/L	Before	16.59	2.36	14,500.00
20 g/L	After	16.22	2.34	14,503.33

Table 2.4. Summary of data collected from disinfection of three types of naturally infested wading boots. The mortality of NZMS and number of live neonates recovered from soles on wading boots after exposure to Virkon® Aquatic concentrations for various exposure times. TNTC=too numerous to count, > 300.

Sole	Concentration (g/L)	Time	Number of replicates	Total number of NZMS	Total dead	Percent mortality (SE)	Number of neonates
		15	3	150	9	6 (3.4)	6
		20	2	116	3	3 (1.9)	5
	0	30	3	108	5	5 (2.7)	8
		15	3	174	174	100 (0)	0
		20	2	233	233	100 (0)	0
Felt	20	30	3	247	247	100 (0)	0
		15	1	967	5	0.5	TNTC
		20	2	267	4	1.5 (0.2)	TNTC
	0	30	1	235	4	2	TNTC
		15	3	152	152	100 (0)	0
		20	1	216	216	100 (0)	0
Neoprene	20	30	3	730	730	100 (0)	0
		15	4	56	0	0 (0)	13
		20	4	48	2	4 (5.9)	30
	0	30	4	113	7	6 (3.8)	31
		15	4	233	233	100 (0)	0
		20	4	207	207	100 (0)	0
Rubber	20	30	4	532	532	100 (0)	0

Table 2.5. Summary of least square means, F and *P*-values for the total number (total) of NZMS colonizing each boot type (sole) and the total number of snails per replicate (pertotal) colonizing each boot type (sole).

	LS mean of NZMS colonizing each sole type			ANOVA	
Model	Felt	Neoprene	Rubber	F statistic	P-value
Total = sole	187.9	648.3	335.0	1459.71	<.0001
Pertotal=sole	73.6	490.0	83.8	1325.48	<.0001

Table 2.6. ANOVA determining significant differences between the total number of aquatic invertebrates (total invert), adult NZMS (adultNZMS), juvenile and neonate NZMS (otherNZMS) and aquatic insects (noAI) colonizing each wading boot sole type (sole) and incorporating the different densities tested, high-density (HD) and low-density (LD). Total numbers of colonizing invertebrates that were attached to each sole type are listed, the exposure times, concentrations, and replicates were combined, as none of these factors effected the colonization of the wading boots. The Tukey's HSD show the significant differences between sole types. Different letters indicate significance at $\alpha = 0.05$.

	ANOVA		Colonizing amount			Tukey's HSD		
Model	F statistic	P-value	Felt	Neoprene	Rubber	Felt	Neoprene	Rubber
total invert=sole	45.32	<.0001	458	292	229	A	B	B
density (HD)+total invert=sole	78.38	<.0001	301	196	151	A	B	B
density (LD)+total invert=sole	1.5	0.2258	157	96	78	A	A	A
adultNZMS=sole	12.09	<.0001	159	114	29	A	A	B
density (HD)+adultNZMS=sole	15.95	<.0001	90	63	18	A	B	C
density (LD)+adultNZMS=sole	17.52	<.0001	69	51	11	A	B	A
otherNZMS=sole	65.11	<.0001	80	22	52	A	B	C
density (HD)+otherNZMS=sole	27.49	<.0001	53	14	29	A	B	C
density (LD)+otherNZMS=sole	41.28	<.0001	27	8	23	A	A	B
noAI=sole	26.56	<.0001	219	156	148	A	B	B
density (HD)+noAI=sole	30.25	<.0001	158	119	104	A	B	B
density (LD)+noAI=sole	10.54	<.0001	61	37	44	A	B	B

Table 2.7. Percent active oxidizer (AO) for each concentration of Virkon® Aquatic with no organic material, 10 g /L peat moss, or 10 g/ L Paradise Creek stream mud for each time tested. NT= not tested.

	10 g/L Virkon® Aquatic			20 g/L Virkon® Aquatic		
Time (h)	No organic material Mean percent AO (SE)	Peat moss Mean percent AO (SE)	Stream mud Mean percent AO (SE)	No organic material Mean percent AO (SE)	Peat moss Mean percent AO (SE)	Stream mud Mean percent AO (SE)
0	12.81 (1.85)	NT	NT	12.37 (3.52)	NT	NT
0.5	NT	12.20 (3.49)	11.73 (0.87)	NT	9.31 (0.78)	10.44 (0.11)
2	NT	10.42 (1.15)	9.66 (0.16)	NT	8.87 (0.46)	9.67 (0.28)
4	10.98 (0.39)	10.10 (0.81)	10.01 (0.42)	10.28 (0.06)	8.34 (0.08)	9.39 (0.24)
24	10.65 (0.52)	7.27 (0.38)	8.35 (0.41)	9.96 (0.22)	6.76 (0.31)	8.51 (0.16)
48	10.79 (0.26)	6.00 (0.27)	4.10 (0.16)	9.30 (0.07)	4.85 (0.12)	7.56 (0.19)
72	11.21 (0.20)	NT	NT	9.29 (0.07)	NT	NT
120	11.23 (0.19)	NT	NT	9.00 (0.01)	NT	NT
168	11.10 (0.53)	NT	NT	9.01 (0.19)	NT	NT

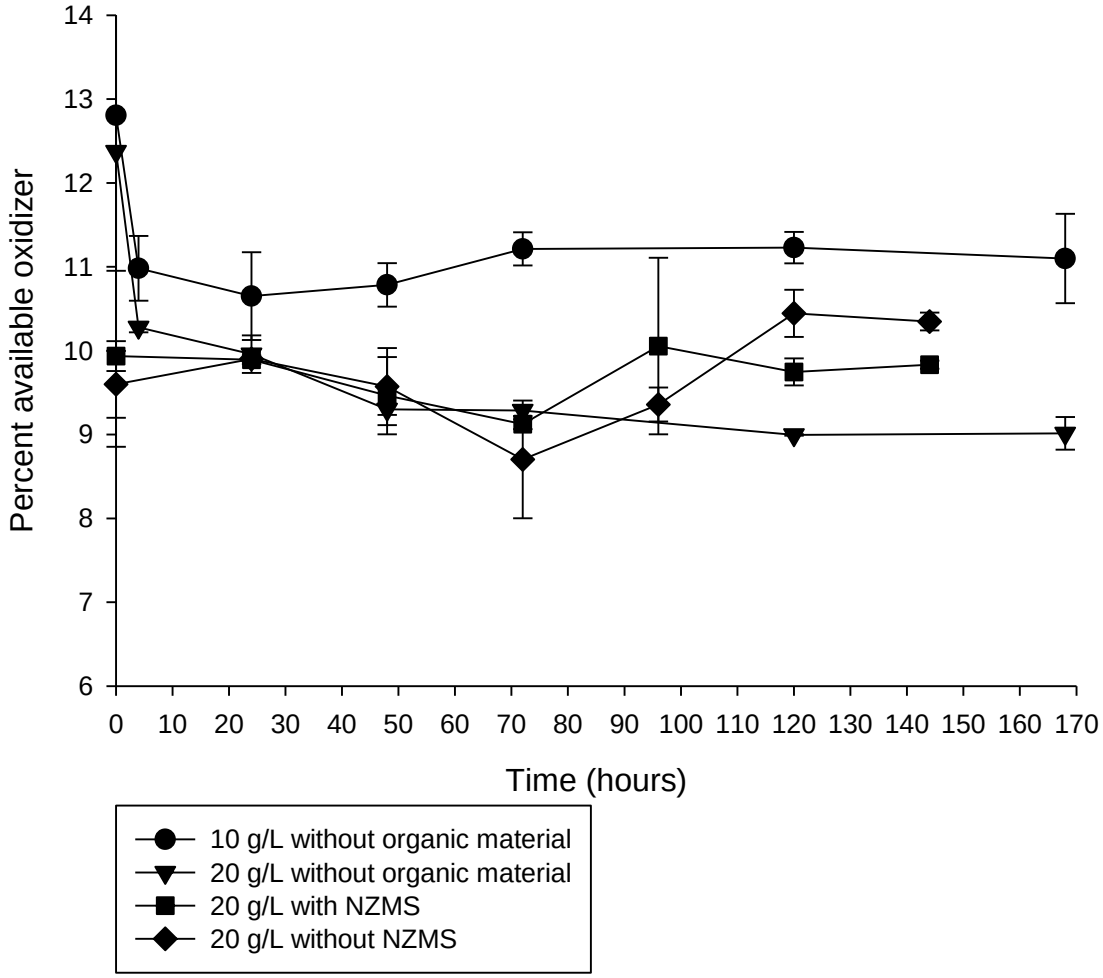

Figure 2.1. The percent available oxidizer (AO) with error bars over time of 10 and 20 g/L Virkon® Aquatic without organic material and with or without 10 NZMS. Virkon® Aquatic is considered active at percent available oxidizers above 9.0.

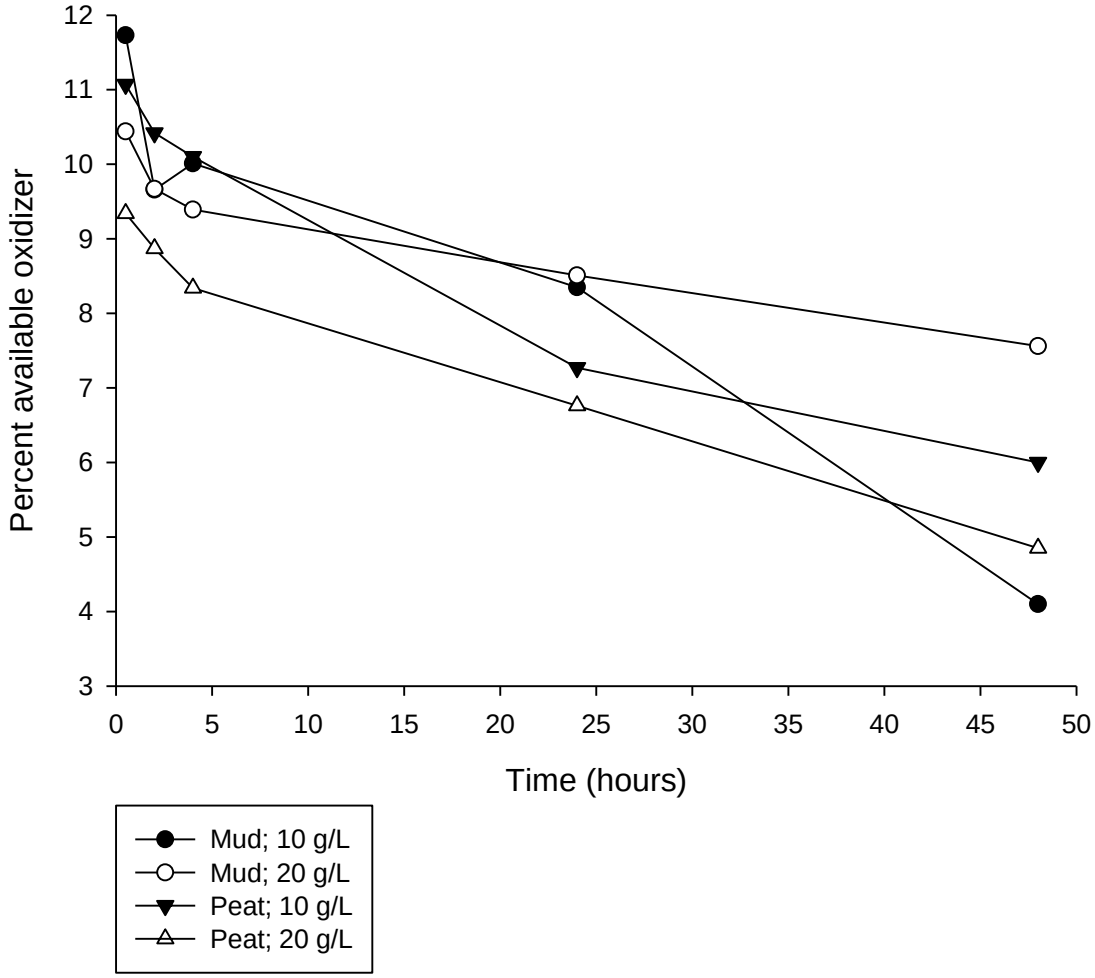

Figure 2.2. The least squares means of the percent available oxidizer (AO) over time of 10 and 20 g/L Virkon® Aquatic with 10 g peat moss (92.92%) or Paradise Creek stream mud (3.87%). Virkon® Aquatic is considered active at percent available oxidizer above 9.0.

Chapter 3: Evaluation of water velocity, hydraulics, and small particle removal efficiency in a mixed cell raceway compared to a Burrows pond rearing system

Abstract

We evaluated an experimental mixed cell raceway to determine its efficiencies of mixing, water velocity, and small particle removal to determine its feasibility of use in a depuration strategy to remove potentially contaminated feces. For comparison, we also measured the same parameters in a Burrows pond with similar dimensions and flow rates. Measurements of hydraulic residence time, particle removal efficiency, and velocity were conducted using salt tracer depletion, removal of particles that simulated feces and target invasive species, and acoustic Doppler velocimetry. The mean hydraulic residence time of the Burrows pond and mixed cell raceway systems were similar; however, the particle removal efficiency of the Burrows pond was relatively poor. Vectors revealed well-defined, counter-rotating cells in the MCR. The substantially higher and more homogenous velocities in the mixed cell raceway system were the likely contributing factor in higher particle removal efficiency. Further tests of these rearing conditions with production fish are needed to determine if the physical conditions result in measurable differences in fish physiology, growth, and performance.

Introduction

Rearing systems affect fish performance specifically fish behavior, growth, and metabolism (Burrows and Chenoweth 1970, Ross et al. 1995, Ross and Watten 1998). Rearing systems are typically evaluated by the quality of the fish produced, but rearing systems can also be evaluated through hydraulic effects (Watten et al. 2000; Rasmussen et al. 2005; De Schryver et al. 2008). A fish rearing unit modification was proposed by Watten et al. (2000) to establish mixed flow reactor behavior into rectangular raceway to improve current velocities and solid scour while eliminating metabolite concentration gradients. This system is referred to as the mixed cell raceway. This system is an improvement from the Burrow's pond rearing system, which also combined mixed flow reactor behavior into a rectangular raceway (Burrows and Chenoweth 1970).

Recently, aquatic invasive species such as the New Zealand mudsnail *Potamopyrgus antipodarum* (NZMS) have become established in source waters for conservation and commercial fish hatcheries. The NZMS are easily transported because they are very small, ranging in size from 80 µm to 6 mm and live in a variety of habitats, such as estuaries, lakes and rivers (Winterbourn 1970; Alanso and Castro-Diez 2008; Bersine et al. 2008). These prosobranch snails have an operculum that closes tightly allowing them to resist desiccation and chemical treatment, surviving transit to new areas (Richards et al. 2004). NZMS are prolific as one NZMS female can populate an area, producing up to 50 neonates in her brood pouch, and reproducing one to six times throughout the year (Møller et al. 2004; Alanso and Castro-Diez 2008). Some fish ingest NZMS (Bersine et al. 2008; Bruce and Moffitt 2010) and NZMS can survive transit through the digestive tract of salmonids (Haynes et al. 1985; Bruce et al. 2008).

Recent federal and state legislation in the Western United States has prohibited the movement of aquatic nuisance species from an infested area to a non-infested area (NISIC 2010; Amy Ferriter, Elizabeth Brown, Eileen Rice, and Larry Dalton, state Aquatic Nuisance Species Coordinators, personal communication). The regulations declare that transported water and fish need to be free of NZMS. Some hatcheries have been closed due to infestations of NZMS (Ken Cline, personal communication) and others have experimented with different tools to minimize their risk of NZMS infestation (Oplinger and Wagner 2010). The mixed cell raceway may be a tool that can be used to minimize the risk of NZMS infestation (Neilson 2008; Bruce and Moffitt 2010). Bruce and Moffitt (2010) recommended using a depuration strategy of feeding infested steelhead or rainbow trout for 96 h and then providing holding in a clean water source for at least 48 h. To properly depurate trout, infested feces need to be removed efficiently so that the risk of re-infection is limited. Hydraulic characteristics can be evaluated to determine the success of the mixed cell raceway in eliminating the risks associated with a NZMS invasion.

The design of the mixed cell raceway as described by Watten et al. (2000) consists of vertical pipe sections extending to the tank floor and positioned at the corner of the cells. The number of cells is variable, but the number is dependent on the relationship of length equaling width of the cells. The vertical pipe sections have jet ports, which direct the water

into the cells tangentially to establish circulation. Water exits each cell through a centrally located drain covered with aluminum screen. A standpipe in the effluent regulates the height of the water in the raceway. Watten et al. (2000) showed that fecal material was purged quickly from the raceway and found that the presence of fish decrease tank mixing. Ebeling et al. (2005) modified the mixed cell raceway for partial reuse with a "Cornell type" dual drain system and found that velocities were high enough to scour and remove solids. Labatut et al. (2007) conducted velocity profiles on each of the cells of a mixed cell raceway that revealed no significant differences occurring between cell velocity contours and vector plots in the cells. The mixed cell raceway was hydraulically active and attained an adequate degree of mixing.

Hydraulics effects are described by either a hydrodynamic model or a reactor model that describes a mixing process (Haan et al. 1994). Tracers can be used to model the hydraulic response based on the principle that inflow equals outflow (Levenspiel 1979). Large particle removal of feed pellets also followed the same assumptions (Summerfelt and Timmons 2000). Velocities within the raceways can be tested to determine where differences in velocities occur and further characterize the raceway's hydraulics. It is important to model the hydraulics of a system to determine how fish will perform and how water particles are moved through the system. Hydraulics also dictates how the feed and feces are distributed throughout the system. If feed is moved out of the system too quickly, fish will not feed, food will be wasted, and the fish will not be satisfied. However, feed accumulation from poor waste removal hydraulics provides media for bacteria and viruses to proliferate, which increases the incidences of disease (De Schryver et al. 2008). Solid accumulation also shows that the system will not be adequate for use in depuration methods to reduce the risk of NZMS within a facility.

The purpose of this study was to evaluate the hydraulic characteristics of the mixed cell raceway to evaluate the waste removal and water quality conditions that could reduce the accumulation of potential harmful species in the rearing systems. For comparison, we also evaluated a traditional Burrow's pond system.

Methods

Studies were conducted at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. In 2008, two Burrows ponds (BP) were modified to create two mixed cell raceway systems (MCR) by removing the center wall and creating four central drains. Piping was installed to transport water from each water inlet to down legs that distributed flow tangentially to establish counter rotating cells (Figure 3.1). Each of the 4 cells was 5.715 m long and 5.18 m wide, which drained into a 30.5 cm diameter drainpipe. The standpipe in the effluent box, from the original Burrows pond, maintained the water height. In 2009, the surfaces of two raceways and two unmodified BP were coated with a concrete coating to reduce the shear stress from deteriorating and rough concrete walls and bottoms. Modeling studies were conducted in one MCR (pond 64; Figure 3.1) and one BP (pond 62; Figure 3.2). Closeable-valve ports were added to the influent piping on BP pond 62, and the MCR pond 64, Figure 3.3 to add salt solutions.

Optimal flows at 2,271 L/min were recommended for both the MCR and BP (Barnaby Watten, USGS Fishery Biologist, personal communications). Equal flow rates were necessary for comparison, as different discharges would change the hydraulic characteristics in the raceways.

Flow Rates

On the MCR, paddle wheel flow meters (Signet Meter Model 3-5500 coupled to a MK515-PO probe) were installed on the inlet pipes perpendicular to flow (Figure 3.3), but the meters failed after October 2009. Flows were monitored using a portable staff gauge in the standpipe area of both experimental units. A 0.61 m length of 2.54 cm diameter PVC pipe was pre-marked and attached securely to the standpipe, to act as a staff gauge. Discharge was determined by using USFWS calculated sheet of flows for Burrow's ponds (Table 3.1). However, different discharge estimates were determined with an equation for discharge under orifice flow validated by the tracer method,

$$Q = C'A\sqrt{2gH} \qquad \text{Equation 3.11}$$

where Q=discharge (L/s);

C'= contraction coefficient;

A= orifice area of stand pipe (m^2);

g= gravity (9.81 m/s^2);

H= head (m) (Haan 1994 and BOR 2001).

The MCR standpipe had an inside diameter of 30.163 cm and the BP was 30.639 cm.

Salt tracer- Discharge was also calculated with a tracer method in both the MCR and BP. A 1,514 L tank was used to provide a concentrated solution of NaCl that was introduced into the test systems (~60 g/L). The concentrated salt solution was added at a constant rate by a double-headed peristaltic pump (Masterflex Model 7545-30) into the ports in the inflow pipes (Figure 3.3) through 1.27 cm diameter tygon tubing. The pumping rate was measured by timing the rate of fill or depletion of a known volume. The conductivity of the test system water was measured and recorded with a YSI 556 MPS multiprobe meter (YSI, Inc., Yellow Springs, Ohio) before and during tests with the salt tracer at 1 min intervals for the duration of tests at the effluent standpipe box of the MCR or BP. The high concentration of salt solution in the source tank was mixed with a sump pump to prevent stratification and conductivity was measured with a YSI Pro-Plus meter (YSI, Inc., Yellow Springs, Ohio). Discharge was calculated from the concentrations using the relationship:

$$Q = q\frac{C1-C2}{C2-C0} \qquad \text{Equation 3.2}$$

where Q=discharge (L/min);

q= the discharge (L/min) of the strong salt solution injected into the flow;

C0= the natural or background conductivity (mS/cm);

C1= the conductivity (mS/cm) of the strong salt solution;

C2= the conductivity (mS/cm) in the effluent after full mixing, which includes background conductivity (Haan 1994 and BOR 2001).

Salt solution was considered fully mixed when the conductivity within the effluent box was consistent for 30 min.

Hydraulic Residence Times

Once the salt solution was fully mixed into the raceway, achieving a flow rate of approximately 2,271 L/min, the valves to the salt ports were closed and the peristaltic pump was turned off. This was the start of the depletion analysis. The conductivity was logged for at least four hours. One trial in the MCR and BP were conducted without fish present on 17 and 24 September 2009. The same procedures for the MCR were conducted in November 2008 to obtain another depletion curve without fish present; however, this was before application of the concrete coating, so MCR walls were rough concrete. On 23 and 24 November 2009, a salt tracer trial was completed in the MCR and BP to determine the depletion curve with juvenile fish present. Salt tracer trials were also completed 17 and 18 March 2010 to determine the depletion curve with large fish.

Fish loading- Steelhead trout (*Oncorhynchus mykiss*) were raised in the MCR and BP raceways from October 2009 to April 2010 from brood stock year 2009. In mid-October 2009, 31,500 juveniles were placed into each of the MCR and BP raceways. The biomass of fish in BP (pond 62) for the 24 November trial was 1288 kg with an average fish length of 156 mm and weight of 39 g. Biomass of fish in MCR (pond 64) during the 23 November trial was 1071 kg; the fish were an average length of 147 mm and weight of 34 g. On 18 March, the biomass of the BP (pond 62) was 1172 kg with an average fish length of 183 mm and weight of 63 g. The MCR (pond 64) steelhead fish on 17 March had a biomass of 1092 kg with an average length of 178 mm and weight of 58 g. One kg of fish biomass was assumed to equal one L of displaced water.

Data modeling and analysis- Depletion curve tracer for each trial was modeled using the proportional change in conductivity over time. The change in conductivity was normalized for each trial by the following equation:

$$NC = \frac{C_i - C_0}{C_{max} - C_0} = \frac{\Delta C}{\Delta C_{max}} \qquad \text{Equation 3.3}$$

where NC is normalized conductivity (0 to 1);

C_i is conductivity in mS/cm at time i;

C_0 is natural or background conductivity (mS/cm);

and C_{max} is the maximum conductivity (mS/cm) or the conductivity before peristaltic pump was shut down.

Flow characteristics were analyzed by determining the mean residence time ($\bar{t}_c$) of the water within the raceways, the variance about the mean residence time (σ^2), and the degree of axial dispersion or mixing ($D/\mu L$). With a step down in the salt tracer concentration, $\bar{t}_c$ and σ^2 were defined as follows (Levenspiel, 1979):

$$\bar{t}_c = \frac{\sum_{i=1}^{n} t_i \Delta C_i}{\Delta C_{max}} \qquad \text{Equation 3.4}$$

$$\sigma^2 = \frac{\sum_{i=1}^{n} t_i^2 \Delta C_i}{\Delta C_{max}} - \bar{t}_c^2 \qquad \text{Equation 3.5}$$

$$\frac{\sigma^2}{\bar{t}_c^2} = 2\left(\frac{D}{\mu L}\right) - 2\left(\frac{D}{\mu L}\right)^2\left[1 - exp^{-\frac{\mu L}{D}}\right] \qquad \text{Equation 3.6}$$

The theoretical retention time,$\bar{t}$, was equal to volume of the tank divided by the discharge rate. Volume was adjusted for the biomass of the fish at each trial. Mean hydraulic residence time, $\bar{t}_c$, was compared to ideal hydraulic residence time ($\bar{t}$) to determine the turnover efficiency, the extent of stagnant regions, SR, and the volume of the stagnant regions, (volume*stagnant regions) as a percentage of volume.

$$\text{SR=1-turnover efficiency=1-}\bar{t}_c/\bar{t} \qquad \text{Equation 3.7}$$

The volume was calculated from the dimensions of each raceway and subtracting the biomass of fish for each corresponding trial. Discharge was calculated from flow rate calculations. Time to complete depletion of salt shows how long the taill was in the depletion curves and efficiency of removal. The normalized concentrations were transformed by taking

the negative natural log of the data to obtain a linear equation. Linear regression analysis was conducted with SAS 9.2 (SAS Institute 2002-2008) using PROC REG. Slope, intercept and r-square were determined for each trial. The slopes of regression lines established were compared using PROC GLM using an analysis of covariance (ANCOVA) with contrast statements to determine significant differences between trials. Paired t-tests were conducted on the calculated hydraulic residence times to test for differences between trials.

Particle Removal Efficiencies

We evaluated the removal of small particles within the MCR and BP to simulate wasted feces and NZMS. Replicated tests were conducted during June 2010 at design flows of 2,271 L/min. To accomplish these trials, bottom drains were plugged with inflatable balls wedged and weighted down; and we replaced the drain screens. The drain screens were modified screens fitted with a 5.08 cm diameter opening in the center of each of the four MCR drains and in the outflow drains of the BP. The screen open area was adjusted to achieve 60% for each modified drain screens in the MCR. The end of a 5.08 cm PVC was placed about 5.1 cm from the bottom of the drain screen and fitted to a manifold filtration system (Figure 3.4 and Figure 3.5). Trash pumps (Gasoline EPT3 Tsurumi Pump, Glendale Heights, IL) were used to remove water at the same rate as normal flow through. The pumps were placed in adjacent raceways. Flows were calibrated to 568 L/min. Two 80 µm plankton nets 30 cm diameter by 90 cm long with the clamped on cod ends with filtered vent holes were used to catch pumped outflow. A 100 µm plankton tow net with the bottom stitched together was placed in the test system standpipe to catch any outflow particulates not removed from the raceways by the pumps.

Sinking cylindrical ABS beads 3 mm in length and 3 mm in outside diameter were added in pulses to quantify solid flushing rates in the MCR and BP. The beads had a specific gravity of 1.05 (Summerfelt and Timmons 2000) similar to that reported of trout fecal material. Clean dry beads were weighed out and then soaked in water before trials.

To start testing, 4,535 g of beads were introduced in a similar manner to scattering feed particles into the MCR, 1,134 g into each cell, over 1 min for the first of two replicated trials. For the third trial, 12,600 g of beads were introduced into the MCR, 3,150 g into each cell.

Beads were collected with the plankton tow nets at intervals of 2.5, 6, 10, 15, and 20 min for trial 1, and 2.5, 6, 10, 15 and 20 min for trial 2 and 1, 5, 10, 15, and 20 min for trial three. Collected beads were placed into containers at each of the intervals and were later dried and weighed to evaluate the cumulative mass removal by time interval.

For each test in the BP, 4,535 g of beads were distributed from the center wall over the entire length of the BP over l min. Beads were collected at 2.5 min intervals until 10 min, 5 min intervals until 20 min and then 10 min intervals until 50 min had elapsed. Collected beads were placed into containers to be dried and weighed.

This pulse response data was analyzed with the mixing cup method (Levenspiel 1979).

$$\bar{t}_c = \frac{\sum_{i=1}^{n} t_i \Delta t_i C_i}{\Delta t_i C_i} \text{/(output/input)} \qquad \text{Equation 3.8}$$

$$\sigma^2 = \left(\frac{\sum_{i=1}^{n} t_i^2 \Delta t_i C_i}{\sum_{i=1}^{n} \Delta t_i C_i} - \bar{t}_c^2\right)\text{/(output/input)} \qquad \text{Equation 3.9}$$

The rate that the beads were flushed from the drains, k, was calculated using the following equation (Summerfelt and Timmons 2000):

$$\%\text{flushing due to enrichment} = \frac{k}{k + Q/V} * 100 \qquad \text{Equation 3.10}$$

Where k can also be found by plotting the negative natural log of the fraction of solids remaining versus time where (Q/V+ k) is equal to the slope parameter.

Individual cell hydrodynamics were modeled by testing one cell of the MCR. Testing started when 1,134 g of beads were distributed into cell 2 of the MCR over 1 min. Beads were collected every 30 s and placed in labeled containers for the first 5 min and then at every min until 17 min. Bead collection in the nets after 17 min was negligible. All four pumps were pumping to maintain a 2,271 L/min discharge. This procedure was repeated twice. Beads were dried and weighed to get mass removal at each time point.

Hydraulic residence time was calculated using Equation 3.8 and variance associated with the calculated hydraulic residence time, Equation 3.9 flow characteristics were analyzed.

Velocity Profiles

We conducted velocity profiles at approximately 2,271 L/min with no fish in the MCR and BP raceways during June 2010. A SonTek Argonaut-Acoustic Doppler Velocimeter (SonTek/YSI, San Diego, CA) was used to analyze velocity at two depth intervals, 0.305 and 0.610 m, along transects in each system, at 0.305 m intervals. Magnitude and standard error of the x, y, and z velocity components and the calculated speed and direction in the x-y plane were reported. The meter measured velocities within 0.001 to 6 m/s with a resolution of ±0.0001 and accuracy of ±1% of measured velocity.

Velocities in the MCR were measured at 0.254 m from the first drain (cross section 1) and second drain (cross section 2) toward the close side of the wall and from the center of drain 1 to the center of drain 2 (cross section 3; Figure 3.1). Interval 9 was not measured because of inadequate access from cross section 1 and cross section 2. Discharge in the MCR was measured with a staff gauge, 8.51 cm, which corresponds to 2,346 L/min, using Table 3.6 that correlates with the salt tracer flow calculations.

Velocities in the BP were measured 2.16 m from both end walls (cross section 1 and 4), 5.817 m from end wall over first drain (cross section 2), and in the middle of the raceway at 11.43 m (cross section 3; Figure 3.2). We were unable to measure interval 7 and 8 in all cross sections and interval 9 on the far end at 20.7 m because of inadequate access. Discharge during these tests in the BP were estimated from staff gauge reading of 7.87 cm, as 2,294 L/min, using Table 3.6 that correlates with the salt tracer flow calculations. Velocities were recorded continuously over an eight min time period producing average readings every fifteen seconds. These measurements were downloaded into a spreadsheet and plotted with Sigma Plot 11.0 (Systat Software, Inc. 2008). Measurements from each cross section were transposed in a similar fashion as Labatut et al. (2007) to model the complete raceway making the contour and vector plots for the complete MCR (Figure 3.1). Average velocities were calculated for each of the BP and MCR. Paired t-tests of average x, y, z, and speed velocities were used to determine significant differences between the average velocities of

the two measured depths. If there were no significant differences, velocities were combined to calculate an average velocity for the raceway. Velocities were measured at one depth in one cross section when the trash pumps were running in the particle removal studies to determine if flow characteristics were similar. Average velocities were compared with paired t-tests.

Results

Flow Rates

We determined that there was variation between the methods of measuring discharge, flow meter, salt tracer, and staff gauge, as presented in Table 3.2, Table 3.3, and Table 3.4. In theory, the three methods should calculate the same discharge for the same raceway in a given trial. Staff gauge measurements have the highest variation when measured and when compared to a table for a calculated discharge. The paddle wheel flow meter and the salt tracer method produced the same discharge on 17 September 2009, 2,271 L/min; however, the paddle wheel flow meter was not available on the Burrow's pond and after October, they were nonfunctional on the MCR. Staff gauge measurements became very useful, as they were easily obtained. Salt tracer discharge determinations, using Equation 3.2, were used for further testing in the MCR and BP, Table 3.2, Table 3.3, and Table 3.4.

Measurements of head over the standpipe were taken with a staff gauge; and readings were converted to discharge in L/min using United States Fish and Wildlife Service (USFWS) provided tables, Table 3.1. However, readings were low and did not match the calculations from the salt tracer discharge or the flow meter discharge. The USFWS tables were from calculations made by Piper et al. (1982), which calculated discharge on Cippoletti and rectangular weirs, not for BP raceways. Equation 3.1 was used to determine the orifice flow based on the area of the standpipe. To determine all parameters of the equation, it was assumed that 7.62 cm of head over the standpipe was equal to approximately 2,271 L/min. The contraction constant was calculated and then tables were made for other measurements of head, Table 3.6. Contraction constant is a measurement of the amount the water after passing over the standpipe edge continues to contract or curve away from the edge (BOR 2001). A non-standard installation will cause the coefficient to vary from given values (Haan

et al. 1994). For the MCR, a contraction constant was calculated as 0.43 and for the BP 0.42 using English units. Newly calculated discharge for staff gauge measurement aligns better with discharge measured with the other methods; see Table 3.2, Table 3.3, and Table 3.4. Equation 3.2 was used to determine the discharge for each trial. Table 3.5 shows the measurements that were made to achieve the required discharge. A hypothetical reading was calculated to determine the high point of conductivity in a fully mixed effluent. Flow was changed to achieve 2,271 L/min. Actual readings were calculated to ensure discharge was near hypothetical.

Hydraulic Characteristics

Hydraulic characteristics were calculated with the empirical data and not from theoretical data. Table 3.7 reports the volume, discharge, and time to complete depletion of salt of each of the seven trials conducted in the MCR and BP with and without fish. Using calculated hydraulic residence time, Equation 3.4; variance associated with the calculated hydraulic residence time, Equation 3.5; the dispersion factor, Equation 3.6 and stagnant regions, Equation 3.7, flow characteristics were analyzed and presented in Table 3.7. Table 3.7 also shows the linear regression line with corresponding r-square value. There were no significant differences between the seven trials at the 95% confidence level based on the calculated residence times.

The hydraulic characteristics were calculated using truncated data; we did not include the long depletion tails and cut the data at 95% removal to eliminate the bias associated with fluctuations in the conductivity measurements at low levels. Table 3.8 shows the same hydraulic characteristics as Table 3.7, but using the truncated data. The calculated hydraulic residence times increased slightly; however, the variance of all trials dramatically decreased. This also decreased the dispersion factor values. When the slopes were compared between each regression line for the independent trials, there were significant differences in the slopes, ($F = 48103$; $P < 0.0001$). Table 3.8 lists the regression equations with the different letters indicating *P*-values less than 0.05. Equations with the same letters were not significantly different from each other at the 95% confidence level. Contrast statements showed that the MCR with no fish in September 2009 was not significantly different from the MCR with small fish, and the BP with no fish trial was not significantly different from

the BP with small fish. Figure 3.6 represents the depletion curve and the natural log of the curve of the salt tracer in the MCR and BP when no fish were present. The depletion curve and the linearized depletion curves for the salt tracer with small fish present are represented in Figure 3.7. Figure 3.8 shows the depletion curve and the linearized depletion curve of the salt tracer testing with large fish present.

Particle Removal Efficiencies

Empirical and theoretical data was used to determine the hydraulic characteristics of the beads flowing through the raceways (Table 3.9). The best analysis of the hydraulics was by calculating k, the rate of removal of the beads from the drain, applying first order kinetics. The percent flushing due to enrichment determined how much of the removal was associated with the mass action of the water flow (V/Q) versus the enrichment of the beads. In the full trials of the MCR, the enrichment of the beads accounted for an average for 90% of the removal rate, whereas in the BP there were not enough beads removed from the system to account for the differences in removal. The BP only had an average of 8% of the beads removed from the system. Most of the beads were piled in the slow velocity areas of the raceway, which was before the inlet pipe jets settled on the bottom middle of the raceway floor. Mean hydraulic residence time and associated variance were calculated accounting for beads not collected, Equation 3.8 and Equation 3.9. This analysis indicates that the beads in the MCR were removed much faster than the water particles with an average retention time of 12.23 min, Table 3.9. In the BP, the water particles were removed much faster than the beads.

When an individual cell in the MCR was loaded with beads, the hydraulic calculations were very similar to the full MCR model Table 3.10. This shows that all four cells were working similarly and cell 2 was not dominant over the other cells. The cell exchange rate shows that there was movement out of cell 2 and into the other cells Figure 3.9.

Velocity Profiles

A comparison of the average velocity and speed with associated standard errors for each MCR and BP at each depth measured are presented in Table 3.11 and Table 3.12. The MCR had higher average velocities and more uniform velocity distributions compared to the BP.

The lowest depths tested, 0.610 m had higher average velocities. However, when paired t-tests were computed there were no significant differences between depths for the MCR or the BP. Average velocities for the BP were 0.1684 m/s and for the MCR the average velocities were 0.2342 m/s.

Mixed cell raceway- Average velocities and standard errors were computed and graphed for each cross-section. The different depths were put on the same graph to compare significant differences between the two depths. Speed as a function of distance from the wall was plotted in the same way. In the velocity plots, X, Y, and Z velocities were plotted as a function of distance from the wall. The general trend to have circular flow should have x velocities with a decreasing or increasing slope and Y and Z velocities close to zero. This was apparent in cross sections 1 and 2 in the MCR, Figure 3.10a and 3.11a. The speeds for these two cross-sections at 0.305 m depth increased around the drain; however, the speed at 0.610 m depth decreased, Figure 3.10b and Figure 3.11b. When velocities were taken longitudanly, as in cross section 3, the Y velocities increased as the distance from the drains increased, and the X and Z velocities were near zero. Figure 3.12a showed the Y velocity increased as the distance from the drain decreased. Speed for cross section 3 was highly variable for both depths measured, Figure3.12b. The contour and vector plots for each depth showed the slower flows around the drain and faster flows near the walls, Figure 3.19 for 0.305 m depth and Figure 3.20 for 0.610 m depth. Figure 3.21 showed the full contour and velocity profiles of the MCR at a depth of 0.305 m, hypothetical measurements were used at the downleg areas to better define the contour velocities. The full contour and velocity profiles of the MCR at a depth of 0.610 m was represented in Figure 3.22 also with hypothetical measurements at the downlegs.

Burrow's pond- In the BP, the water movement should be in the X direction. The closer the measurements were made to the inlet jets the higher the X velocities. The X velocities showed an increasing slope in cross section 1, which showed high flows in 0 to 2.5 m and low flows in the last part 2.5 to 5 m, Figure 3.13a. Y and Z velocities were close to zero, indicating low flow in all directions for the last part of the BP. The speeds for the two depths showed that flows were faster in the first section and slower in the second section, Figure 3.13b. In cross section 2, which was located over a drain in the first section, showed

increasing X velocities as distance from the wall increases; negative values indicated direction of flow, Figure 3.14a. This also showed that the velocities above the drain did not significantly increase. Y and Z velocities were near zero for both depths measured in cross section 2. The speed of cross section 2 did show that the speed decreased over the drain in the first section, Figure 3.14b. In cross section 3, the middle of the BP, the X velocity increased as the distance from the middle wall increased at both depths measured¸ so water was moving faster on the edges in the X direction of the BP, Figure 3.15a. The Y and Z velocity were near zero at both depths, Figure 3.15a. Speed in cross section 3 increased as the distance from the middle wall increased, Figure 3.15b. Cross section 4 was a mirrored profile of cross section 1, Figure 3.16a, where X velocities were slower in the first section than the second section. Y and Z velocities were very near zero and speed was slow in the first section and fast in the second section, Figure 3.16b. The four cross-section results was indicative of the flow pattern in the BP. Contour and vector plots for each depth showed the flows slow down as they get further from the inlet jets, Figure 3.23 for 0.305 meter depth and Figure 3.24 for 0.610 meter depth. These two contour and velocity plots do have hypothetical measurements for the water out of the jets.

Trash pump velocity comparisons- There were no significant differences between the trash pump velocities and the measured velocities with normal flow through the MCR or BP; speed was also not significantly different (Table 3.11 and Table 3.12). Figure 3.17 showed that the X, Y, and Z velocities and speed did not significantly differ at each interval in the MCR. In the BP, the flows created with the trash pumps were not significantly different from the normal flow; Figure 3.18 showed the X, Y, and Z directional velocities and the speed for the same depth measured.

Discussion

In this study, we analyzed the hydraulic characteristics of a mixed cell raceway and compared our model with those obtained from testing a Burrows pond of similar size. Our analysis determined that the fluid response to a step down in tracer conductivity in the MCR was similar to the BP response. Using the truncated depletion data, all of the hydraulic characteristics became very similar, showing less significant differences between the

different treatments. Using the full data set, we found that the variance was larger for all of the BP trials compared to the variance of the MCR trials. The large variance combined with the longer amount of time to deplete the salt completely showed that the BP was not as effective as the MCR in removing the complete volume of the raceway. The rate at which the BP removed the salt tracer from the water column was slower than that of the MCR, making the rate closer to the ideal hydraulic residence time.

The dispersion factor indicates the amount of mixing that occurred in the system. $D/\mu L \leq 0.001$ indicates that no mixing occurred in the raceway (Levenspiel, 1979). For closed vessels, the dispersion factor is dependent on the variance. Large variance indicates a large amount of dispersion and mixing, whereas a small variance is indicative of less dispersion (Levenspiel 1962). The values calculated in the salt depletion analysis with the truncated data still had a significant dispersion factor indicating a large amount of mixing in all trials in the MCR and BP.

Linear analysis of the salt tracer depletion showed that large fish effected the movement of the salt tracer from both the MCR and BP. The juvenile fish did not have a significant effect on the depletion curve, as it was not significantly different from the depletion curve without fish present. The trial with rough concrete walls in the MCR was also significantly different from all other depletion curves. Data analysis trends that might be determined by comparing the hydraulic characteristics were 1) similarities between rate of removal of the salt tracer between the BP and MCR and 2) increasing mean hydraulic residence time and dispersion factors, and decreasing stagnant regions as fish were added and grew in size (Rasmussen et al. 2005). Trend 1 was rejected because the ANCOVA and contrasts determined that the depletion curves were significantly different between the MCR and BP at the 95% confidence level. Trend 2 occurred in the MCR; as fish increased in size the hydraulic residence time was closer to ideal, dispersion factor increased, and the stagnant regions decreased. However, discharge inconsistencies could have the greatest effect on the hydraulic calculations and associated trends.

The bead depletion tests produced mean particle residence times that showed the mean amount of time the beads stayed in the water column and were removed by the drain. In the

MCR, the mean hydraulic residence time was 12.23 min, averaged over all three trials, and the average amount of beads removed was 88.6%. In the BP, the mean hydraulic residence time was 237 min, which was much greater than the ideal hydraulic residence time. Only an average of 8% of the beads was removed from the raceway. The rate of removal associated with the force of the drains was calculated and determined by k. The drain rate was only calculated for the MCR, an average of 0.249, which corresponded to 90.73% of the beads being removed by the forces of the drain pulling them out of the water column faster than the water current could take them down the drain. In the BP, k was so low that the only beads that were transported out of the system were with the water column and less mass of beads than expected were removed with the trash pumps from the drains. Most of the beads settled before being flushed down the drain. The mean particle residence time also shows that the beads stayed in longer than the tank turnover rate. There was not enough velocity to remove the beads from the raceway floor.

Bead tests conducted in one cell of the MCR showed similar results as the full model. The differences were attributed to the cell exchange rate. Beads distributed into cell 2 were found in cell 1, 3, and 4, with the majority of beads in cell 1 and the least amount in cell 4. This exchange rate could be attributed to beads being thrown into the jet streams increasing the likelihood of the beads being carried into other neighboring cells. However, this does not explain the beads that traveled into cell 4. The design hydraulics of the MCR does incorporate cell exchange rates (Watten et al. 2000). Watten et al. (2000) found that there was significant cell-to-cell movement with Cl^- tracer. However, the cell-to-cell movement was not significant for the bead movement in this study. There was movement between the cells, but not a significant amount. Cell exchange rates needs to be accounted for in feeding strategies, as feed will move from the cell it was thrown and into other cells.

The velocities of the MCR were higher than the velocities in the BP. Average velocity could be used to estimate the scouring velocity, safe velocities for fish, and shear stress. These calculations could be used to determine parameters of each raceway that relates to the removal of waste materials. Watten et al. (2000) determined the minimum fish size that could be loaded into the MCR using safe velocity calculations. The safe allowable velocities were found using the following equation:

$$V_{safe} \leq \frac{10.5}{length^{0.37}}$$ Equation 3.11

where, V_{safe} was expressed in body lengths per second and length was expressed in centimeters (Youngs and Timmons 1991). The V_{safe} for the BP was 2 cm at 0.16 m/s average velocity and 3.5 cm for the MCR using 0.23 m/s as the average velocity.

Scouring velocity calculations were designed by Schwab et al. (1966) and used to report minimum velocities needed to remove particles of a certain diameter (Youngs and Timmons 1991). Using the mean velocity of the raceway, the following equation was used to determine the maximum diameter that the velocity could move towards the drains (Watten et al. 2000):

$$V_{sc} \geq \frac{1}{2} d^{\frac{4}{9}} (G-1)^{1/2}$$ Equation 3.12

Where V_{sc} was scouring velocity (ft/s), *d* was particle diameter in mm, and G was the specific gravity of the material. Specific gravity of feces was 1.19 as reported by Watten et al. (2000). The BP velocities, 0.16 m/s, could remove feces that were 8.1 mm in diameter. Velocities in the MCR, 0.24 m/s, had the ability to remove feces that were 17.03 mm in diameter. The scouring velocities of the BP should easily remove feces. The beads had a specific gravity of 1.05, so velocities in the BP should remove beads that were 36.38 mm and the MCR velocities should remove beads 76.43 mm. The average scouring velocities of the BP should have easily removed the beads from the raceway.

Peterson (1999) found that particle removal is more strongly related to particle density than particle size. Benthic shear stress calculations are used to determine the amount of velocity to keep particles in suspension. Using the tables produced by Peterson (1999), the benthic shear stress of the BP is 0.0735 N/m^2, using 0.16 m/s surface speed, 1 m depth of water column, and an adjustment for roughness, k_s, of 0.01 m. The MCR benthic shear stress is 0.131 to 0.1975 N/m^2 for velocities of 0.20 and 0.30 m/s, with the same k_s and depth as used in the BP calculations. A lower value of roughness, k_s=0.001 m will lower the benthic shear stress values by 75%; approximately 0.042 N/m^2 for the BP and 0.075 to 0.17 N/m^2 for the MCR. The amount of benthic shear stress required to move suspended organic material,

including feed pellets and the beads is approximately 0.01 N/m^2. The velocities associated with the MCR and BP should be able to remove suspended organic material and feed pellets from the raceway floors.

Some possible sources of errors with some justification are described as follows. In determining the flow rates, staff gauges were used, which are rough estimations of the discharge. The long tails associated with the salt depletion curves increased the errors in the hydraulic residence calculations (Levenspiel 1979). By truncating the tails at 95% of the depletion curve variance was reduced and the accuracy the calculations increased. Sources of errors that occurred when doing the bead depletion studies in the MCR trials were that some beads were accidently dropped out of collection cups and not replaced, so efficiencies were underreported in the MCR. There were also organic particles that were in with the beads, which even after drying were still prevalent. However, the dried weight of these organics was minimal when compared to the weight of the beads. Changing the balance of the velocities out of each downleg jet could increase flow to all areas, decrease stagnant regions, and ensure turnover rate was close to ideal. This would also lower the amount of time required for complete depletion. Sources of error associated with the velocities were due to the influence of using a few measurements to characterize the entirety of the raceways. A portion of the grid system was measured, as time was a limiting factor. The use of hypothetical equations and mean velocities were not the most accurate to determine the scouring velocities and safe fish velocities.

The testing was conducted at DNFH, which controlled the flow, feed, and fish during the reported studies. We noticed that flows would be turned down from 2271 L/min (600 gpm) to about 1703 L/min (450 gpm) in each of the BP and MCR. DNFH also had variable head, which increased the variation of flow in each raceway. Discharge could have been different based on how many raceways were running at a particular time in different areas of the hatchery. Since DNFH was built in the 1960's, the concrete had cracks and there were many leaks in the raceways. Water could leak out of the system or into other raceways. This amount should be negligible and not affect any of the hydraulic calculations, but this could be a source of bacterial and viral contamination.

Steelhead trout were raised in the MCR and BP. The behavior observed while conducting the other tests were noted. In the MCR, the fish aligned with the flow, they were found to be distributed throughout the circular current and equally distributed amongst the cells. They would also play, swimming in and out of the vortex of each cell. Sick fish were pulled to the drain when they were not strong enough to withstand the MCR velocities. In the BP, fish were congregated around the outlet jets and in high velocity areas, as shown in Figure 3.23 and Figure 3.24. Sick fish would laze around in the slow velocity areas, saving their strength to swim away from the personnel collecting sick or dead fish. Watten et al. (2000) suggested that forced swimming occurring in the MCR should condition fish to consume less oxygen because it increases white mussel activity, improves cardiac output, and enhanced oxygen carrying capacity in the blood, thus increasing stamina.

The MCR can be used in combination with a depuration strategy to actively remove NZMS from the water column. The high velocities and the low particle residence times designates the MCR as an ideal tool for removing NZMS infested feces. Haynes et al. (1985) demonstrated that NZMS could remain in place at velocities of 30 cm/s. In the lab, we determined that the specific gravity of NZMS, with an average length of 3.77 mm, was 1.42. Using Equation 3.12, the scouring velocity required to move NZMS was 17.8 cm/s. Only the MCR has velocities high enough to create this much scouring velocity. Peterson (1999) devised an equation to determine the required amount of shear stress needed to suspend a particle:

$$\tau_{cr} = 0.11803\rho\Delta^{0.8}g^{0.8}d^{0.4}v^{0.4} \qquad \text{Equation 3.13}$$

where ρ was the density of water (998 kg/m^3), Δ was the relative specific gravity, g was the acceleration due to gravity (9.81 m/s^2), d was the diameter of the particle, and v was the viscosity of water (10^{-6} m^2/s). In the lab, we determined that the specific gravity of NZMS with an average length of 3.77 mm was 1.42. Therefore, the required shear stress to suspend NZMS was 0.156 N/m^2. Previously reported shear stresses for the BP determined that the BP velocities would not remove any of the NZMS. However, depending on the amount of roughness associated with the bottom of the MCR, velocities could be high enough to create enough shear stress to remove NZMS. Velocities approaching 30 cm/s would be adequate in

creating that amount of shear stress. It was also important to note that NZMS in high enough densities would increase the roughness, thus creating higher shear stresses. The beads also showed that the NZMS would be removed relatively quickly out of the system. NZMS defecated from trout would be removed from the system quickly as their settling velocity would be higher than the beads, but lighter than live NZMS. If snails were attached to the walls of the raceway, it was hypothesized that a salt or a potassium permanganate treatment would loosen the snails from the walls allowing the velocities to remove the NZMS. This was based on hypothetical equations; further testing needs to be completed to determine if NZMS are completely removed from the MCR.

The MCR is a more efficient raceway design because of higher velocities that efficiently remove wastes, lowering the risk of disease and the amount of physical cleaning conducted by hatchery personnel. Wastes are removed with high dispersion creating discharge that is more consistent. Large fish help the mixing processes in the MCR and BP removing water particles out at a faster rate. The MCR also has high enough velocities to remove NZMS.

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Table 3.1. USFWS flow calculations used to determine discharge at Dworshak National Fish Hatchery for the Burrow's ponds; DOSP=depth over standpipe.

DOSP (cm)	L/min
5.08	1400.45
5.40	1487.51
5.72	1574.56
6.03	1661.62
6.35	1752.46
6.67	1839.51
6.99	1926.57
7.30	2013.62
7.62	2100.68
7.94	2187.73
8.26	2274.79
8.57	2361.84
8.89	2452.68
9.21	2539.74
9.53	2626.79
9.84	2713.85
10.16	2800.90

Table 3.2. Discharge in liters per min (L/min) using staff gauge, Figure 3.24 (USFWS) and Equation 3.1 (new calc), and salt tracer methods, Equation 3.2, without fish present for each test system.

	Staff gauge			Salt tracer	
Time	Reading (cm)	USFWS (L/min)	New calc. (L/min)	C2 (mS/cm)	Discharge (L/min)
MCR Flow Results 17 September 2009:					
Initial (1030)	7.62			0.025	
1100	7.62			0.126	
1130	7.62			0.229	
1200	7.62			0.259	
1230	7.62			0.274	
1300	7.62			0.278	
1330	7.62			0.279	
1400	7.62	2101	2254	0.278	2271
BP Flow Results 24 September 2009:					
Initial (1030)	6.35			0.025	
1100	6.89			0.173	
1130	6.89			0.233	
1200	6.89			0.262	
1230	6.89			0.279	
1300	6.89			0.286	
1330	6.89			0.288	
1400	6.89	1900	2150	0.289	2234

Table 3.3. Discharge in liters per min (L/min) using staff gauge, Figure 3.24 (USFWS) and Equation 3.1 (new calc), and salt tracer methods, Equation 3.2, with small fish present for each test system.

MCR Flow Results 23 November 2009:

	Staff gauge			Salt tracer		
Time	Reading (cm)	USFWS (L/min)	New calc. (L/min)	C2 (mS/cm)	Discharge (L/min)	Comments
Initial (1030)	6.10			0.026		
1100	6.10			0.197		
1130	6.35			0.304		11:40 turned flow up
1200	6.89			0.333		
1230	7.62			0.314		
1300	7.62			0.307		
1330	7.62			0.306	2315	
1400	7.62	2101	2245	0.24		Started to run out of salt before shut down occurred
BP Flow Results 24 November 2009						
Initial(1000)	7.62			0.027		
1030	7.62			0.201		
1100	7.62			0.278		
1130	7.62			0.302		
1200	7.62			0.311		
1230	7.62			0.313		
1300	7.62	2101	2270	0.312	2240	

Table 3.4. Discharge in liters per min (L/min) using staff gauge Figure 3.24 (USFWS) and Equation 3.1 (new calc), and salt tracer methods, Equation 3.2, with large fish present for each test system.

	Staff gauge			Salt tracer		
Time	Reading (cm)	USFWS (L/min)	New calc. (L/min)	C2 (mS/cm)	Discharge (L/min)	Comments
MCR Flow Results 17 March 2010:						
Initial (1020)	6.35			0.031		
1050	6.35			0.167		
1120	6.35			0.221		
1150	6.35			0.246		Flow turned up at 12:00
1220	7.62			0.234		
1250	7.62			0.221		
1320	7.62			0.217		
1350	7.62			0.216		
1420	7.62	2100	2254	0.214	2277	
BP Flow Results 18 March 2010:						
Initial (1020)	8.64			0.033		
1050	8.64			0.127		
1120	8.64			0.167		
1150	8.64			0.184		
1220	8.64			0.189		
1250	8.64			0.191		
1320	8.64			0.193		
1350	8.64			0.192		
1420	8.64	2400	2420	0.191	2427	

Table 3.5. Discharge (Q; L/min) for each time testing MCR and BP using the salt tracer method calculated with Equation 3.2. Actual reading indicates the calculated discharge with conductivity readings when testing stopped and hypothetical readings are to determine if the discharge is correct by back calculating for C2 at the effluent.

				Salt conductivity (mS/cm)			
Date	Raceway	Q (L/min)	q (L/min)	C0	C1 (high)	C2 (effluent)	Reading
		2280	6.32	0.025	91.54	0.278	Hypothetical
17-Sep	MCR	2271	6.32	0.025	91.54	0.279	Actual
		2266	6.32	0.025	93.5	0.285	Hypothetical
24-Sep	BP	2234	6.32	0.025	93.6	0.288	Actual
		2269	7.65	0.027	82.2	0.303	Hypothetical
23-Nov	MCR	2315	7.65	0.027	84.8	0.306	Actual
		2268	7.23	0.027	88.77	0.309	Hypothetical
24-Nov	BP	2240	7.23	0.027	88.94	0.313	Actual
17-Mar	MCR	2277	7.23	0.032	80.118	0.214	Actual & hypothetical
		2474	4.66	0.033	82.27	0.201	Hypothetical
18-Mar	BP	2227	4.66	0.033	82.57	0.191	Actual

Table 3.6. Newly calculated flows, using Equation 3.1 for the MCR and BP. DOSP is depth over standpipe measured in inches and centimeters to achieve discharge, Q, in gpm and L/min.

Mixed cell raceway				Burrows pond			
DOSP (in)	Q (gpm)	DOSP (cm)	Q (L/min)	DOSP (in)	Q (gpm)	DOSP (cm)	Q (L/min)
2	486	5.08	1840	2	490	5.08	1854
2 1/8	501	5.40	1897	2 1/8	505	5.40	1911
2 1/4	516	5.72	1952	2 1/4	519	5.72	1966
2 3/8	530	6.03	2006	2 3/8	534	6.03	2020
2 1/2	544	6.35	2058	2 1/2	548	6.35	2072
2 5/8	557	6.67	2108	2 5/8	561	6.67	2124
2 3/4	570	6.99	2158	2 3/4	574	6.99	2174
2 7/8	583	7.30	2207	2 7/8	587	7.30	2222
3	596	7.62	2254	3	600	7.62	2270
3 1/8	608	7.94	2300	3 1/8	612	7.94	2317
3 1/4	620	8.26	2346	3 1/4	624	8.26	2363
3 3/8	632	8.57	2391	3 3/8	636	8.57	2408
3 1/2	643	8.89	2435	3 1/2	648	8.89	2452
3 5/8	655	9.21	2478	3 5/8	659	9.21	2496
3 3/4	666	9.53	2520	3 3/4	671	9.53	2538
3 7/8	677	9.84	2562	3 7/8	682	9.84	2580
4	688	10.16	2603	4	693	10.16	2622

Table 3.7. Hydraulic characteristics, using Equation 3.4 through Equation 3.7, of the mixed cell raceway and burrows pond without steelhead and small and large steelhead present. Linear regression was applied to the negative natural log of the normalized conductivity (NC).

	Mixed cell raceway				Burrows pond		
	No fish Nov 08	No fish Sep 09	Small fish Nov 09	Large fish March 10	No fish Sep 09	Small fish Nov 09	Large fish March 10
Volume (L)	116210	116210	115139	115118	113800	112510	116842
Discharge (L/min)	2271	2271	2315	2277	2234	2240	2427
Complete depletion (min)	227	227	201	214	299	314	239
$\bar{t}_c$ (min)	39.68	38.08	37.31	39.04	40.22	40.54	36.72
σ^2 (min)	1360.39	1091.71	1143.30	1303.04	1415.48	1396.26	1119.69
$\bar{t}$(min)	51.17	51.17	49.74	50.56	50.94	50.23	48.14
Turnover efficiency	0.78	0.74	0.75	0.77	0.79	0.81	0.76
SR	0.22	0.26	0.25	0.23	0.21	0.19	0.24
Volume of SR (L)	26097	29714	28713	26292	23664	21668	27734
$\sigma^2/\bar{t}_c^2$	0.86	0.75	0.82	0.85	0.87	0.85	0.83
D/μL	2.20	1.09	1.61	2.04	2.41	1.96	1.70
F(t)=-ln(NC)	0.02714time-0.1218	0.03014time-0.1392	0.02922time-0.0841	0.02756time-0.0612	0.02646time-0.0513	0.02676time-.0698	.02968time-.0797
R^2	0.9992	0.993	0.999	0.9982	0.9999	0.9999	0.9998

Table 3.8. Hydraulic characteristics with 95% of the depletion curve, using Equation 3.4 through Equation 3.7, of the mixed cell raceway and burrows pond without fish and small and large steelhead present. Linear regression was applied to the negative natural log of the normalized conductivity (NC) to determine if there were significant differences between each trial, 95% of the data was used. Superscripted letters represent differences at the 95% confidence level; values with the same common letters do not differ at the 95% confidence level.

	Mixed cell raceway				Burrows pond		
	No fish Nov 08	No fish Sep 09	Small fish Nov 09	Large fish March 10	No fish Sep 09	Small fish Nov 09	Large fish March 10
Volume (L)	116210	116210	115139	115118	113800	112510	116842
Discharge (L/min)	2271	2271	2315	2277	2234	2240	2427
95% depletion (min)	115	104	105	107	115	115	103
$\bar{t}_c$ (min)	40.26	38.58	37.74	38.97	40.42	40.73	36.99
σ^2 (min)	224.60	76.63	103.42	178.14	173.49	161.62	129.02
$\bar{t}$(min)	51.17	51.17	49.74	50.56	50.94	50.23	48.14
Turnover efficiency	0.79	0.75	0.76	0.77	0.80	0.81	0.77
SR	0.21	0.25	0.24	0.23	0.20	0.19	0.23
Volume of SR (L)	24770	28575	27701	26453	23230	21233	27099
$\sigma^2/\bar{t}_c^2$	0.14	0.05	0.07	0.12	0.11	0.10	0.09
D/μL	0.07	0.03	0.04	0.06	0.06	0.06	0.05
F(t)=-Ln(NC)	0.02667(NC) -0.10357[a]	0.2956(NC)- .1166[b]	0.02879(NC)- 0.06651[b]	0.02756(NC)- 0.06183[c]	0.02643(NC)- 0.05077[d]	0.02669(NC)- 0.06809[d]	0.02941(NC)- 0.06903[e]
R^2	0.9996	0.9992	0.9998	0.9994	0.9999	0.9999	0.9999

Table 3.9. Hydraulic characteristics, using Equation 3.8 through Equation 3.10 of the bead pulse trials in the mixed cell raceway and Burrows pond.

	Mixed cell raceway				Burrows Pond		
	Trial 1	Trial 2	Trial 3	Average (SE)	Trial 1	Trial 2	Average (SE)
Volume (liters)	108160	108160	108160	108160	103168	103168	103168
Percent collected	97.3	97.7	70.8	88.6 (15)	6.0	9.9	8.0 (2.8)
k/min	0.425	0.152	0.170	0.249 (0.15)	-0.021	-0.021	not valid
Percent flushing due to enrichment	95.287	87.890	89.023	90.73 (3.98)	-2651.87	-1734.58	not valid
$\bar{t}_c$ (min)	16.692	10.142	9.870	12.23 (3.86)	452.829	94.507	273.67 (253.37)
$\sigma 2$ (min)	263.018	163.757	152.643	193.14 (60.77)	5614.081	3689.936	4652.01 (1360.6)
$\bar{t}$(min)	47.62	47.62	47.62	47.62	46.04	46.04	46.04

Table 3.10. Hydraulic characterization using Equation 3.8 through Equation 3.10 of an individual cell in the mixed cell raceway.

	MCR Cell 2		
	Trial 1	Trial 2	Average (SE)
Volume (liters)	27040	27040	27040
Percent collected	93.832	72.403	83 (15)
k/min	0.28985	0.07605	0.183 (.15)
Percent flushing due to enrichment	77.532	47.518	62.52 (21.22)
$\bar{t}c$ (min)	20.6996	23.1192	21.91 (1.71)
$\sigma 2$ (min)	71.6785	90.1018	80.89 (13.03)
$\bar{t}$(min)	47.6213	47.6213	47.62

Table 3.11. Average velocities (m/s) and speed (cm/s) with associated standard errors for the BP at depths of 0.305 and 0.610 m below the surface and the average of all depths, velocities taken with trash pump (TP) is included.

Depth (m)	Velocity X	SE	Velocity Y	SE	Velocity Z	SE	Speed	SE
0.305	-0.0034	0.0143	-0.0056	0.0380	-0.0016	0.0021	17.0397	0.7585
0.610	0.0050	0.0188	0.0097	0.0453	0.0007	0.0021	16.6445	0.5367
Average	0.0008	0.0059	0.0021	0.0108	-0.0004	0.0016	16.8421	0.2794
0.610 TP	-0.0173	0.1365	0.0618	0.0249	0.0052	0.0138	14.2070	5.7417

Table 3.12. Average velocities (m/s) and speed (cm/s) with associated standard errors for the MCR at depths of 0.305 and 0.610 m below the surface and the average of all depths, velocities taken with trash pump (TP) is included.

Depth (m)	Velocity X	SE	Velocity Y	SE	Velocity Z	SE	Speed	SE
0.305	-0.0038	0.0050	0.0765	0.1070	-0.0068	0.0047	24.6723	2.0584
0.610	-0.0068	0.0047	0.0598	0.1098	-0.0108	0.0047	22.1617	0.3261
Average	-0.0053	0.0021	0.0681	0.0118	-0.0088	0.0028	23.4170	1.7753
0.305 TP	0.0019	0.2253	-0.0154	0.0353	0.0003	0.0094	21.9441	6.1506

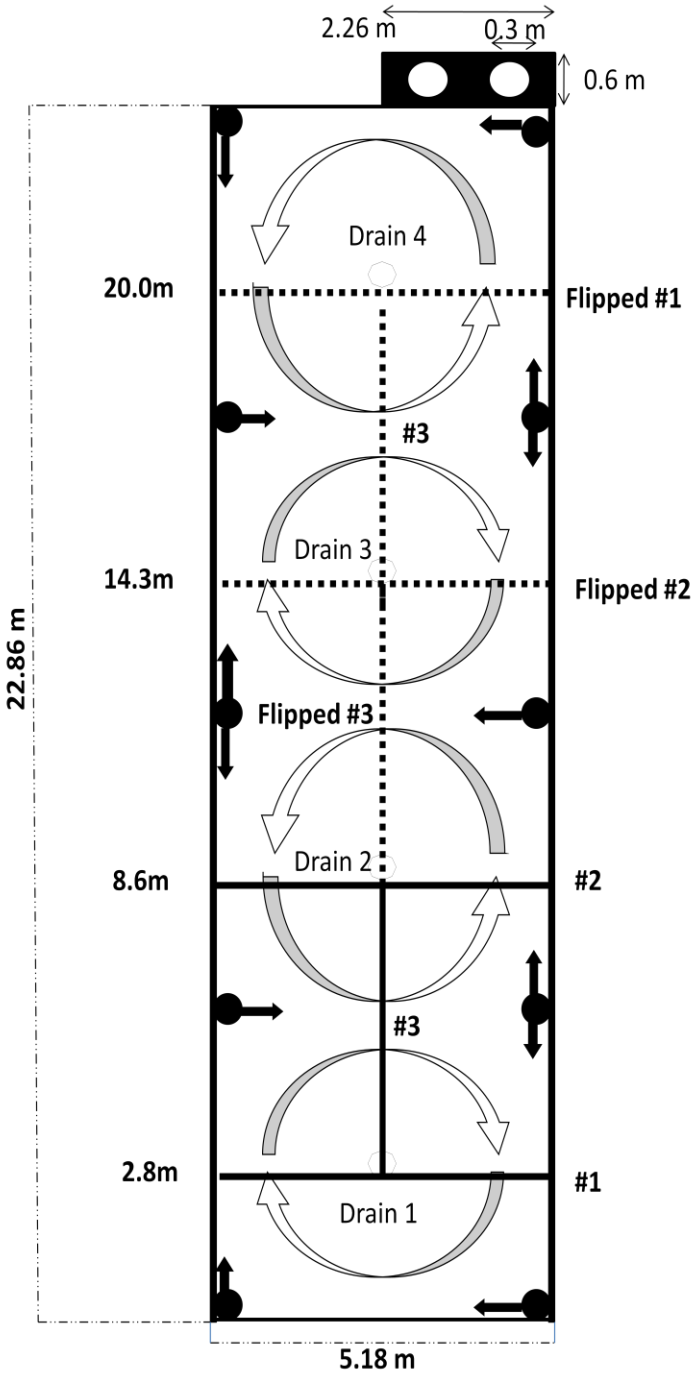


Figure 3.1. Schematic of MCR with cross-sections for each velocity measurement transect (solid lines labeled #1, #2, and #3) and transposed cross-sections with directionality associated with them (dotted lines). Black arrows from the black circles are the direction water is being jetted out through the downlegs (back circles); the white arrows indicate the directional flow of water down into the drains (white circles) and out through the standpipe box (black box and white circles represent the standpipes).

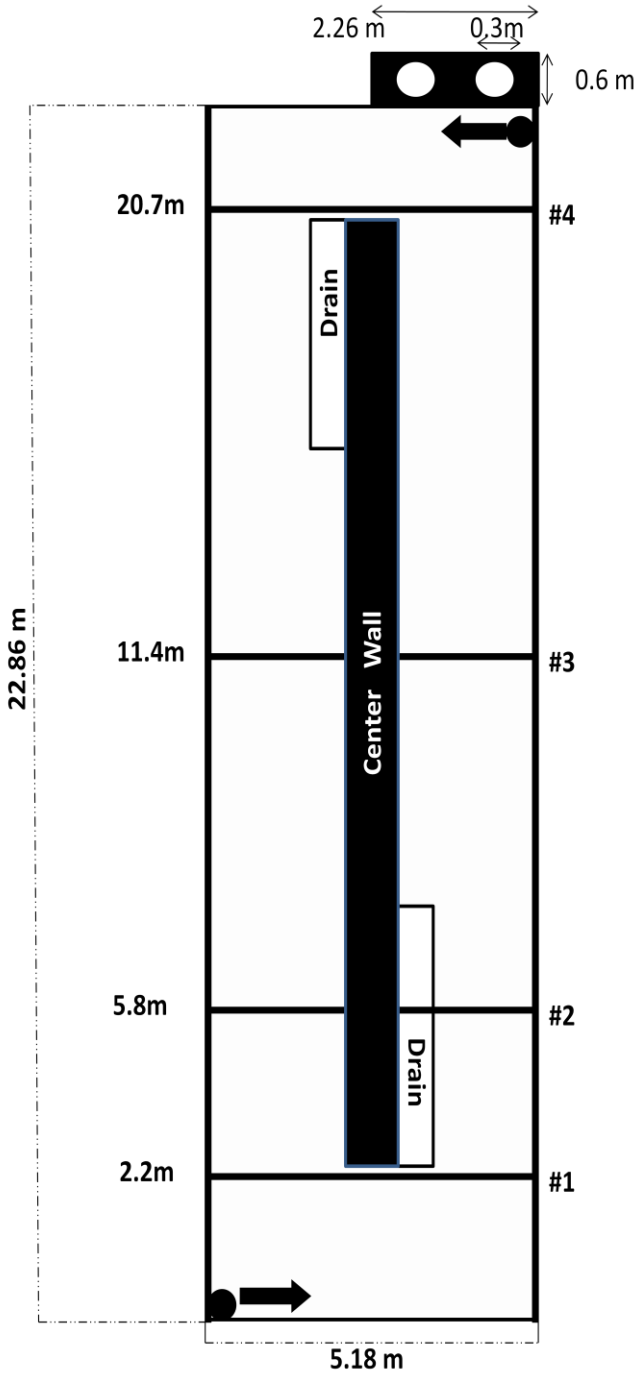


Figure 3.2. Schematic of BP with cross-sections for each velocity measurement transect (solid lines labeled #1, #2, #3 and #4). Black arrows from the black circles are the direction water is being jetted out through the inlet pipe (back circles). The water flows around the tank and exits through the drains and out through the standpipe box (black box and white circles represent the standpipes). The center wall dimensions are 16.76 by 0.2032 m.

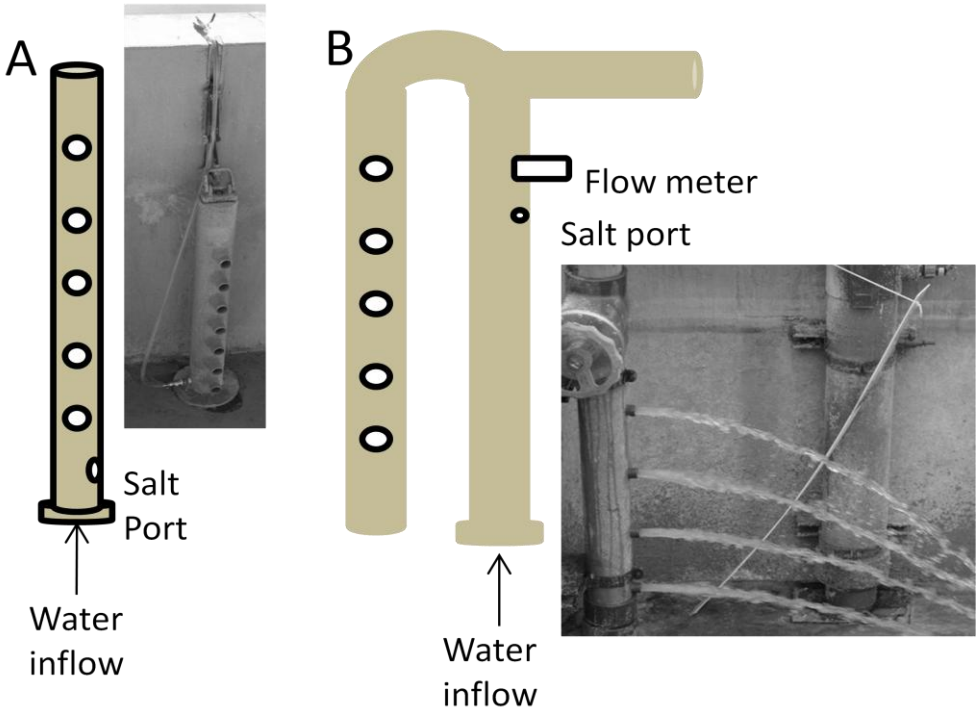
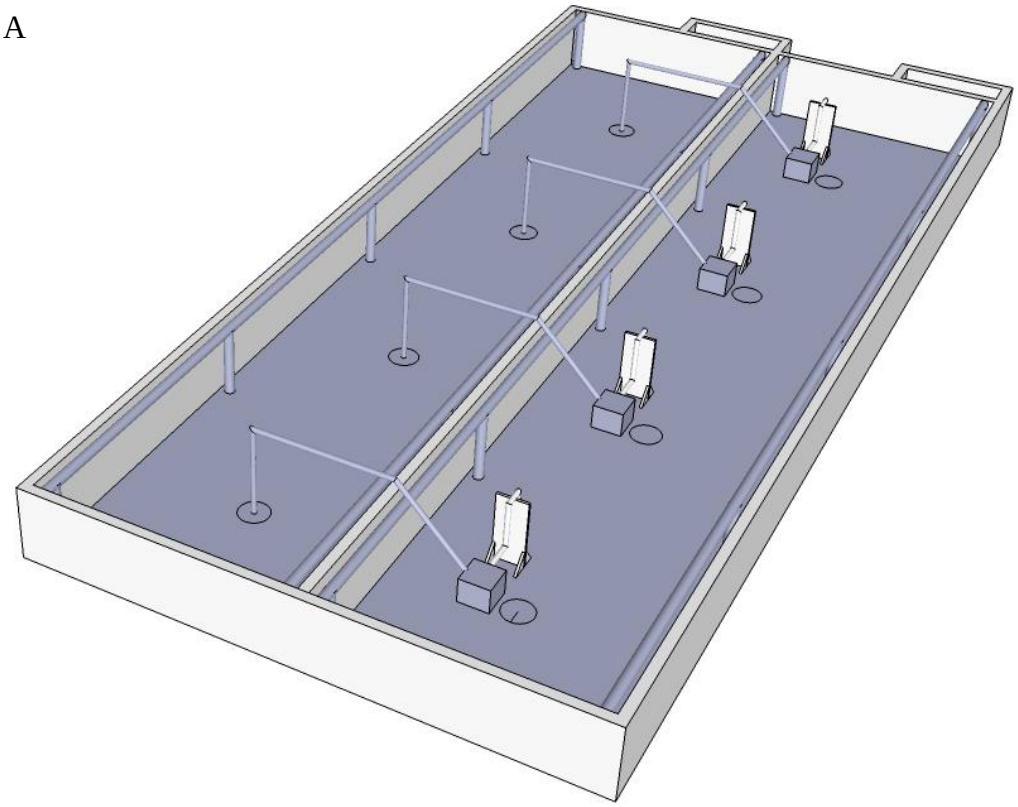


Figure 3.3. Schematic of A) inlet pipes of Burrow's Pond and B) downlegs of Mixed Cell Raceway. Jets are represented by open circles, salt port, where salt is added is labeled. The paddle wheel flow meter is also labeled and the arrow indicates water inflow through the system.

A



B

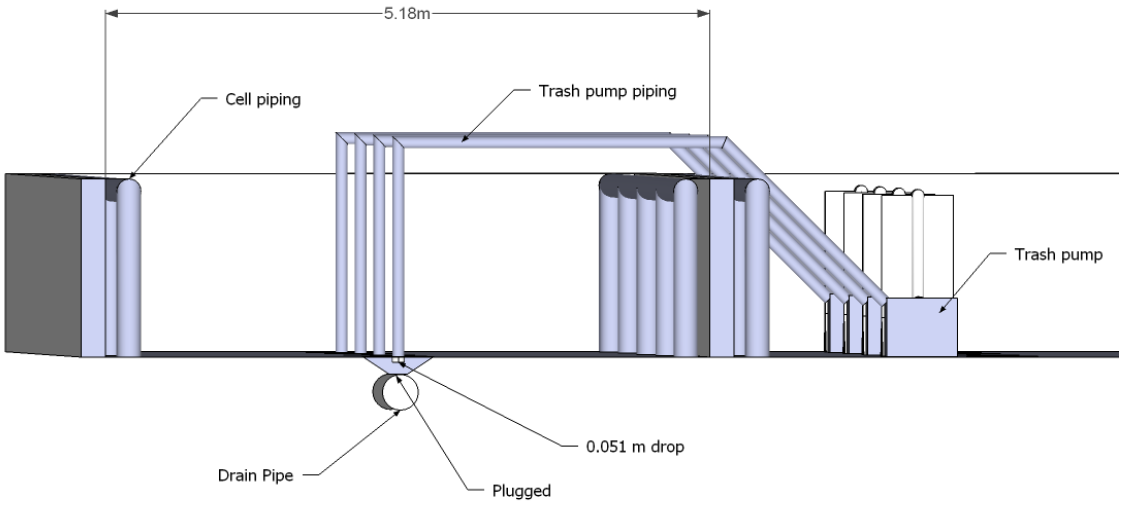


Figure 3.4. Schematic of the MCR with the drains plugged and the pumps draining the water during the particle removal study; A) is the plan view and B) is the profile view.

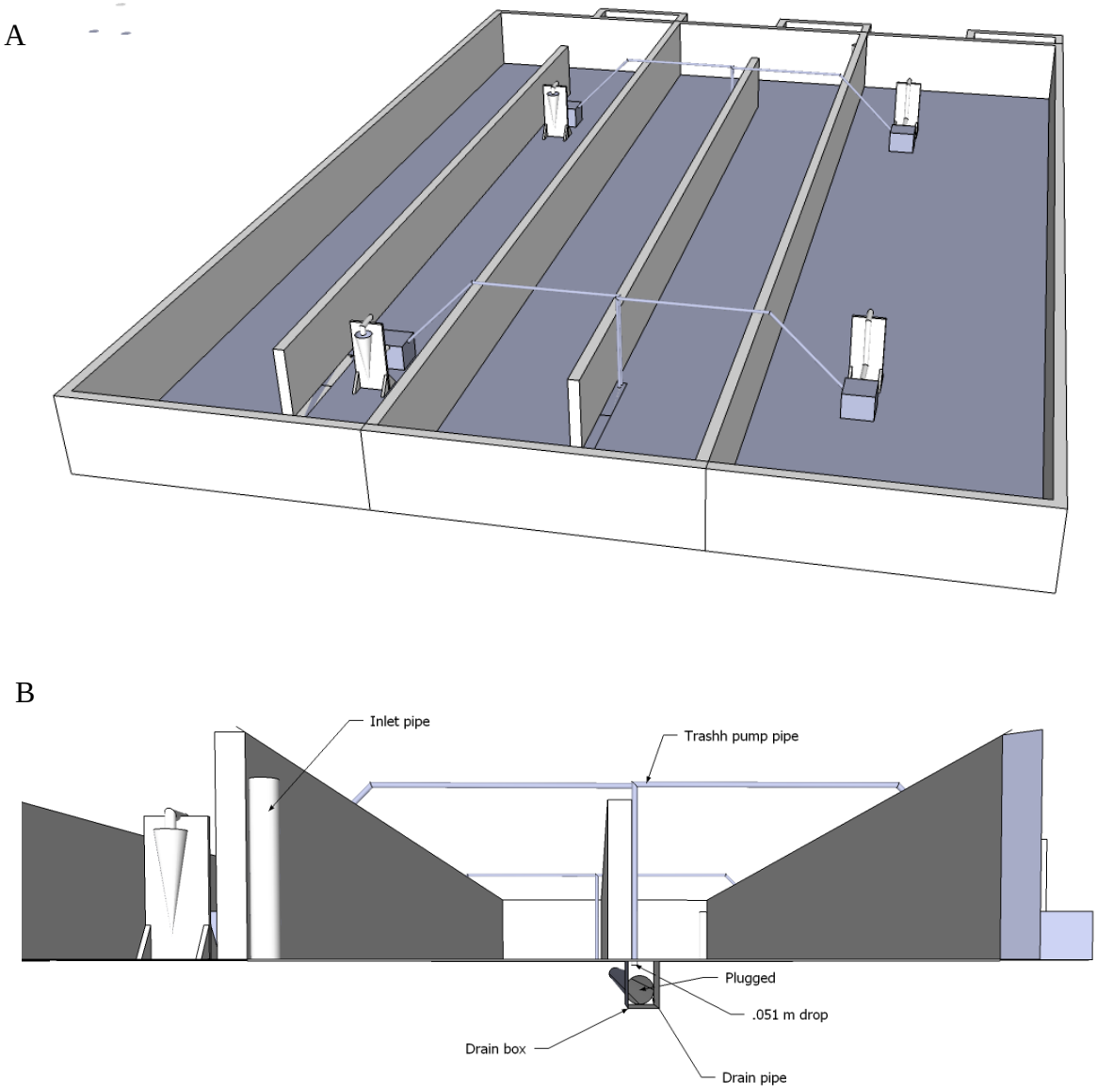


Figure 3.5. Schematic of the BP with the drains plugged and the pumps draining the water during the particle removal study; A) is the plan view and B) is the profile view.

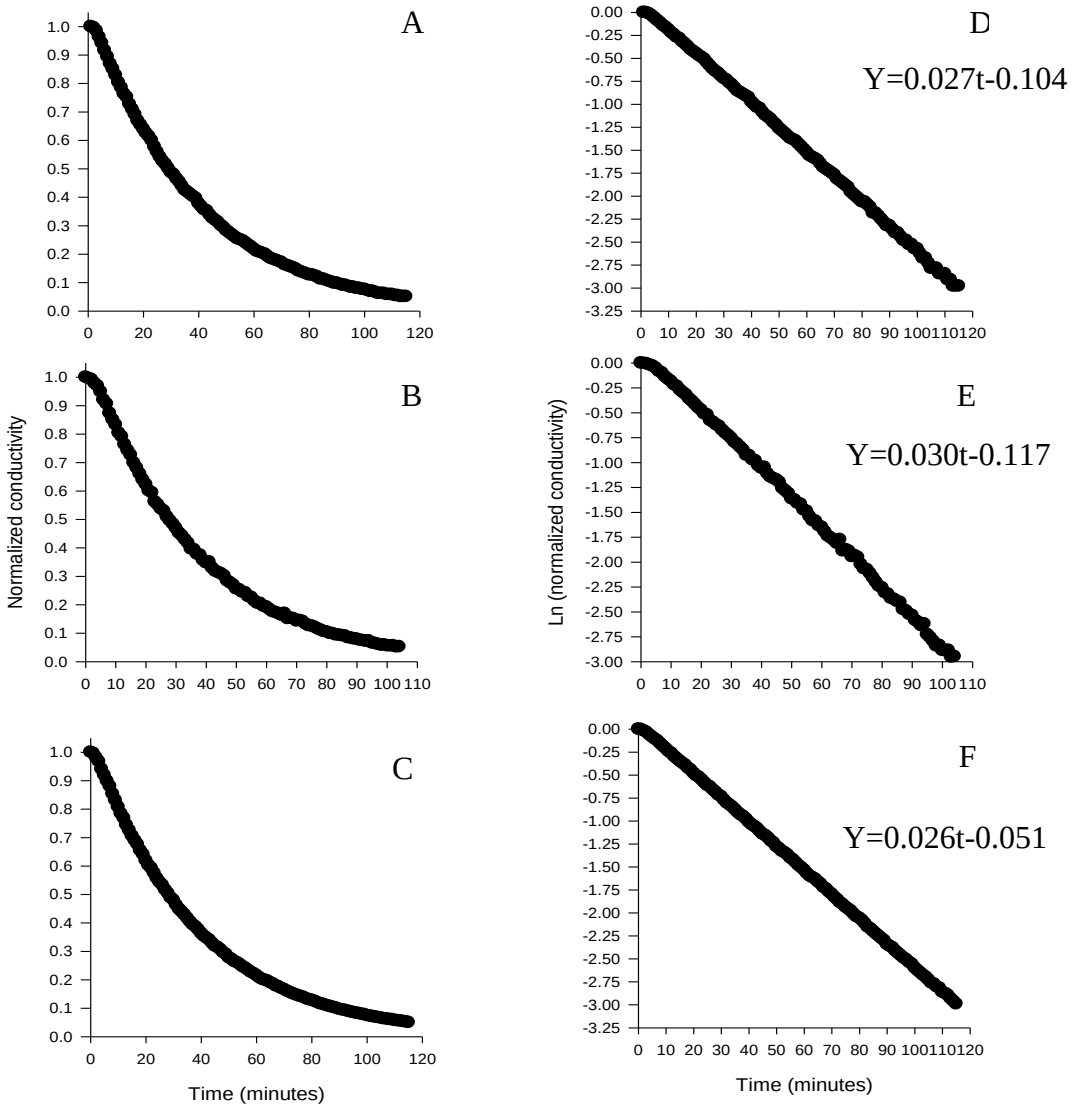


Figure 3.6. Depletion curve with 95% of the data for testing conducted in November 2008 (A) and 17 September 2009 (B) in the mixed cell raceway and for testing conducted on 24 September 2009 (C) in the burrows pond all without fish present. Linear regression was applied to the negative natural log of the normalized conductivity (NC) to determine if there were significant differences between each trial, D) testing date November 2008, E) 17 September 2009, and F) 24 September 2009. Linear regression models are shown for each trial.

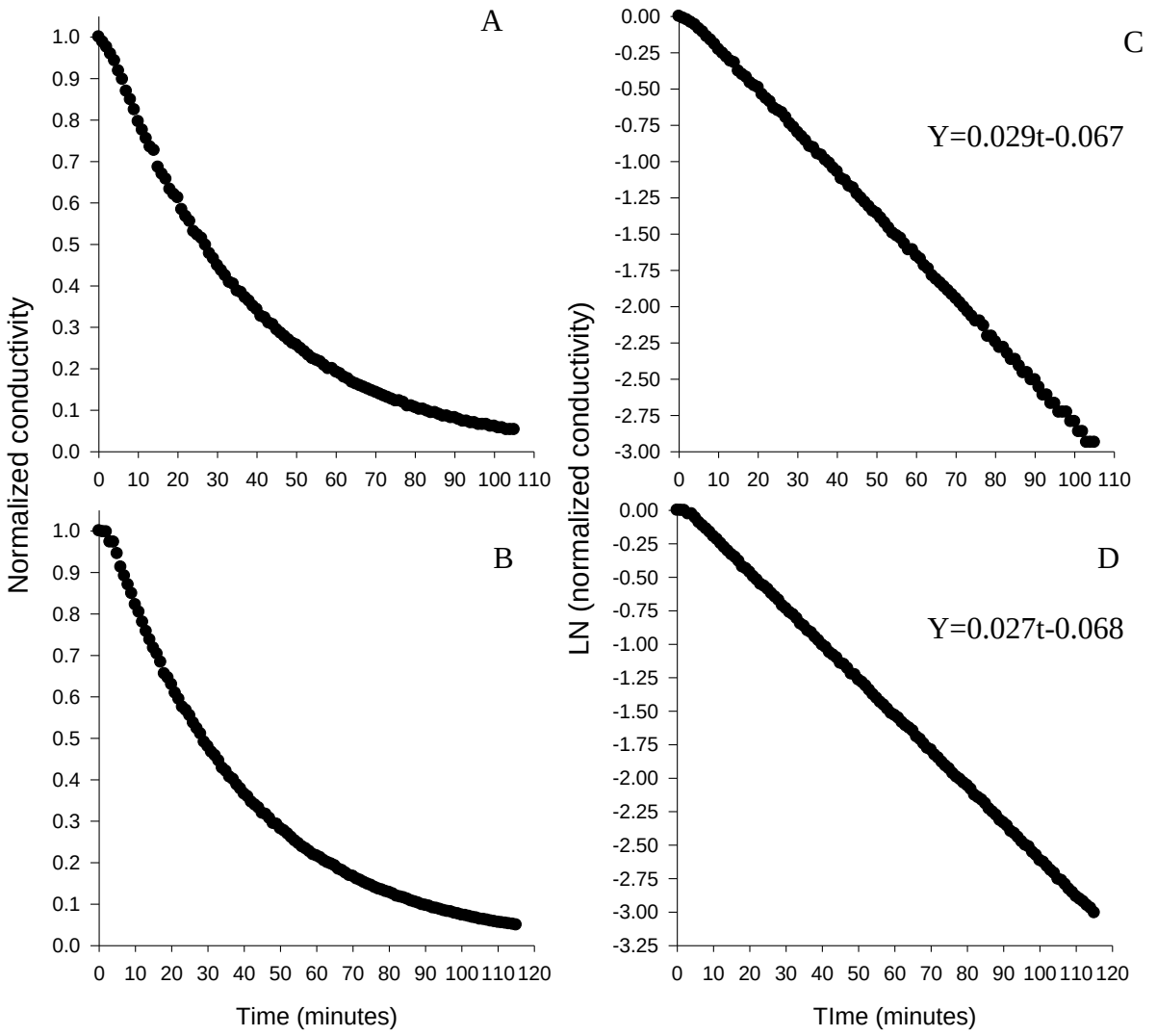


Figure 3.7. Depletion curve with 95% of the data for testing conducted in 23 November (A) in the mixed cell raceway and for testing conducted on 24 November 2009 (B) in the burrows pond all with small fish present. Linear regression was applied to the negative natural log of the normalized conductivity (NC) to determine if there were significant differences between each trial, C) testing date 23 November 2009 and D) 24 November 2009. Linear regression models are shown for each trial.

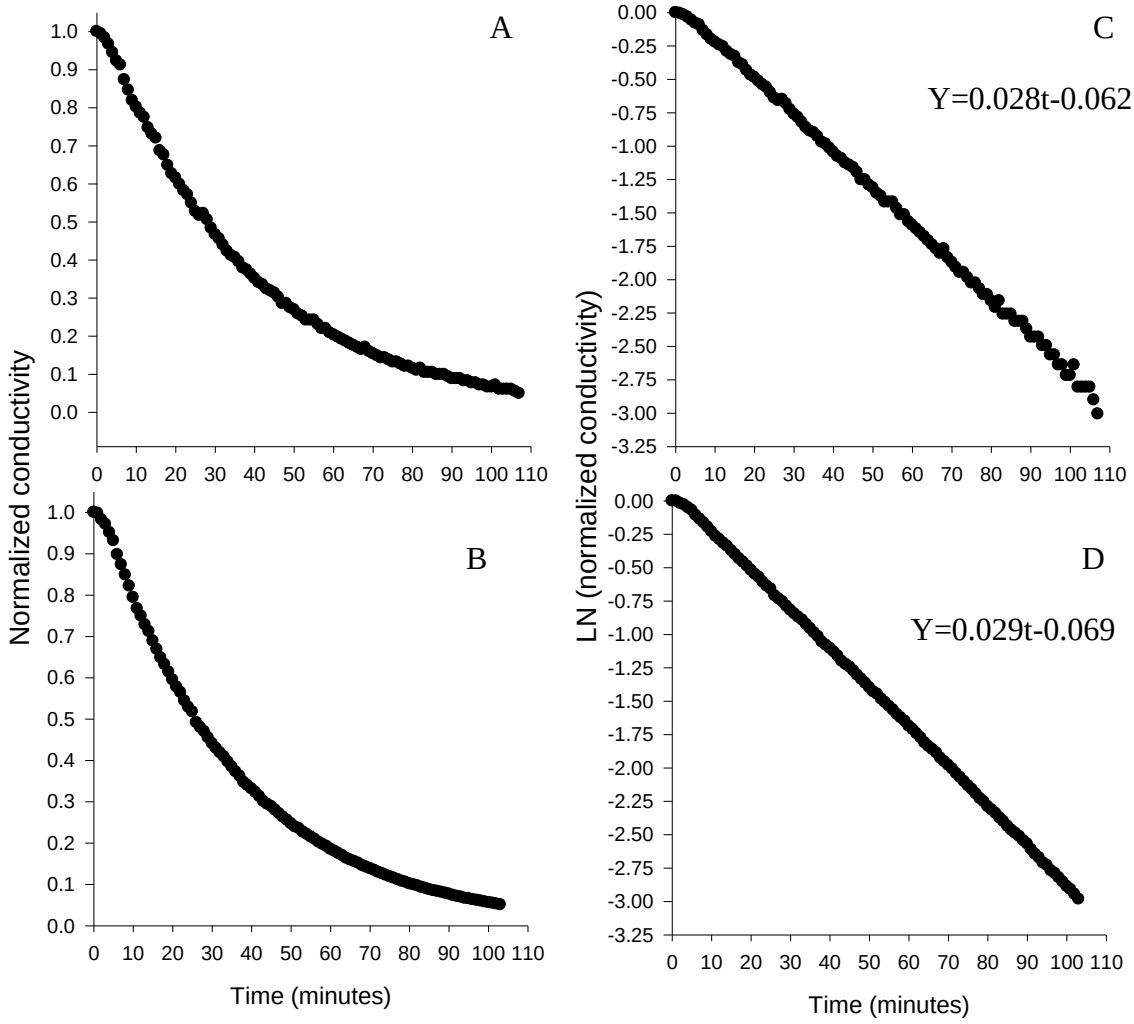


Figure 3.8. Depletion curve with 95% of the data for testing conducted in 17 March (A) in the mixed cell raceway and for testing conducted on 18 March 2010 (B) in the burrows pond all with large fish present. Linear regression was applied to the negative natural log of the normalized conductivity (NC) to determine if there were significant differences between each trial, C) testing date 17 March and D) 18 March 2010. Linear regression models are shown for each trial.

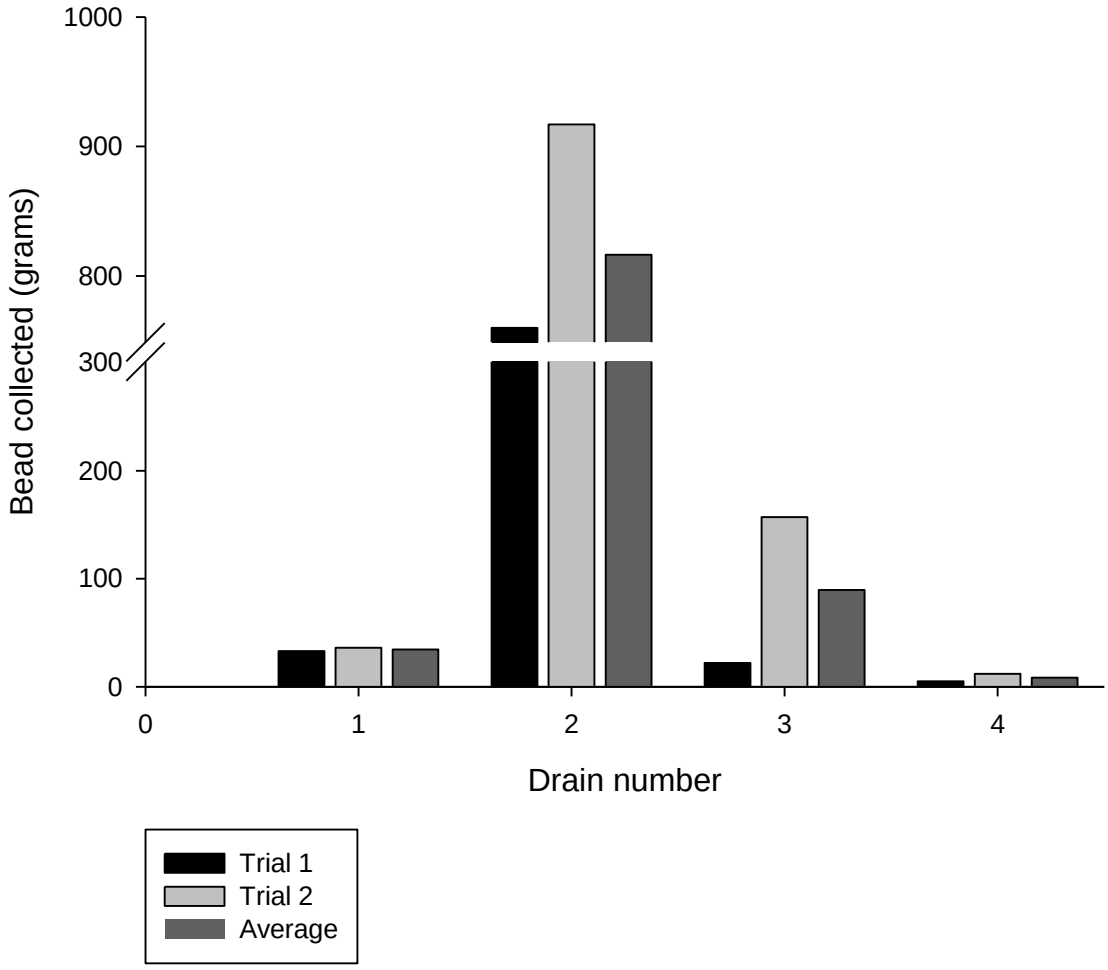


Figure 3.9. Amount of beads collected while testing the individual cell hydrodynamics of cell 2 in the MCR for trial 1, trial 2, and the average of the two trials are represented by the different colored bars. 1,134 g of beads was distributed in cell 2 only; all pumps were running and one net was collecting beads at drain 1, 3 and 4.

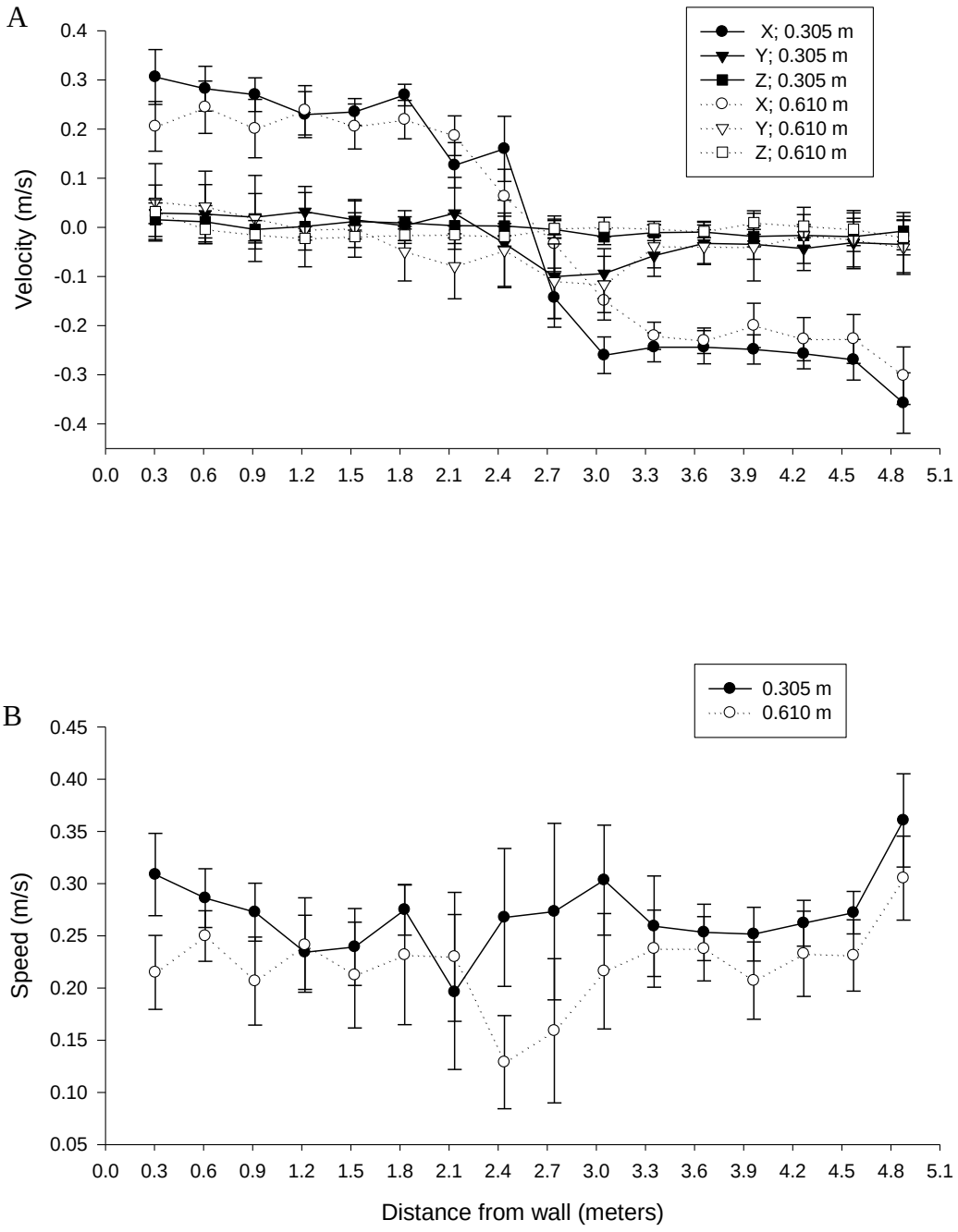


Figure 3.10. Velocity of cross section 1 in MCR with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the MCR cross section 1 at 0.305 and 0.610 meters from the surface (B).

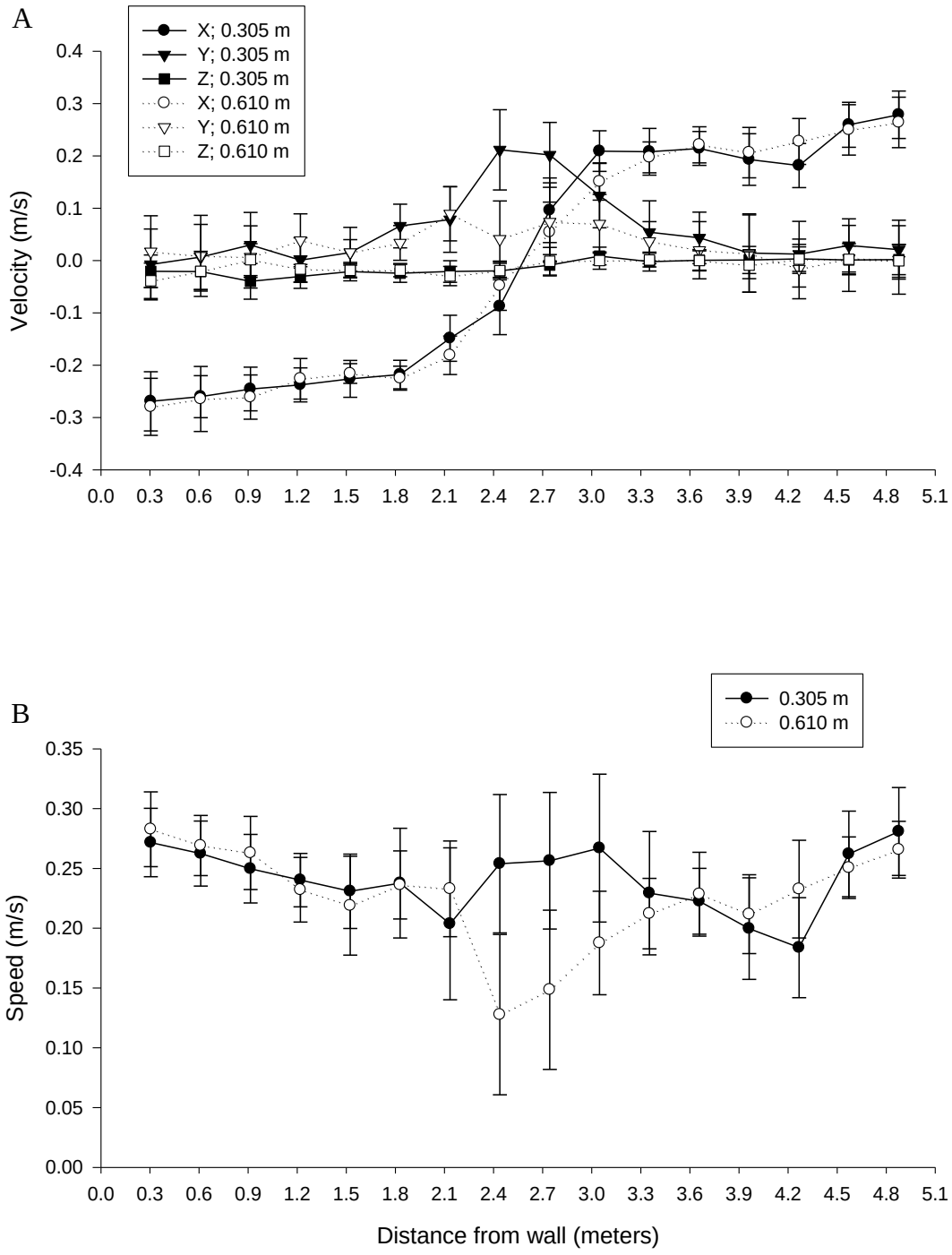


Figure 3.11. Velocity of cross section 2 in MCR with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the MCR cross section 2 at 0.305 and 0.610 meters from the surface (B).

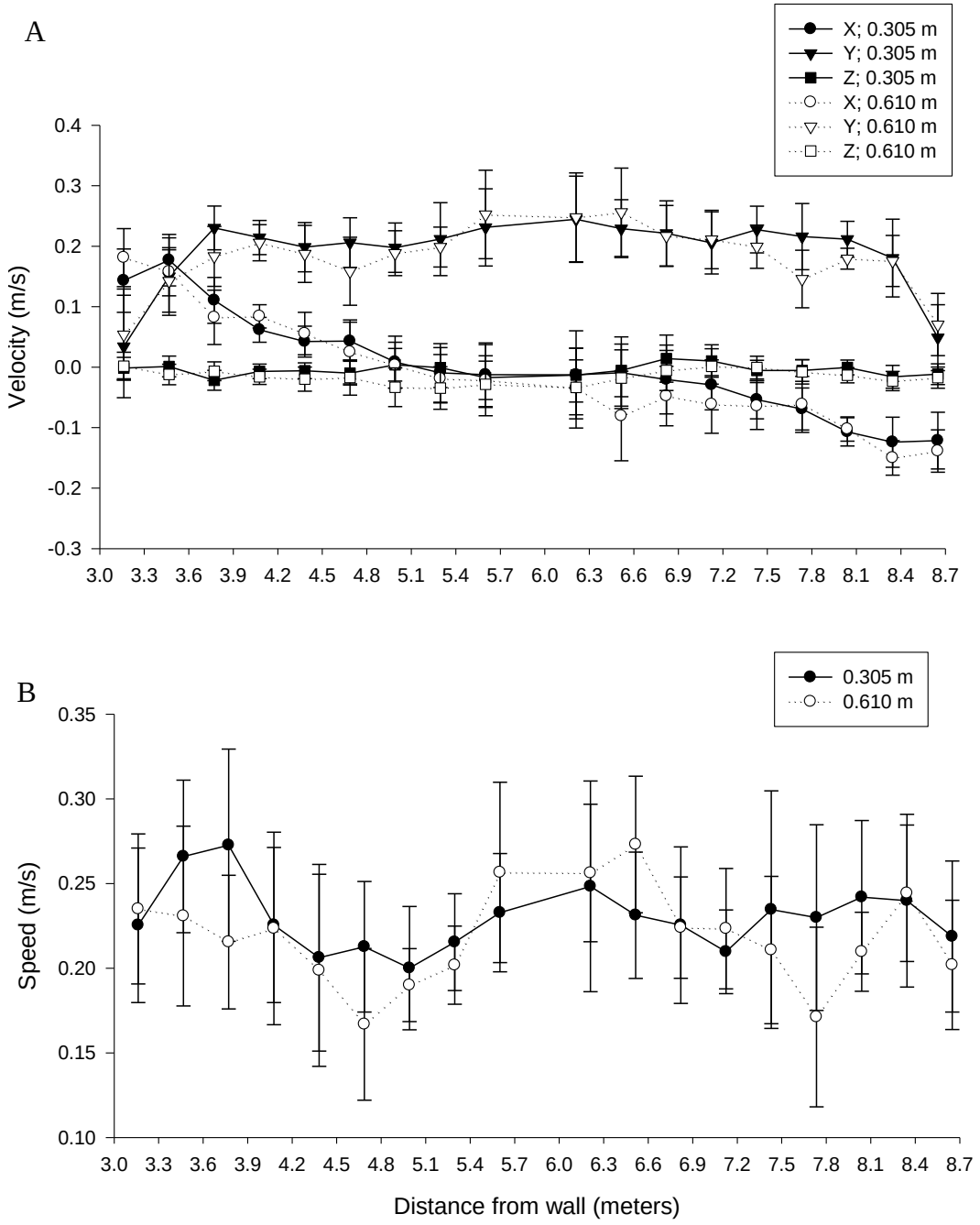


Figure 3.12. Velocity of cross section 3 in MCR with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the MCR cross section 3 at 0.305 and 0.610 meters from the surface (B).

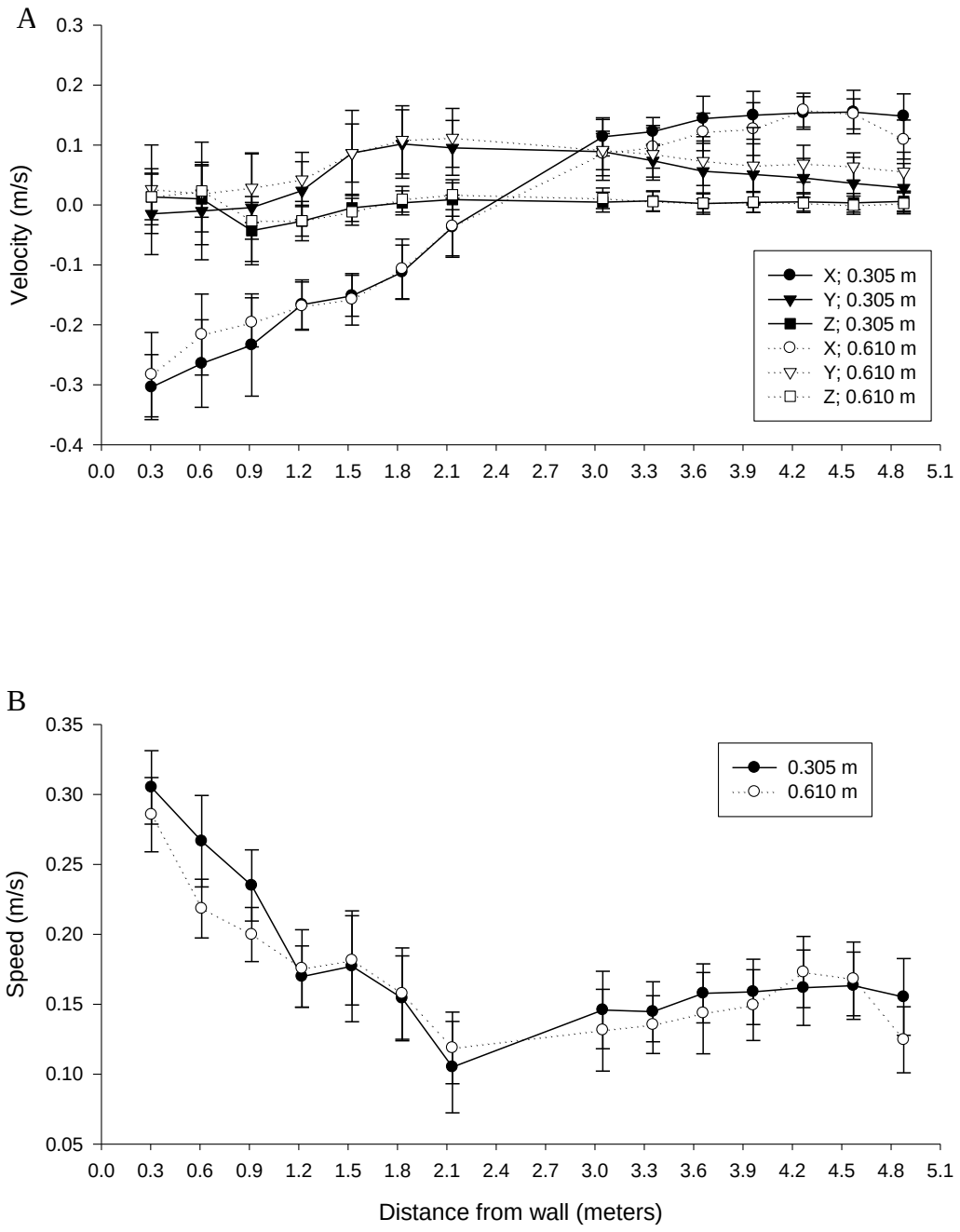


Figure 3.13. Velocity of cross section 1 in BP with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the BP cross section 1 at 0.305 and 0.610 meters from the surface (B).

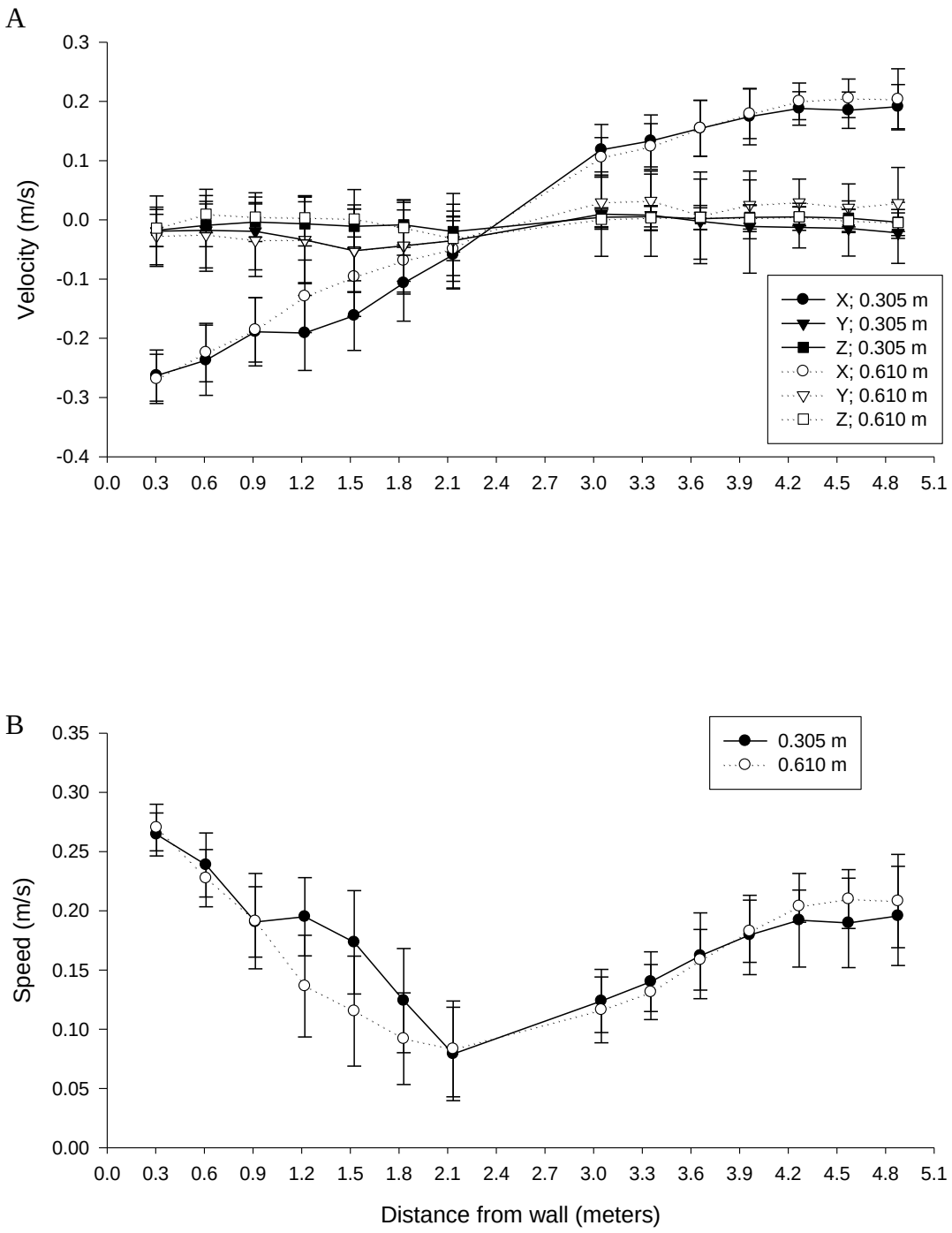


Figure 3.14. Velocity of cross section 2 in BP with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the BP cross section 2 at 0.305 and 0.610 meters from the surface (B).

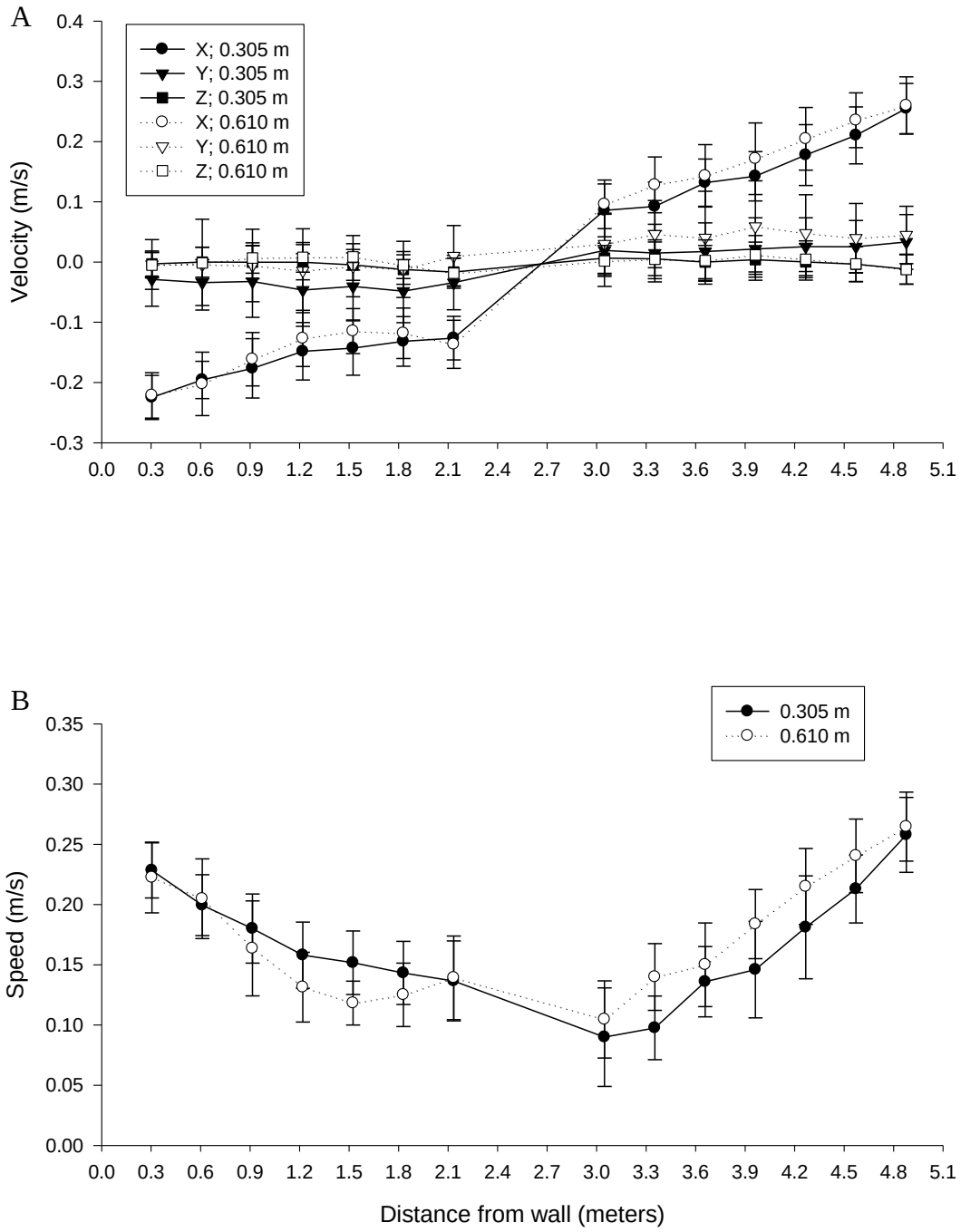


Figure 3.15. Velocity of cross section 3 in BP with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the BP cross section 3 at 0.305 and 0.610 meters from the surface (B).

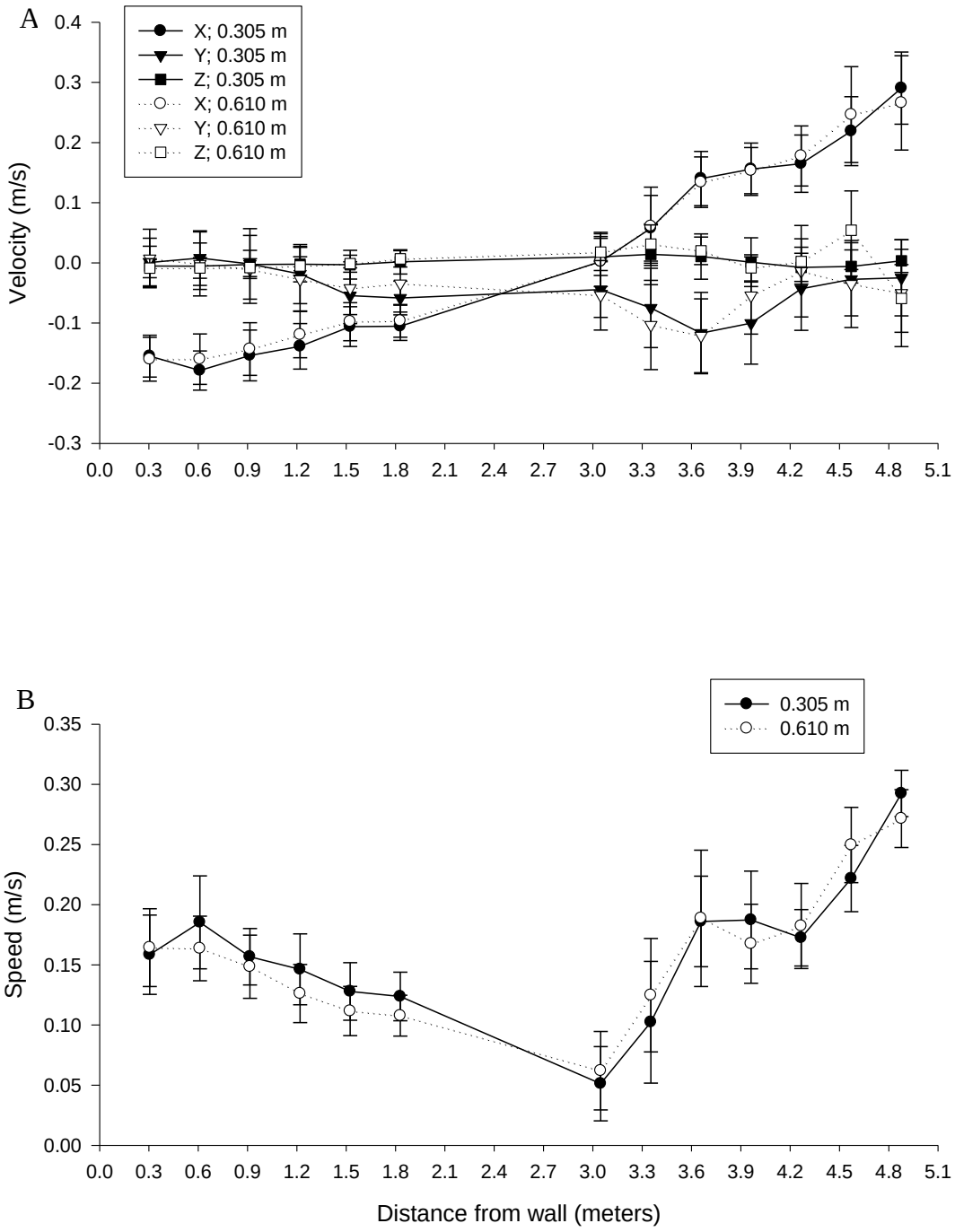


Figure 3.16. Velocity of cross section 4 in BP with standard error bars in the X, Y, and Z directions at 0.305 and 0.610 meters from the surface (A) and speed in meters per second with standard error bars for the BP cross section 4 at 0.305 and 0.610 meters from the surface (B).

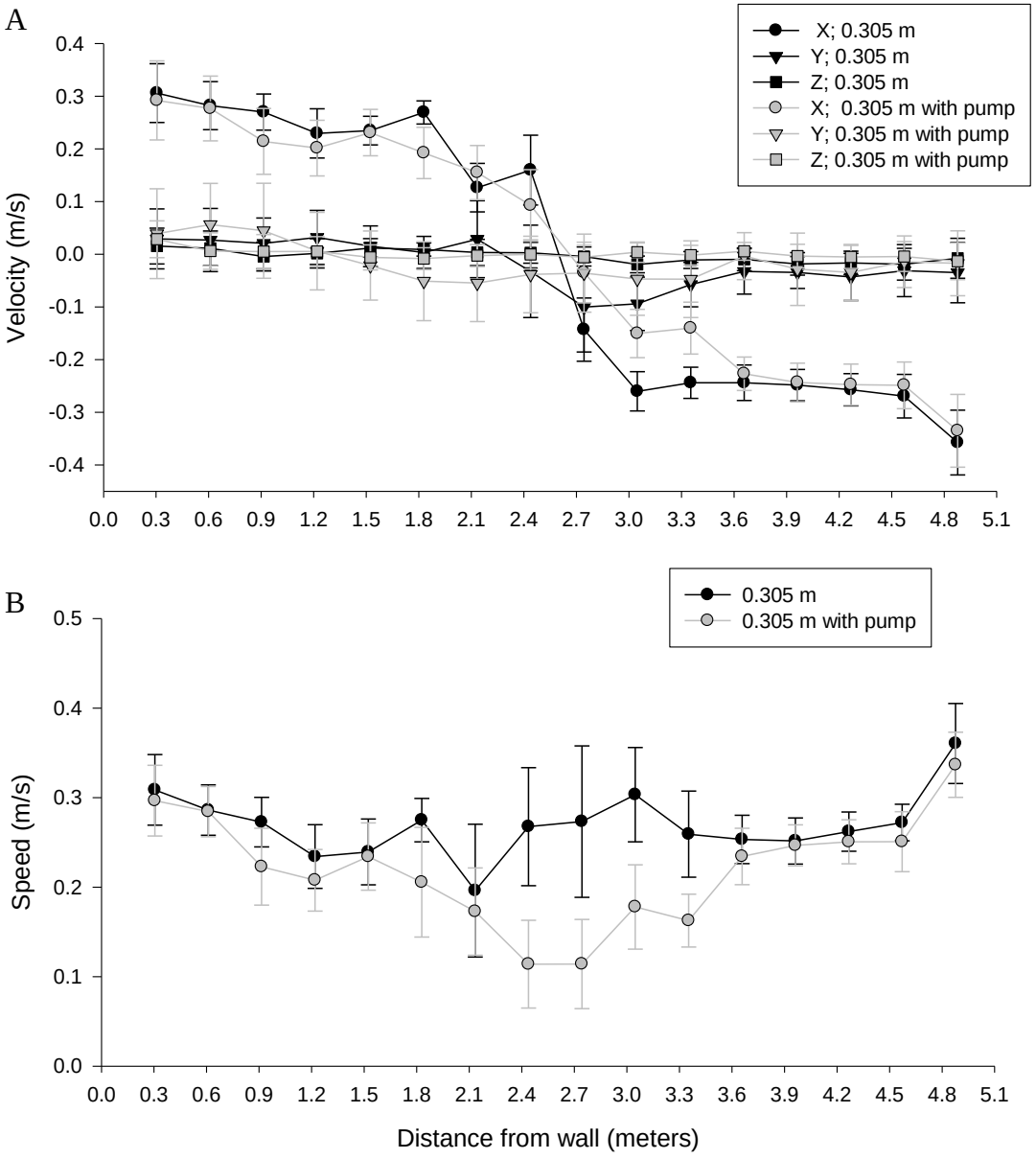


Figure 3.17. Velocity of cross section 1 in MCR with standard error bars in the X, Y, and Z directions at 0.305 meters from the surface and with trash pumps (TP) (A) and speed in meters per second with standard error bars for the MCR cross section 1 at 0.305 meters from the surface and with trash pumps (B).

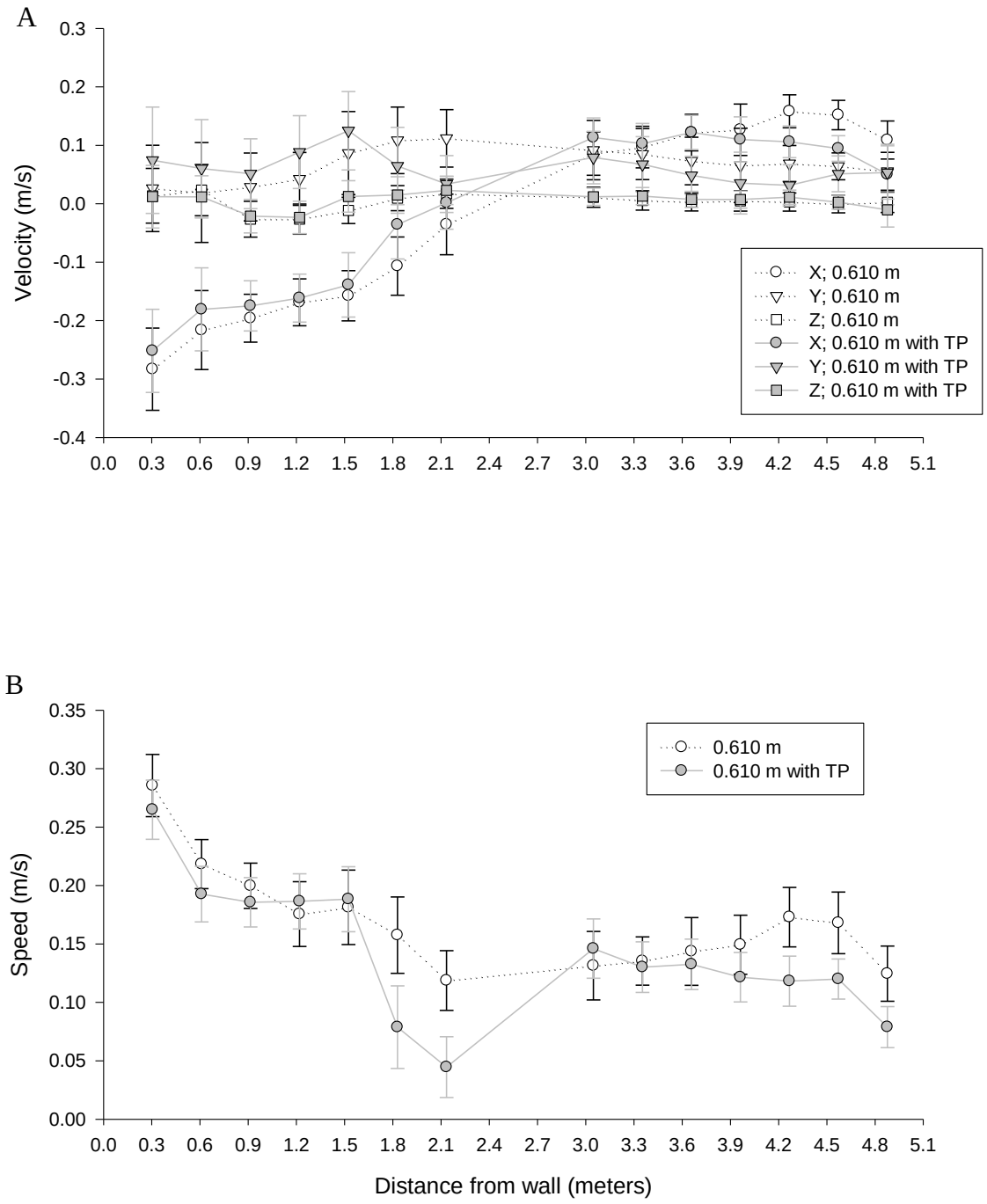


Figure 3.18. Velocity of cross section 1 in BP with standard error bars in the X, Y, and Z directions at 0.610 meters from the surface and with trash pumps (TP) (A) and speed in meters per second with standard error bars for the BP cross section 1 at 0.610 meters from the surface and with trash pumps (B).

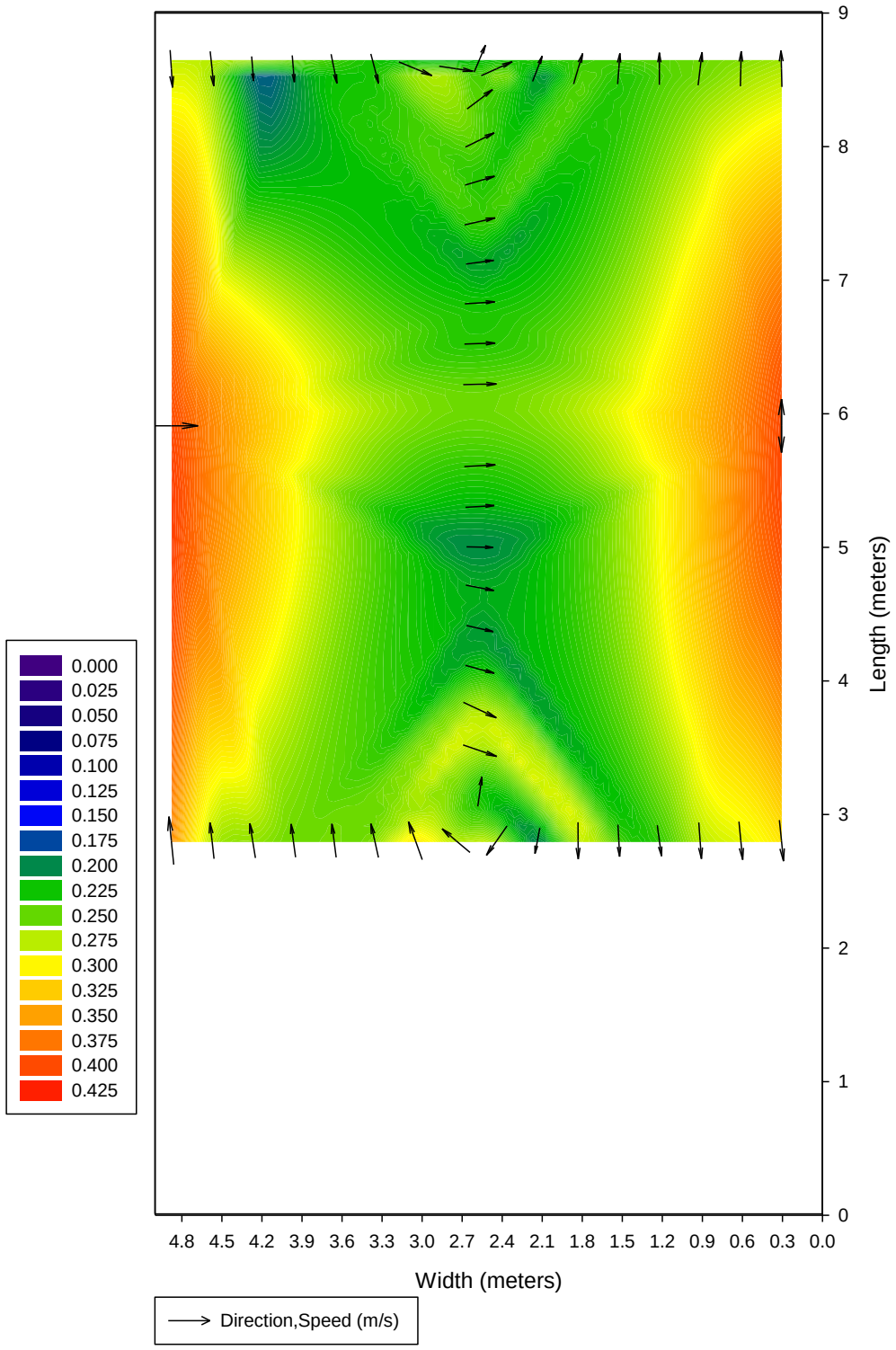


Figure 3.19. Velocity contours (m/s) and vector plots at 0.305 m from the surface in the MCR.

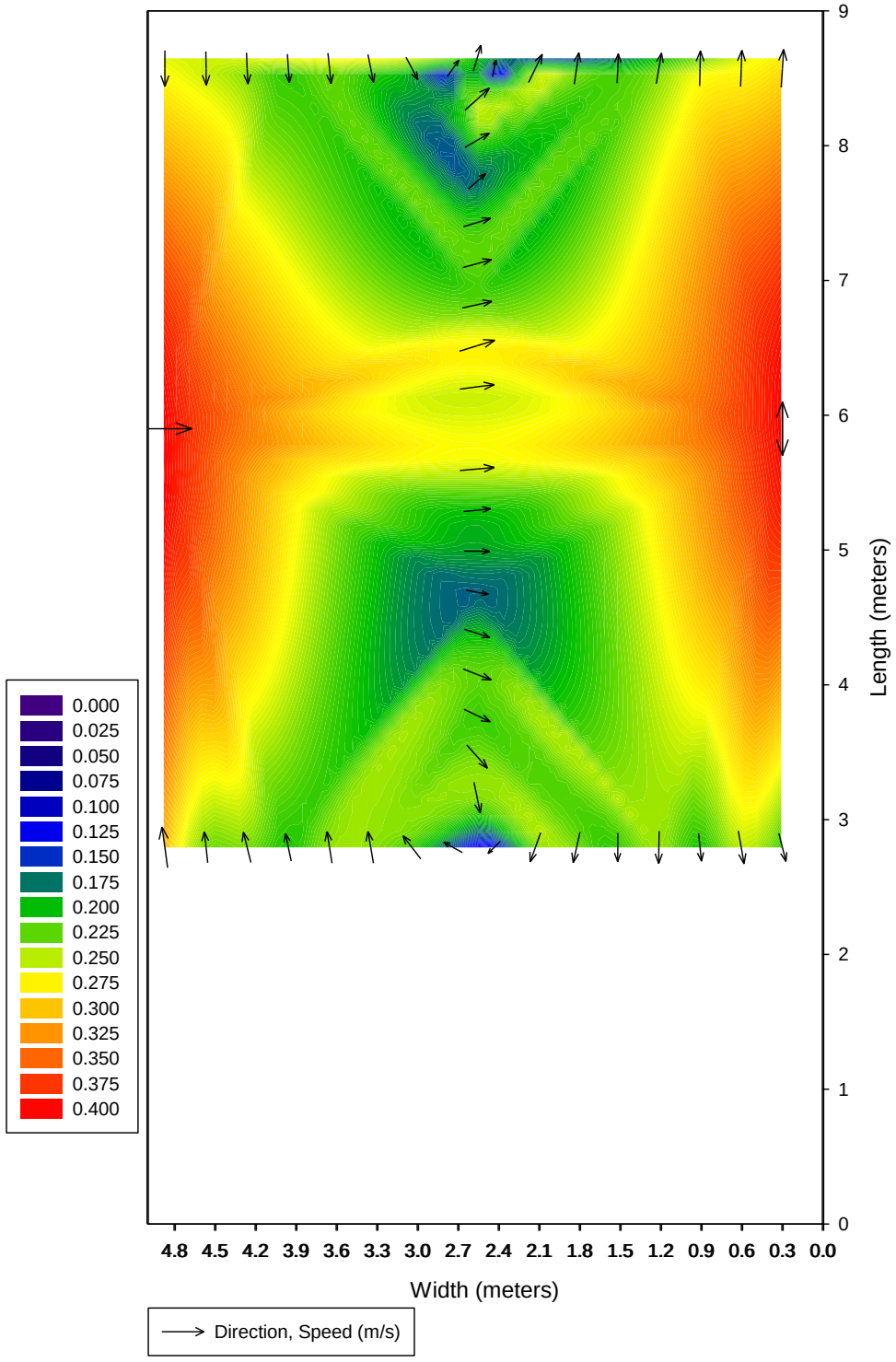


Figure 3.20. Velocity contours (m/s) and vector plots at 0.610 m from the surface in the MCR.

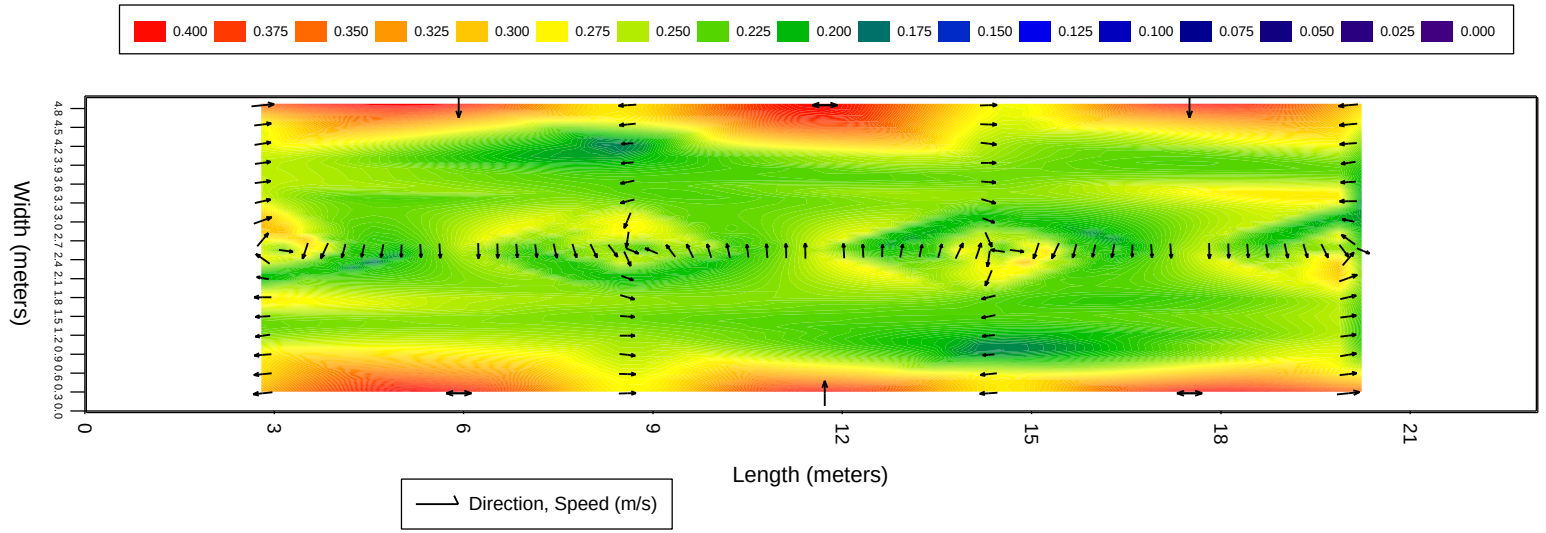


Figure 3.21. Velocity contours (m/s) and vector plots at 0.305 m from the surface in the MCR with measured and hypothetical measurements.

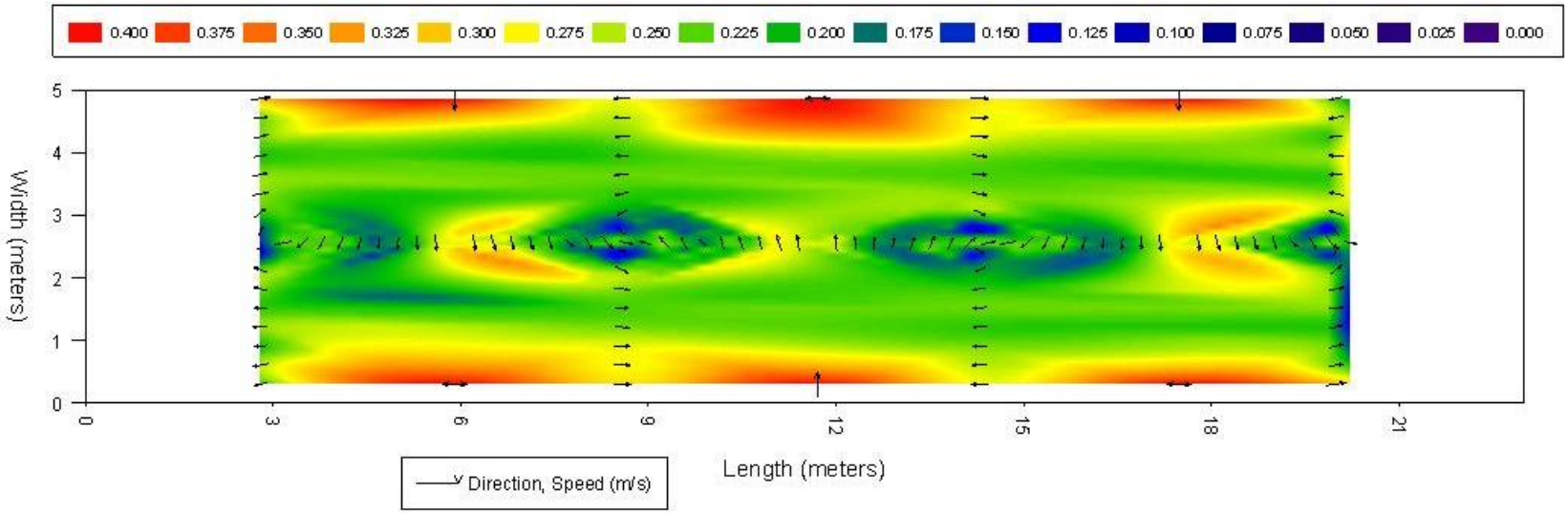


Figure 3.22. Velocity contours (m/s) and vector plots at 0.610 m from the surface in the MCR with measured and hypothetical measurements.

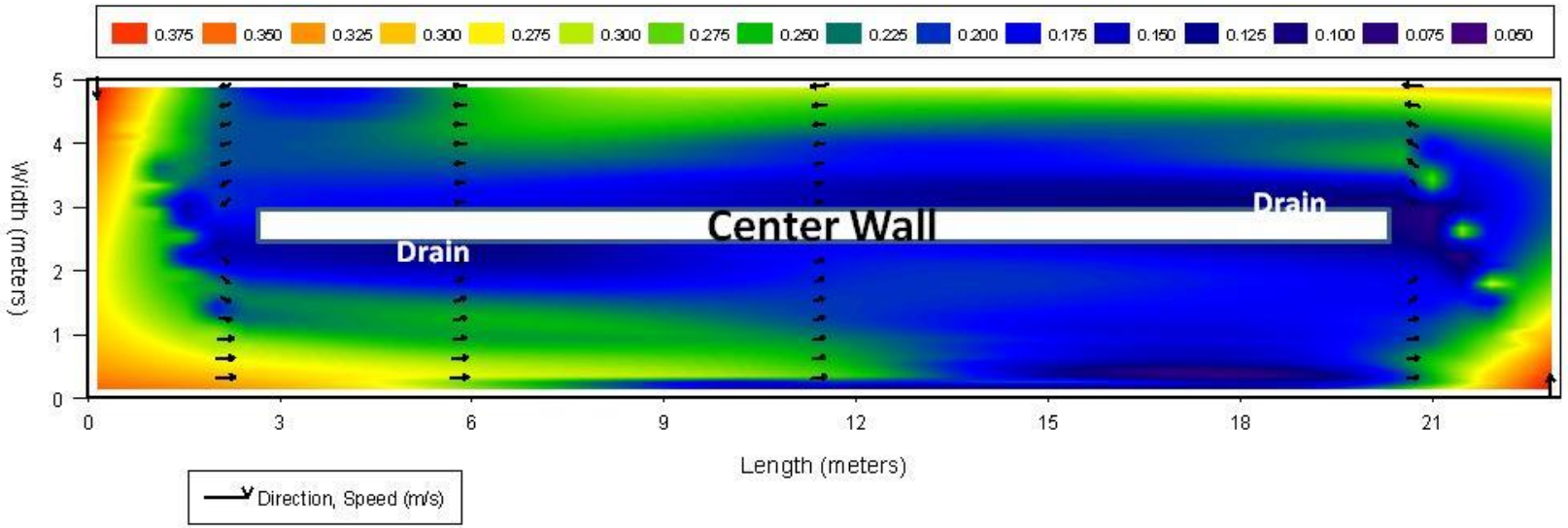


Figure 3.23. Velocity contours (m/s) and vector plots at 0.305 m from the surface in the BP.

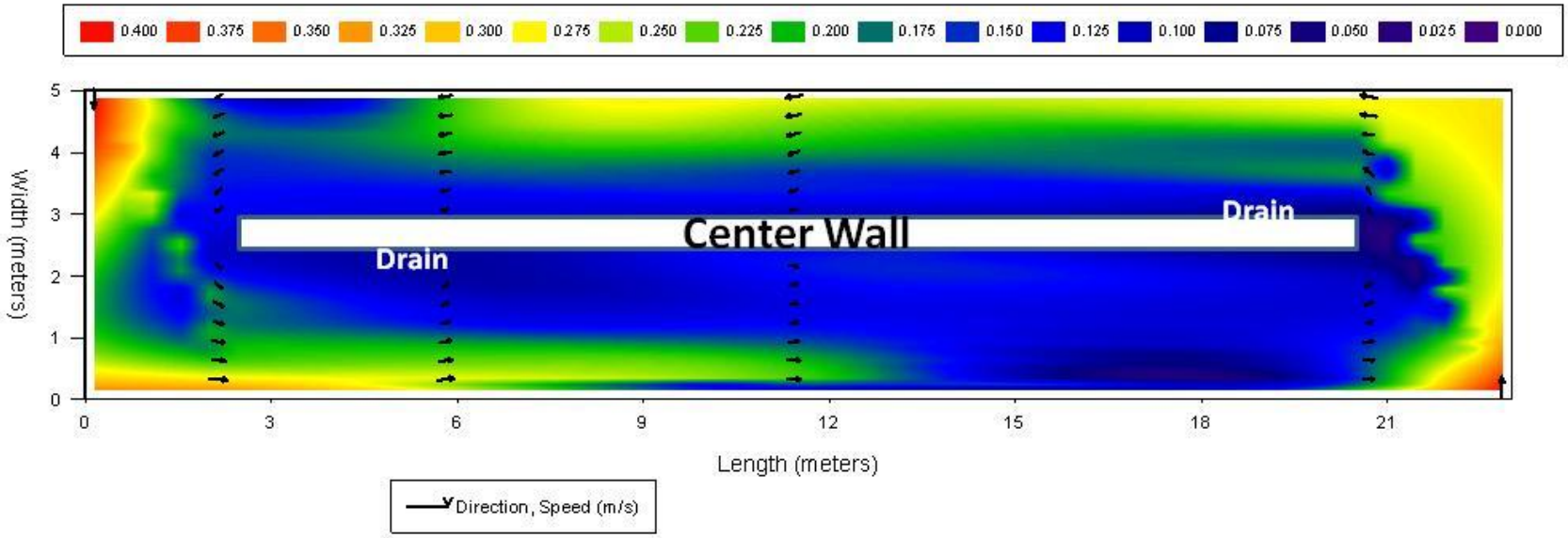


Figure 3.24. Velocity contours (m/s) and vector plots at 0.610 m from the surface in the BP

Chapter 4: Decision Guide to Assess Risk, Options for Management and Control of Existing or Potential Infestations of New Zealand Mudsnails in Fish Hatcheries

Abstract

We provide a decision guide framework and data summary that incorporates risk assessment, risk management, and decision analysis for preventing, eliminating, or controlling a NZMS infestation at a fish hatchery facility. This document is a living document, which should be updated and changed to include information based on the needs of the user, and incorporate new information through adaptive management tools. Although these tools were developed for infestations of NZMS at fish hatcheries, the principles of the assessment and decision framework can be applied to locations outside of fish hatcheries and to other species of concern.

Introduction

The ecological impacts from invasion are the most poorly understood aspect of biological invasions (Kulhanek et al. 2011). This coupled with limited research about the parameters influencing biological organisms make risk analysis complex (Dahlstrom et al. 2011; Kulhanek et al. 2011). When risk analysis is applied to aquatic invasive species, it becomes more difficult. There are a limited number of risk analyses focused on aquatic invasive species from international, regional, and national entities with effective applications (Dahlstrom et al. 2011). Combining risk analysis frameworks using decision trees, scientific data, and being transparent will increase the effectiveness of a risk analysis applied to a facility with a risk of being infested with aquatic invasive species.

Risk is encountered in every aspect of life. We assess the risk associated with actions, and manage or control that risk with the actions and choices we make. Risk analysis is applied to many situations to inform and determine the importance of regulatory and technological decisions, setting priorities among research needs, and developing approaches costs and benefits (Committee on Improving Risk Analysis Approaches Used by the US EPA

2009). The U.S. Environmental Protection Agency (EPA) was one of the first to apply risk assessment to the environment, using the 1983 National Research Council report *Risk Assessment in the Federal Government: Managing the Process*, also known as "the Red Book" (National Academy of Sciences 2008). However, the science of risk assessment is increasingly complex because it has been improved by analytical techniques (Committee on Improving Risk Analysis Approaches Used by the US EPA 2009).

One method for determining risks of invasive species is through guidelines and examples developed by the Council of the Commission for Environmental Cooperation (CEC; Mendoza et al. 2009). Guidelines were modeled after the review process developed by the Aquatic Nuisance Species Task Force, Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process (Hammond 2009). The CEC work group produced a guidelines document that is useful, flexible, and easily understood (Mendoza et al. 2009). The guidelines provide a structure for a risk assessment of a particular species, incorporating scientific, cultural, and other data, cost-benefit analysis, and pathway assessments. The assessment incorporates uncertainty as part of the ranking process, as a qualitative measure; however, other methods or detailed instructions need to be incorporated to determine and decrease uncertainty of the data included in the assessment. Another limitation is there are no forms to fill out to describe the risk assessment to others.

The HACCP planning process has been used for conducting risk assessments for invasive species at fish hatchery facilities. The HACCP process was developed for food production to ensure product purity in the early 1960s (USFWS 2004). The International Organization for Standardization has adopted HACCP as a way to formally standardize a risk assessment analysis for food hygiene (ISO 2005). The NOAA Sea Grant and the USFWS have suggested this method for investigating risk of aquatic invasive species infestations (USFWS 2004). For aquatic nuisance species in hatcheries, the process provides a series of forms to document and organize in detail activities at a facility to determine potential unwanted organisms that may affect the activity (USFWS 2004). The critical control points are then determined, with direction for control measures and monitoring to determine if control measures were adequate. Limitations of the HACCP approach include the lack of risk assessment to determine the likelihood of infestation at the site. In addition, focus only on human activities

can overlook potentially other pathways of infestation. The HACCP plan for Hagerman National Fish Hatchery (HNFH) documents the activity of raising steelhead at their facility because they are trying to minimize their risk of transporting NZMS to new areas (HNFH 2002). However, their plan does not address the risk posed by the pathway of visitors entering the facility, or animals carrying NZMS onto or off their facility. Finally, any management program must have monitoring steps in place to report the effectiveness of control measures, and plan for an adaptive management that uses new information to improve control and monitoring in the future.

Uncertainty influences our decisions and the actions involved in analyzing risk. Uncertainty is a state of having limited knowledge where it is impossible to exactly describe existing state or future outcome. For example, there is uncertainty regarding control measure research; water chemistry, such as pH, hardness, and specific conductivity, along with flow restrictions, which change the effectiveness of different control strategies. Adequate head is needed to operate a hydrocyclone filtration, which has a 367 L/min flow capacity (Nielson 2008). Other examples of uncertainties in the control measures are the discrepancies between studies testing the efficacy of chemical treatments to kill NZMS. Hosea and Finlayson (2005) found and recommended that a 50% concentration of Formula 409® was adequate to obtain 100% mortality of NZMS. Schisler et al. (2008) found that the 50% concentration was not adequate to obtain 100% mortality; a 100% solution of Formula 409® was needed. Another aspect of uncertainty is applying small-scale laboratory studies to field studies and then to the real world practical setting. Uncertainty can be minimized, accounted for, and decreased when used in a risk analysis. Uncertainty can be quantified and analyzed through qualitative measures by using weight of evidence techniques (Suter II and Cormier 2011). There are many methods of determining the probability of an occurrence, such as repeated sampling, observations sampling, and use of prior distributions (Harwood and Stokes 2003).

Several studies have addressed a diversity of control measures that can be applied to insure hatchery waters and fish can be protected from infestations of New Zealand mudsnails *Potamopyrgus antipodarum* (NZMS). The National Management and Control Plan provided a summary of this information through 2007 (Aquatic Nuisance Species Task Force and National Invasive Species Council 2007). However, research on control measures has

continued. These include depuration strategies (Bruce and Moffitt 2010), filtration methods (Nielson 2008), use of low or high temperatures or chemical disinfectants to kill NZMS (Dwyer et al. 2003; Cheng and LeClair 2011; Oplinger and Wagner 2011; Stockton this thesis), copper, electrical, and velocity barriers (Oplinger and Wagner 2009 a & b; VanDyke 2010; Hoyer 2011). Some of the experiments, especially the chemical disinfectants studies, have added more control measures that achieve 100% mortality or effectiveness in limiting NZMS (Oplinger and Wagner 2009a; Oplinger and Wagner 2011). However, some of these studies have conflicting results, which confused the public on what and how to use the research for their personal use, especially when disinfecting their own contaminated gear.

Fish hatchery managers are concerned about NZMS. NZMS are easily transported because they are very small, ranging in size from 80 µm to 6 mm and live in a variety of habitats, such as estuaries, lakes and rivers (Winterbourn 1970; Alanso and Castro-Diez 2008; Bersine et al. 2008). A freshwater prosobranch, NZMS have an operculum that closes tightly allowing them to resist desiccation and chemical treatment (Richards et al. 2004). They are parthenogenic, so females are abundant and only one NZMS will populate an area (Richards et al. 2004; Alanso and Castro-Diez 2008). Some fish eat NZMS (Bersine et al. 2008). NZMS will survive the digestive tract of salmonids (Haynes et al. 1985; Bruce and Moffitt 2010).

Federal regulations, laws, policies, and plans have required that infestation of NZMS within the control of Federal agencies, such as fish hatcheries, be managed. The Lacey Act makes transport of NZMS from certain states to other states, which restricts possession, an offense of the Lacey Act (Aquatic Nuisance Species Task Force and National Invasive Species Council 2007). The Federal Executive Order 13112 of 1999, created the National Invasive Species Council and Invasive Species Advisory Committee, which in turn created the National Management and Control Plan for the New Zealand Mudsnail (USOFR 1999; Aquatic Nuisance Species Task Force and National Invasive Species Council 2007). Federal fish hatchery managers have employed Hazard Analysis and Critical Control Point planning (HACCP) to assess their facilities for unwanted organisms, specifically used to limit aquatic invasive species (USFWS 2004).

States have created aquatic nuisance species management plans. Some states, such as California and Colorado have closed facilities that are infested with NZMS. Others have just monitored their state to find populations and incorporated the NZMS into watch lists in their aquatic nuisance species management plans. Idaho is requiring HACCP documentation from infested hatcheries before obtaining permits to move fish in the state (Phil Mamer, Idaho Fish and Game, Eagle, Idaho, personal communications).

This paper proposes a risk assessment and risk management framework and decision tree for preventing and controlling a NZMS infestation at a fish hatchery facility. We utilize the Guidelines used by the CEC as an approach for assessing the risk potential of an infestation of NZMS, the HACCP planning risk assessment tool for utilizing control measures, and deterministic weight of evidence for ranking certainties associated with research conducted on establishment characteristics, gear decontamination methods, and control measures. This document analyzes the current research, monitoring and disinfection information, and management regulations. Current risk assessment procedures have been combined to utilize a holistic approach in managing for NZMS at a fish hatchery.

Methods

We chose the Guidelines from CEC as the model for determining the NZMS risk potential to establish, colonize, and survive to be consistent with other risk assessment approaches completed for other invasive species. The HACCP planning was utilized to stay consistent with a risk assessment process that fish hatchery managers were familiar with in evaluating their facilities. Weight of evidence tools were incorporated to assess and quantify the uncertainty of the science incorporated into the risk assessment and risk management of NZMS.

Guidelines for Risk Assessment

The framework pertaining to NZMS risk assessment is essentially the same as that developed by the CEC in The Trinational Risk Assessment Guidelines for Aquatic Alien Invasive Species (Mendoza et al. 2009). Risk potential is divided into two components, probability of infestation and consequences of infestation. In this document, each component

is treated equally to determine the risk potential. These two components are divided into seven basic elements that utilize scientific, technical, and other relevant information into the risk assessment. The probability of infestation component of the risk assessment has four steps that are averaged together.

Step 1: assessment of pathways- This is when the managers determine the pathways that the NZMS can utilize successfully. There has been much research and discussion about potential pathways associated with the transport of NZMS to new areas.

Step 2: entry potential-Each pathway has a qualitative probability associated with it of NZMS surviving in transit. This considers the probability of NZMS in the specific pathway entering the fish hatchery.

Step 3: colonization potential-The colonization potential considers many of the specific environmental characteristics around the hatchery facility to determine the likelihood of the area supporting the colonization and establishment of the NZMS. Factors that are considered are stream/water body, water quality, and human mediated characteristics.

Step 4: spread potential-This estimates the probability of the NZMS spreading beyond the colonized area. Natural dispersal and density are the characteristics considered for NZMS potential.

For the consequences of establishment, the CEC guidelines methods were followed where value and uncertainty were kept quantitative. This section has the remaining three steps to complete the risk potential assessment. The consequences of infestation are where the costs and potential benefits are assessed of the NZMS infesting the hatchery. These consequences are going to differ based on the rules, regulations, and culture of each state managing NZMS. The assessments can also lead to positive effects under each category, not just negative effects.

Step 5: economic impact potential-The purpose of this step is to assess the economic impact to the hatchery if NZMS become established. Characteristics to consider are the costs of direct control and management, loss in production, fines, or specialized personnel costs.

Step 6: environmental impact potential-This step assesses the environmental impact that NZMS will cause if established at or near your facility. Characteristics to consider are ecosystem degradation, modification, or destabilization, reduction of biodiversity, reduction, or elimination of endangered/threatened species, loss, or reduction in quality of preferred habitat conditions for native species, or consequences of control actions.

Step 7: social and cultural influences-The last step in this risk assessment is to estimate the effects to social and cultural practices if NZMS are introduced to your facility. Characteristics to consider include effects on cultures of national and regional importance and social effects not easily captured under the economics step.

Risk Analysis Processes and Control Measures

The same principles and format as the US Fish and Wildlife Service HACCP planning process is used to assess the fish hatcheries risk of infestation. The only change is that we are evaluating a pathway instead of an activity. However, the HACCP planning process should occur for every pathway that is deemed necessary to manage or prevent for the introduction of NZMS. Control measures specific to preventing, managing, or eradicating NZMS is discussed.

Uncertainty Analyzed with Weight of Evidence

Uncertainty of the research pertaining to the characteristics of determining NZMS potential to colonize and survive in an area, the control measures, and the disinfectants were assessed using deterministic factors. Deterministic factors were assessed on the strength and credibility of the science, specifically the factors related to the effectiveness, protocols, type of sources, key environmental indicators, and applicability to use in a fish hatchery setting. Criteria-guided judgment was used to rank the certainty of the chemical disinfectant studies. Number of replicate trials and temperatures tested, the number of trials completed by different investigators procuring similar results and determining if the presence of neonates was evaluated were the factors that determined the certainty of the chemical for use as a gear disinfectant. The literature regarding the physical disinfection treatments and control measures were analyzed using case-specific logic. Weights were assigned to each factor and

then the weights of each factor were combined to rank the certainty of the physical disinfection treatment or control measure.

Literature Review

An extensive literature review was conducted on NZMS to determine the factors influencing the NZMS likelihood of colonizing and surviving in an area around a fish hatchery. Only peer-reviewed articles, theses, and reports were used to determine the factors associated with colonization of NZMS. Literature regarding monitoring, disinfection, and control measures in field and hatchery applications from peer-reviewed journals, theses, agency or consulting firm reports, and personal communication were evaluated. Literature from the United States, Canada, Europe, New Zealand, and Australia was found to be available and pertinent.

Results

The risk analysis process takes the risk assessor through all of the processes of a risk assessment as shown in Figure 4.1. A risk assessment is conducted to determine the fish hatcheries risk of harboring NZMS. The next step is to determine if the hatchery is infested with NZMS by implementing monitoring and disinfection procedures. Then, it is time to make a decision of how to manage for NZMS based on the risk assessment, infestation status, and regulations pertinent to the fish hatchery. Figure 4.2 is a decision tree that shows the pathways through each of these steps. The potential options are to keep at status quo, prevent, manage, or eradicate. Once a decision has been made the HACCP planning process is conducted to determine where the pathway can be controlled. The HACCP process identifies critical control points to effectively use control measures to reduce the risk of infestation. The last step is to monitor and repeat risk analysis to increase certainty of controlling NZMS infestations. Forms were designed to help conduct the risk assessment processes. The first step is to determine the risk potential of the organism.

Step 1: Assessment of Pathways

Literature review suggested many methods for NZMS to colonize are area. The following is a list of potential pathways that apply to fish hatchery facilities: fish collection processes,

fish transport processes, field crews and associated gear, effluent ponds, anglers and associated gear, utilizing open water sources, aquarium trade, allowing visitors on the premises, ballast water, birds and mammals, boaters, and other recreationalists (Bowler 1991; Schreiber et al. 2003; Aquatic Nuisance Species Task Force and National Invasive Species Council. 2007; Loo et al. 2007 a & b; Alanso and Castro-Diez 2008; Bersine et al. 2008; Bruce et al. 2008).

Step 2: Entry Potential

Forms were made incorporating all potential pathways NZMS utilize to get into a region and fish hatchery facility. The risk assessor needs to consider the following elements when assessing the fish hatcheries risk of being affected by the pathway. The elements are the organism's ability to hitchhike, survive during transit due to environmental conditions, the life cycle stage of the organism in transit, the number of potential organisms associated with the pathway, or deliberately introduced (Aquatic Nuisance Species Task Force and National Invasive Species Council. 2007; Loo et al. 2007 a & b; Vinson et al 2007). This form helps the risk assessor determine the likelihood of that pathway affecting the facility, ranking the likelihood as either uncertain, very high, high, medium, low, or very low. Uncertainty is incorporated into the ranking as the fish hatchery manager or risk assessor deems appropriate.

Step 3: Colonization Potential

The colonization potential was divided into three categories that are weighted equally when ranking the risks. Literature was analyzed to determine the likelihood of NZMS colonizing an area based on the information presented by the study. Much of the literature ranked the likelihoods or success of the NZMS surviving or adapting to the conditions tested.

The following are the stream/water body characteristics, the likelihoods are listed in Table 4.1:

- Flow rate-NZMS dislodgement probability was higher on bedrock and cobbles than on gravel and pebbles, staying in place at velocities greater than 160 cm/s (Holomuzki and Biggs 1999).

- Expansion rate- dependent on flow rate- NZMS will expand out to other areas downstream and upstream from source populations at flows below 30 cm/s (Haynes et al. 1985; Richards et al. 2001).
- Spread rate- dependent on flow rate- The rate of colonization upstream from original site is increase as flow increases with 30 cm/s as the maximum flow tested (Haynes et al.1985).
- Depth- NZMS have been found at various depths in the Great Lakes; however, as the depth increases the density of NZMS decreases (Levri et al. 2008).
- Stream Order- Higher order streams are more likely to support NZMS colonization (Vinson et al. 2007).
- Elevation-NZMS have not been found often at high elevations, much more frequently at elevations of 1000 meters or less (Vinson et al. 2007).
- Light intensity- There is no phototactic responses in NZMS (Bowler 1991; Levri and Fisher 2000; Oplinger et al. 2010).
- Habitat type- NZMS can be found in a variety of habitats, runs, riffles, eddies, edges and vegetation (Richards et al. 2001; Vinson et al. 2007).
- Substrate type- NZMS are most likely found in gravel and vegetation and less likely in sediment within the habitat (Richards et al. 2001; James 2006)
- Connectivity-Since NZMS will survive the digestive tract of actively eating trout, fish can spread the snails to other connected waters (Haynes et al. 1985; Loo et al. 2007b).

The following are the water quality characteristics; the likelihoods are listed in Table 4.2:

- Season- Densities of NZMS are the greatest in spring, summer, and fall months (Winterbourn 1970; James 2006).
- Temperature- Optimal temperature for NZMS is 18ºC (Dybdahl and Kane 2005). Maximum temperature that NZMS were found to be surviving for more than 96 h was 32.4±2.5ºC (Cox and Rutherford 2000). Minimum temperatures that NZMS were found to survive in the lab and field was -4°C for a 3-7 day exposure (Hylleberg and Seigismund 1987; James 2006).

- Specific conductivity- Significant reductions in survival and growth occur at low levels of specific conductivity (Herbst et al.2008).
- Calcium concentration- NZMS growth was inhibited in calcium free water (Thomas et al. 1974; Hunter 1989; Herbst et al.2008).
- pH- NZMS grew fastest and had the highest fecundity at mid range pH levels (Hunter 1989).
- Phosphorus ratios- Size and reproduction of the NZMS is dependent on the amount of phosphorous present in the system (Tibbets 2010).
- Salinity- NZMS can survive a wide range of salinities, from 0 to 15 parts per thousand (Duncan 1966). The optimum salinity for NZMS is 5 parts per thousand (Jacobsen and Forbes 1997).

The following are the human mediated characteristics; the likelihoods are listed in Table 4.3:

- Disturbance/pollution-NZMS have been found in highly disturbed areas and less so in undisturbed areas (Bowler 1991; Bowler and Frest 1992). NZMS grew and survived in sediment rich waters (Broekhuizen et al. 2001). NZMS can survive higher concentration of ammonia levels over a long-term and short-term duration compared to other aquatic invertebrates (Alonso and Camargo 2003; 2009). Estrogen pollution stimulated embryo production at low doses and caused inhibitory effects at higher doses in the laboratory (Jobling et al. 2003).
- Angling/stocking- Angling and stocking practices are indicators of potential NZMS populations (Loo et al. 2007b; Vinson et al. 2007).
- Birds- NZMS are carried from one river system to another (Haynes et al. 1985; Loo et al. 2007b).

Step 4: Spread Potential

Ranking was determined by how close and dense the nearest known population of NZMS is from the facility. These characteristics were ranked as uncertain, very high, high, medium, low, and very low depending on a generalized scale. Uncertainty was incorporated into the descriptions of each ranking level (Loo et al. 2007 a & b).

Monitoring and Disinfection Methods

Information regarding monitoring methods is included in the decision document for hatchery managers to assess their facilities. A description of each method is included in the decision document so managers can choose which methods are most applicable to the area being monitored. Disinfection techniques are also described; Table 4.7 and Table 4.8. Uncertainty and ranking procedures were conducted.

Risk Analysis Processes and Control Measures

Forms from the risk analysis process were used to assist with conducting the risk management. The risk analysis planning process is completed and control measures are utilized at critical control points. The following control measures were included that can limit the risk of infestation with NZMS.

Hydrocyclone-The effectiveness of hydrocyclonic filtration as a technique to remove all life stages of NZMS was tested at Hagerman National Fish Hatchery (Nielson 2008; Neilson et al. *in review*). They found the hydrocyclone very effective in filtering all sizes of NZMS from an infested water source. The filtrate was diverted to a smaller area, and isolated. This underflow could be either placed directly into a chemical treatment or filtered further to remove all snails. Tests used flow rates of 367 and 257 L/min. Hydrocyclones can be combined together in manifold systems to handle larger volumes of water. The test system at HNFH used gravity feed to provide the necessary head for filtration, but pumps could power systems without adequate gravity head pressures. Collaborating with an engineer or a company that sells hydrocyclones will be necessary to obtain the correct size and establish the correct head and pressure for the flow of water.

Drum filters- Drum filters have not been evaluated for NZMS, but many suppliers have suggested them as feasible. Some of the constraints of these systems are fouling and the need for power to operate. This process does not kill the snails, so if there are areas that the snail could crawl around and get into the filtered water this would not be an effective control measure. Collaborating with the company that can install these will be essential to lower the risk of failure. Summerfelt and Penne (2005) shows a drum filter removing very small to large particles efficiently.

Sieves-Richards (2008) described a method of taking rainbow trout eggs from the wild to be used in a hatchery and filtering out NZMS through an action similar to panning for gold. This method would be simple to use to gently sieve the eggs with a <2 mm sieve and rinse using the circular motion of panning for gold. The snails would fall away from the eggs when agitated and potentially be washed through the sieve. Eggs could then be separated from larger NZMS. Richards (2008) also suggests transporting the eggs promoting agitation to ensure all snails are removed from the eggs. The contaminated water can be treated when back at the hatchery. Oplinger and Wagner (2009a) also suggested sieving eggs to remove NZMS; however, chemicals may be needed to remove neonates.

Screens-Mesh screens have been used in hatcheries in Montana to filter their NZMS infested spring water (Eileen Ryce, personal communications). They were effective in controlling downstream spread into the hatchery. Mesh sizes of greater than 80 micrometers are recommended to catch drifting neonates (Nielson 2008).

Freezing temperatures-Freezing temperatures, below negative 2ºC, are required over a 2 to 3 day period to kill New Zealand mudsnails (James 2006). Field trials have been completed at Capitol Lake in Washington, where the water was drained from the lake and the littoral areas were frozen for several days with air temperatures below negative 2ºC. The event was 98% successful, as some New Zealand mudsnails were found in treatment sites up to 4 days later (Cheng and Le Clair 2011; Johannes 2011).

Boiling water- Studies by Dwyer et al. (2003) show there are easy and effective ways to eradicate NZMS using hot water. NZMS in this study were exposed to three different temperatures for three intervals of time. Results indicate that all NZMS exposed to 45ºC (113ºF) for 60 s died. However, using 50ºC (122ºF) water killed 100% of the NZMS in 15 s. This may be difficult to achieve in an outdoor setting, however, pressure washing with 140ºF water would kill and remove NZMS.

Fire-A flamethrower was used by Ken Cline to remove NZMS from his facility in 2007. The concrete walls were not damaged and the snails sounded like popcorn when they were heated to such high temperatures (Ken Cline, personal communications).

Ultraviolet light-UV will eventually kill snails but it may take up to several weeks (Wong and Wagner 1956). There has been no research conducted on NZMS to test the effectiveness of UV.

Ozone-Ozone induces NZMS retract into their shells as shown in studies by Capual (2006 unpublished data). No mortality was seen by treatment with ozone. Ozone could be used to remove snails from an area, with a treatment to kill them after collection.

Depuration strategies-Oplinger et al. (2010) also found that NZMS would pass through a rainbow trout's digestive tract live after 48 h after consumption. Haynes et al. 1985 found that rainbow trout fed willingly on NZMS. The NZMS survived the passage through the digestive tract live and produced neonates within 24 h. No depuration strategies were assessed. Bruce and Moffitt (2010) recommend evaluation of feeding fish heavily for 96 h and then depurating for at least 48 h in a NZMS clean water source before transportation. Studies conducted by Bruce and Moffitt (2010) found that both rainbow trout and steelhead consumed NZMS, with rainbow trout consuming almost twice as many NZMS as steelhead. They also determined that a maintenance diet increased the total consumption of snails, even though starved fish consumed snails as well. Another study conducted in the same lab shows that snails did live through the digestive tract of rainbow trout within 12-24 h of ingestion (Bruce et al. 2009). No live NZMS were found in the digestive tract or feces after 48 h after ingestion (Bruce et al. 2009). The success to a depuration strategy is having a clean water source. Sealey et al. (2009) showed that tilapia when fed NZMS they are likely to grind and digest them not allowing for survival out of the digestive tract. There was 100% survival of NZMS regurgitated.

Mixed cell raceway-Studies conducted in a mixed cell raceway (Stockton this thesis chapter 3), illustrated velocities and particle removal rates. The testing that was completed used 10 pounds of plastic beads to mimic NZMS, which were removed from the raceway in 20 min. The velocities, an average of 23 cm/s, were sufficient to remove NZMS based on theoretical calculations. Further testing with NZMS is needed to increase the certainty of this control measure, especially if combined with depuration strategies.

Barriers-Movement of snails can be monitored by cameras. Myrick (2009) describes a low cost, easy to use camera system for monitoring NZMS.

Copper barriers- Preliminary analysis by Myrick and Conlin (*in press*) showed that cooper and copper based materials served as NZMS deterrents by reducing their crawling distance under static conditions. Copper strips (roof flashing) were used in Oplinger and Wagner's study (2009b) in 1, 2, 3, and 4 inch strips. They found that snails crossed the strip with no significant difference from the control. Copper concentrations in the water samples were below 0.008 mg/L. Further studies found that copper sheeting or copper mesh were the most effective surfaces of deterring upstream migration (Hoyer 2011). The copper based barriers need to be constructed of either material containing 99.9% pure copper with a minimum distance of 250 cm. Copper based painting was also investigated by Hoyer (2011); however, the paints were not as effective as the copper sheeting and mesh. All levels of copper that were leached into the water were less than the Environmental Protection Agencies minimum requirements. Water chemistry of the surrounding water did make a difference in the crawling distances observed on the copper barriers (Hoyer 2011). The copper barrier must be maintained and cleaned to reduce fouling and maintain effectiveness.

Electrical barriers- Erin VanDyke (2010) and Randy Oplinger (2008) investigated using electrical barriers to inhibit upstream movement of NZMS. Water temperature, conductivity, amount of electrode in the water, and spacing of the electrodes affects the electrical current flow through the water. Stainless steel is the best metal to use, because it corrodes slower than the other metals. VanDyke (2010) found that it was very important to seal the electrode to the substrate so that the NZMS would not find a way around the electrical field. Pulsed electrical current will work as well. System needs to be monitored and maintained to ensure effectiveness.

Velocity barriers- Testing conducted by Holomuzki and Biggs (1999) determined that most NZMS were dislodged from gravel, cobble, and pebble substrates at near bed levels measured velocities of 150 cm/s. NZMS were still found in crevices of the rocks at the high velocities. Near bed velocities of 150 cm/s were achieved with flow rates of 50 cm/s; the shear stress created by the gravel, cobble and pebbles created higher velocities. During

testing with copper substrates, Hoyer (2011) determined that velocities greater than 33 cm/s were needed to dislodge NZMS from smooth surfaces. The velocity needed to remove NZMS from a pipe has not been researched. Oplinger and Wagner (2009b) found that terrestrial travel was very unlikely for the NZMS, so minimal efforts are needed in preventing terrestrial movement of NZMS.

Aeration barriers-A wall of bubbles produced by an aeration pump might be effective in deterring the upstream migration of NZMS. Pumped air around the pipes opening forming a thick wall of bubbles could be an effective aeration barrier (Larry Dalton, personal communication).

Suction dredge/sand wand-This tool is very similar to a vacuum, which sucks up sediments and puts them on to the bank. These tools have been used to remove NZMS from areas. The use of the sand wand was effective in reducing populations of NZMS, but was not 100% effective (Sepulveda 2011).

Disturbance cleaning- The tools that are used to clean the raceways, specifically a pond scrubber, which is effective in removing algae from the bottoms of the raceways (Bryan Kenworthy, personal communication).

Potassium permanganate- Using potassium permanganate as a drip system into raceways is an example of chemical treatment. Oplinger and Wagner (2010) determined that at a 2 mg/L concentration of potassium permanganate was not effective in controlling or eliminating NZMS from the raceway. A 2 mg/L concentration of potassium permanganate is used to help suppress disease outbreaks. Research conducted in the Moffitt lab found that potassium permanganate was a good snail relaxer; it was observed that the NZMS were gaping after a 1 mg/L exposure time (Stockton this thesis chapter 1).

Bayluscide- Bayluscide a molluskicide with nicolosamide as its active ingredient can be used as a drip treatment within raceways to remove NZMS. McMillin and Trumbo (2009) reported that a 1 mg/L nicolosamide concentration over 8 h resulted in a 98% mortality of NZMS. Bayluscides half-life in water was 1.8 days and 2.6 days in sediment. Bayluscide is also toxic to fish at the same concentrations as mollusk species.

Hydrogen peroxide- Hydrogen peroxide at 3 and 6% was found to be an effective chemical that eradicated NZMS. When used in the field to kill NZMS in the springs of Loa hatchery, there was not 100% mortality achieved as determined by Oplinger (2010). Within a couple of weeks, NZMS densities were detectable in the springs.

Sodium chloride- When exposed to NaCl NZMS will contract into their shell, allowing a salt solution to be added to the raceway and removing the snails. Oplinger et al. (2010) showed that 60% of NZMS would contract into their shells and roll off a surface when exposed to a sodium chloride solution of 11.0 g/L. Lower concentrations produced a lower percentage of snail contraction. Nearly all snails that contracted into their shells did so within the first 10 s of exposure.

Carbon dioxide-Pressurized CO2 treatment is effective at decontaminating NZMS collected in filtered water. Studies conducted by Nielson (2008) showed that pressurized CO2 is more effective than non-pressurized CO2. Non-pressurized CO2 is effective at killing NZMS; however, it takes more time and more CO2.

Decontamination station- Disinfection stations are only as good as the disinfectant used; see disinfection section for description and evaluation of chemicals and physical methods that remove NZMS from gear. A decontamination station can be used at entrances to force people into decontaminating shoes and other gear before entering the facility.

Supplemental documentation for form 5 of the risk planning was included in the decision document to evaluate how ranked control measures affected the risk level of the pathway it was applied to as a critical control point. The other supplemental documentation was a form that encourages hatchery personnel to fill out to indicate that monitoring was completed.

Uncertainty Analyzed with Weight of Evidence

The forms created in the decision document incorporate the uncertainty for the literature used. There is flexibility within these forms for the risk assessor to determine their own certainty regarding the characteristics, control measures, and disinfectants. The characteristics related to colonization and survivals were weighed determining the credibility

and strength of the literature. The case specific method identified the following parameters to evaluate that were consistent with all reviewed literature.

- Lab/field- Categorization of where the trials were conducted to relate to the effectiveness of applying the same procedures to a field setting at a hatchery.
- Source type- The information's source; peer-reviewed sources, thesis, reports, or personal communications.
- Temperature- Temperature can have an effect on the characteristics applying to the biological reaction of NZMS.
- Source population- Many of the studies were conducted on other populations than the US1 isolates (Dybdahl and Drown 2011), so genetic variations could account for uncertainty in the likelihood of colonization and establishment.
- Calculated/ provided- The determination if the characteristic ranking was provided as part of the literature (provided) or gleaned from the data provided by the source (calculated).
- NZMS or other- Some of the characteristics that are important in determining the likelihood and establishment of NZMS are not yet available. Surrogates can be used however, there is a high uncertainty associated with using this data.

For chemical and physical decontamination methods, the following parameters were analyzed: number of replicate trials and temperatures tested, the number of trials completed by different investigators procuring similar results, and determining if the presence of neonates was evaluated.

The control measures sources were evaluated using the following parameters:

- Percent effective-the degree to which the control measure was found to be effective.
- Calculated/ provided-the determination if the effectiveness was provided as part of the literature (provided) or gleaned from the data provided by the source (calculated).
- Lab/field- categorization of where the trials were conducted to relate to the effectiveness of applying the same procedures to a field setting at a hatchery.

- Neonates-categorization of whether or not the presences of neonates were assessed in the outcome and conclusions of the trials. Stressed and dying adults will release neonates immediately so their presence can increase the likelihood of an infestation.
- Source type- the information's source; peer reviewed sources, thesis, reports, or personal communications.

Example with Deterministic Weighting Factors

Case-specific logic was used to weigh the parameters for analysis of the credibility and strength of the literature. For the characteristics of determining if NZMS were to colonize and survive weights were chosen for each parameter evaluated in the literature. The values of the parameters are as follows:

- Lab/field: field=0.90, lab=0.75 and 0.5 theoretical
- Source type: peer reviewed=0.9, thesis=0.8, report=0.7, and personal communication=0.6
- Temperature: 1-(|tested temp-facility temp|*0.1)
- Source population: if source population is different, estuary vs. stream, or US vs. Europe, etc=0.5
- Calculated/ provided: provided=0.9 and calculated=0.8
- NZMS or other: if not NZMS use 0.5 otherwise use 1

Certainty=1-((0.9 field, 0.75 lab or 0.5 theoretical)*(source: 0.9 peer reviewed, 0.8 thesis, 0.7 report, or 0.6 personal communications)* 1-(|tested temp-facility temp|*0.1)*(0.5 if source population is different)*(0.9 for provided or 0.8calculated)*(0.5 if not NZMS)).

Equation 4.1

Evaluated stream and water body characteristics from each source are presented in Table 4.4; water quality characteristics in Table 4.5; and human mediated characteristics in Table

4.6. These values can be modified as new research justifies the applicability to a field setting or ecosystem. Monitoring areas consistently will provide information about influential characteristics determining the NZMS ability to colonize and survive in areas.

The certainty of decontamination methods was divided into two groups, physical and chemical. These were evaluated based on testing parameters and final rankings of chemical disinfectants are presented in Table 4.7 and physical disinfectants in Table 4.8. As more research is conducted to increase the number of trials conducted, the number of different groups testing the method, or neonate survival is assessed, the certainty of the chemical will increase. Probability values were assigned to determine the rankings of the physical disinfectants.

- Replicates and numbers tested: 1 if number of NZMS >100; 0.75 if lab; and .7 if number NZMS tested is <100.
- Source type: peer reviewed=0.9, thesis=0.8, report=0.7, and personal communication=0.6.
- Neonates: assessed=1, not assessed=0.5.

Certainty= (1 if number of NZMS >100; 0.75 if lab; and .7 if number NZMS tested is <100)*(1.0 neonates assessed or 0.5 not assessed)*(source: 0.9 peer reviewed, 0.8 thesis, 0.7 report, 0.6 personal communications or 0.5 theoretical). Equation 4.2

Table 4.9 shows the numerical categorization of the ranking system used for determining the certainty ranking of the physical treatments and control measures.

Control measure certainty was evaluated and the following values were provided to each option in the parameters:

- Effectiveness (1-0): effectiveness of the control measure eliminating the NZMS population
- Calculated/ provided: provided=0.9 and calculated=0.8

- Lab/field: field=0.90 and lab=0.75
- Neonates: assessed=1, not assessed=0.5
- Source type: peer reviewed=0.9, thesis=0.8, report=0.7, personal communication=0.6, and theoretical=0.5

Certainty=Effectiveness*(0.9 for provided or 0.8calculated)*(0.9 field or 0.75lab)*(1.0 neonates assessed or 0.5 not assessed)*(source: 0.9 peer reviewed, 0.8 thesis, 0.7 report, 0.6 personal communications or 0.5 theoretical) Equation 4.3

Evaluated control measures are presented in Table 4.10. Table 4.9 shows the weighted numerical categorization of the ranking system used for determining the certainty rank for each control measure. The resulting certainties of the control measures are organized in Table 4.11. These rankings can be modified as new research justifies the applicability of the control measure to use in the pathway at the facility. Monitoring increases the knowledge about the certainty of a control measure.

When numbers were assigned to ranking orders, uncertain received a 6, the highest ranking, because we assumed that this uncertainty was from ignorance (Harwood and Stokes 2003). If the risk was deemed very high, we wanted the next largest number, 5, to represent that risk in a probability calculation. Lower numbers are used for lower qualitative risk rankings, with very low equal to 1.

Combining certainty of the control measure, characteristic, or disinfection method with the risk assessment was intricately accomplished. When the likelihood of the characteristics that determine the colonization and survival of NZMS were combined with the weighted certainty, the likelihood rating stays dominant and the weighted certainty was added to it. The characteristics with associated weighted certainty from each grouping were averaged together to get the risk ranking, Form 2. Control measure certainty will affect the pathways at the facility. Figure 4.3 shows how the certainty of the control measure will affect the pathway. A very high ranking of the pathway needs a control measure that is ranked as very high to reduce the risk of the pathway bringing in NZMS. A high ranked control measure

will effectively reduce the pathways that have a medium or lower risk associated with it. Pathways ranked as high risk, need a very high control measure, everything below that will be either ineffective or undetermined risk ranking. Monitoring needs to be conducted to improve the state of the science. Figure 4.3 is part of the risk analysis form 5 as a supplemental document in the decision document. The other supplemental document is for hatchery personnel to fill out each month to document that monitoring was completed as required on risk analysis form 5.

Discussion

Developing a framework and decision trees for conducting risk assessment and risk management with uncertainty and incomplete information is a challenge. There are many methods used to conduct risk assessments, evaluate risk management, and analyze uncertainty. Management and policy decision weigh heavily on conducting these risk assessments for fish hatchery facilities. The advantages of using the three techniques discussed in this paper are managers use these methods to evaluate risks with invasive species, the methods are simple and comprehensive, and the methodologies are clear and transparent. Use of a tool helps managers provide a formal way of combining information from a variety of sources and documenting their risk analysis efforts and procedures. These qualities make decisions made with this framework and decision trees more defensible.

Peer review and testing of this process is needed before this process is adopted. Test should be conducted by fish hatchery managers, resource managers, policy makers, and research scientists. Their evaluation would be instrumental in ensuring that all of the characteristics are related to colonization and survival of the NZMS, that there are not significant characteristics missing, and determining the characteristics is feasible in an area. The same evaluation by the managers can be conducted for the control measures, decontamination and monitoring methods. Monitoring of areas to strengthen the certainty of the characteristics in an ecosystem also needs to be conducted. Determining the applicability of control measures and disinfectant needs to be monitored and documented. These actions would strengthen the values chosen to assess the certainty of the literature. Bayesian belief

networks could be used after monitoring has been conducted to strengthen those assumptions (Varis and Kuikka 1999; Marcot et al. 2001).

Fish hatcheries and aquaculture facilities are highly vulnerable to infestations because they have constant flows at constant temperatures with many nutrients available. They are also located near recreational areas, utilize open water sources, transport fish, and use other pathways that unwanted organisms utilize. It is very important for these scrutinized facilities to assess, manage, and communicate risk. All three components need to be part of a transparent process as risk analysis incorporates complex ecological, economic, legal, political, and social issues (Hill 2009). Risk analysis is the most effective when there is open and continuous communications among risk assessors and affected stakeholders (Hill 2009).

Keeping current and incorporating the new information is a difficult challenge. The transparent, clear, and simple method of analyzing existing data should allow others to follow the same process to incorporate new data. Adaptive management provides a systematic tool for incorporating new information with previous, existing information. The basic adaptive management process is straightforward: one chooses a management action, monitors the effects of the action, and adjusts the action based on monitoring results; and throughout this process, uncertainty is inherent (USDOI 2010). During the adaptive management process, changes are expected, learning is emphasized, and objectives revised. Through scheduled monitoring and documentation the risk analysis process conducted in this document is an adaptive process.

This document is a living document, which should be updated and changed to fit current information and the needs of the user. This approach is not only useful for NZMS infestations, but can be applied to other species of concern. Not only is it useful for hatcheries, but this approach can be used in field applications, other industries, or facilities. The characteristics and certainties associated with each species of concern would have to be investigated to be relevant. Control measure evaluations would also need to be conducted to understand new tools and the associated certainties.

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Table 4.1. Literature review for stream and water body characteristics to determine the likelihood of NZMS survival.

	Likelihood of NZMS survival			
	Low	Medium	High	Source
Flow rate	>100 cm/sec	70-100 cm/sec	<70 cm/sec	Holomuzki and Biggs 1999
Flow rate- expansion rate	>30 cm/sec	30-10 cm/sec	<10 cm/sec	Haynes et al. 1985; Richards et al. 2001
Flow-upstream spread rate	<7 cm/sec	7-10 cm/sec	10-30 cm/sec	Haynes et al. 1985
Depth	>25 m	15-25 m	<15 m	Levri et al. 2008
Stream order	1	2	>2	Vinson 2007
Elevation	>1500 m	500 m	1000 m	Vinson 2007
Light intensity	No effect	No effect	No effect	Bowler 1990; Levri and Fisher 2000; Oplinger et al. 2010
Habitat type	Run/Riffles	Edge	Eddies & Vegetation	Richards et al. 2001; Vinson 2007
Substrate type	Sediment	Mix	gravel and vegetation	Richards et al. 2001; James 2007
Connectivity to downstream populations (fish movement)	Low	Medium	High	Haynes et al. 1985; Loo et al. 2007

Table 4.2. Summary of likelihood of survival of NZMS for various water quality factors. Ranking was determined by examination of published studies and reports. [a] applicable to daily mean temperature and daily maximum temperature (±5˚C); [b] 96 h exposure [c] depends on salinity, need less than 15‰ Salinity; [d] information based on snails, not specifically NZMS

	Likelihood of NZMS survival			
	Low	Medium	High	Source
Season	Winter	Spring through Fall	Spring through Summer	Winterbourn 1970; James 2007
Temperature (min)[a,b]	<-2˚Cc	0-2˚C	2-4˚C	Hylleberg and Siegismund 1987; James 2007
Specific conductivity[c]	0-25 µS/cm	25-200 µS/cm	>200 µS/cm	Herbst et al. 2008
Calcium concentration[d]	0-10 mg/l	10-50 mg/l	>50 mg/l	Thomas et al. 1974; Hunter 1989; Herbst et al. 2008
pH	1 to 4	4 to 6	6 to 8	Hunter 1989
	Low	Medium	High	
Phosphorus ratios (SE)	C:N 12.0 (0.42); C:P 1119 (178); N:P 92.8 (11.9)	C:N 8.1 (1.19); C:P 271 (45.7); N:P 36.7 (8.36)	C:N 7.9 (0.64); C:P 203 (19.3); N:P 27.9 (2.13)	Tilbets et al. 2010
Salinity	>15‰ S	0-15‰ S	5‰ S	Duncan 1966; Jacobsen and Forbes 1997

Table 4.3. Literature review for human mediated characteristics to determine the likelihood of NZMS survival.

	Likelihood of NZMS survival			
	Low	Medium	High	Source
Disturbance/ pollution	None to Low	Low to Medium	Medium to High	Bowler 1990; Frest and Bowler 1992; Broekhuizen et al. 2001; Jobling et al. 2003; Alonso and Camargo 2003; Alonso and Camargo 2009
Angling/stocking	Low	Medium	High	Loo et al. 2007; Vinson 2007
Birds/flyway	Low	Medium	High	Haynes et al. 1985; Loo et al. 2007

Table 4.4. Parameters associated with weighting the literature with case-specific logic for stream and water body characteristics that were evaluated to determine the likelihood of NZMS colonizing and surviving in an area, Step 3: Colonization Potential. PR=peer review, FWR= freshwater river; FWL= freshwater lake.

	Source	Lab/ field	Source type	Temp.	Source population	Location	Calculated/provided
Flow rate	Holomuzki and Biggs 1999	Lab	PR	19	FWR	New Zealand	Calculated
Flow rate- expansion rate	Haynes et al. 1985	Lab	PR	5-10˚C	FWR	England	Provided
	Richards et al. 2001	Field	PR	12-16˚C	FWR	Idaho	Supports above
Flow-upstream spread rate	Haynes et al. 1985	Lab	PR	5-10˚C	FWR	England	Calculated
Depth	Levri et al. 2008	Field	PR		FWL	Ontario, USA	Provided/calculated
Stream order	Vinson et al. 2007	Theoretical	Report			USA	Provided
Elevation	Vinson et al. 2007	Theoretical	Report			USA	Calculated
Light intensity	Bowler 1991	Field	PR	15	FWR	Idaho	Provided
	Levri and Fisher 2000	Lab	PR	23	FWL	New Zealand	Provided
	Oplinger et al. 2010	Lab	PR		FWR	Utah	Provided
Habitat type	Richards et al. 2001	Field	PR	12-16˚c	FWR	Idaho	Provided
	Vinson et al. 2007	Field	Report		FWR	Utah	Provided
Substrate type	Richards et al. 2001	Field	PR	12-16˚c	FWR	Idaho	Supports
	James 2006	Field	Thesis		FWR	Idaho	Provided
Connectivity to downstream populations (fish movement)	Haynes et al. 1985	Lab	PR		FWR	England	Calculated
	Loo et al. 2007b	Theoretical	PR			Victoria, Australia	Supports

Table 4.5. Parameters associated with weighting the literature with case-specific logic for water quality characteristics that were evaluated to determine the likelihood of NZMS colonizing and surviving in an area, Step 3: Colonization Potential. PR=peer review, FWR= freshwater river; FWL= freshwater lake; BW= brackish water.

	Source	Lab/ field	Source type	Endpoint	Temp. (˚C)	Salinity	Source population	Location	Calculated/ provided	NZMS or other
Season	Winterbourn 1970	Field	PR		8-19˚C		FWR/L	New Zealand	Provided	NZMS
	James 2006	Field	Thesis				FWR	Idaho	Provided	NZMS
Temperature (max.)	Cox and Rutherford 2000	Lab	PR	Mortality	16		FWR	New Zealand	Provided	NZMS
Optimal temperature	Dybdahl and Kane 2005	Lab	PR	Growth, repro, pop growth rate	12,18, 24	0-32	FWR/ estuary	ID, OR, MT	Provided	NZMS
Temperature (min)	Hylleberg & Siegismund 1987	Lab	PR	Mortality	3	0-30	FWR	Denmark	Provided	NZMS
	James 2006	Lab	Thesis	Mortality	8, 15		FWR	Idaho	Provided	NZMS
Specific conductivity	Herbst et al. 2008	Lab	PR	Mortality	15-20˚C		FWR	California	Provided	NZMS
Calcium concentration	Thomas et al. 1974	Lab	PR	Growth and fecundity	26		Lab	British Isles	Calculated	Other snail
	Hunter 1989	Lab	PR	Growth and fecundity	19-23	Medium cond.	FWR	Massachusetts	Calculated	Other snail
pH	Herbst et al. 2008	Lab	PR	Mortality	15-20˚C		FWR	California	Support	NZMS
	Hunter 1989	Lab	PR	Growth and fecundity	19-23	Medium cond.	FWR	Massachusetts	Calculated	Other snail
Phosphorus ratios	Tilbets et al. 2010	Lab	PR	Growth rate, repro. rate	22-24		FWR	Arizona	Provided	NZMS
Salinity	Duncan 1966	Lab	PR	Growth and fecundity	?		FWR / BW	England and Poland	Support	NZMS
	Jacobsen and Forbes 1997	Lab	PR	Repro., feeding and growth rate, size at birth	15		BW / FWR	Denmark	Provided	NZMS

Table 4.6. Parameters associated with weighting the literature with case-specific logic for water quality characteristics that were evaluated to determine the likelihood of NZMS colonizing and surviving in an area, Step 3: Colonization Potential. PR=peer review; FWR= freshwater river.

	Source	Lab/field	Source type	Endpoint	Temp.	Salinity	Source population	Location	Calculated / provided
Disturbance	Bowler 1991	Field	PR		15		FWR	Idaho	Provided
Disturbance	Bowler and Frest 1992	Field	PR		15		FWR	Idaho	Provided
Sediment pollution	Broekhuizen et al.2001	Lab	PR		20		FWR	New Zealand	Calculated
Estrogen pollution	Jobling et al. 2003	Lab	PR	Growth and embryo production	Ambient		Lab	Germany	Provided
Short term N toxicity	Alonso and Camargo 2003	Lab	PR	Mortality	20	High conductivity	FWR	Spain	Provided
Long term N toxicity	Alonso and Camargo 2009	Lab	PR	Mortality	15.3	High conductivity	FWR	Spain	Provided
Angling/ Stocking	Loo et al. 2007b	Theoretical	PR					Victoria, Australia	Provided
	Vinson et al. 2007	Theoretical	Report				FWR	Utah	Provided
Birds/flyway	Haynes et al. 1985	Lab	PR				FWR	England	Supports
	Loo et al. 2007b	Theoretical	PR					Victoria, Australia	Provided

Table 4.7. Literature reviewed chemicals used to disinfect monitoring gear with associated certainty ranking. Chemicals with less than 100% mortality were not evaluated; their certainty ranking would be low.

Chemical	Conc.	Exposure time (min)	Percent mort.	Rep at each temp (# snails tested)	Neonate	Temp. (C)	Corrosive to gear	Source
Certainty Rank: Very High								
Virkon® Aquatic	2%	20	100%	9 (90)	Yes	8,15,22	Low	Stockton this thesis
Sparquat 256	3.10%	5	100%	4 (100)	Yes	15	Not reported	Schisler et al. 2008
Formula 409 All Purpose Cleaner	Undiluted	10	100%	4 (100)	Yes	15	Medium	Schisler et al. 2008
Certainty Rank: High								
Ammonia	Undiluted	15	100%	4 (100)	No	5,15	Not reported	Hosea and Finlayson 2005, Oplinger & Wagner 2009a
Copper sulfate	504 mg/L Cu	15	100%	4 (100)	No	5,15	Low	Hosea and Finlyason 2005, Oplinger & Wagner 2009ab
Pine-Sol	Undiluted	15	100%	4 (100)	No	5,15	Medium	Hosea and Finlayson 2005, Oplinger & Wagner 2009ab
Formula 409 Degreaser and Disinfectant	Undiluted	15	100%	4 (100)	No	15	Medium	Hosea and Finlayson 2005, Oplinger & Wagner 2009ab
Hydrogen peroxide	3% & 6%	15	100%	4(100)	No	unknown	Not reported	Oplinger & Wagner 2009b; Oplinger 2010
Hyamine 1622	1350, 1,940 & 3,880 mg/L	15	100%	4(100)	No	unknown	Not reported	Oplinger & Wagner 2009b; Oplinger and Wagner 2011

Table 4.7. continued.

Chemical	Conc.	Exposure time (min)	Percent mort.	Rep at each temp & conc. (# snails tested)	Neonate	Temp. (C)	Corrosive to gear	Source
Certainty Rank: Medium								
Copper sulfate	252 mg/L Cu	5	100%	4(40)	No	15	Low	Hosea and Finlayson 2005
Roccal	500 mg/L	15	100%	5(100)	No	18	Unknown	Oplinger and Wagner 2011
Pine-Sol	50% dilution	5	100%	4(40)	No	5,15	Medium	Hosea and Finlayson 2005
Benzethonium chloride	1940 mg/L	5	100%	4(40)	No	5,15	Medium	Hosea and Finlayson 2005
Benzalkonium chloride	450 mg/L	15	100%	5(100)	No	18	Unknown	Oplinger and Wagner 2011
Stepanquat	500 mg/L	15	100%	5(100)	No	18	Unknown	Oplinger and Wagner 2011

Table 4.8. Literature reviewed physical methods used to disinfect monitoring gear with associated certainty ranking determined by weighting each with specific-case logic. Certainty ranking from weights was determined with Table 4.9.

	Certainty ranking	Source	Percent effective	Calculated/ provided	Neonates	Lab/field	Type of data	Certainty weight
Freezers	Medium	Richards et al. 2004	100	Provided	No	Lab	Peer review	0.3375
	High	Hylleberg & Siegismund 1987	100	Provided	No	Lab	Peer review	0.45
Boiling water	Medium	Dwyer et al. 2003	100	Provided	No	Lab	Peer review	0.315
Desiccation	Medium	Richards et al. 2004	100	Provided	No	Lab	Peer review	0.315

Table 4.9. Weighted numerical categorization of the ranking system used for determining the certainty ranking of the physical treatments and control measures.

Ranking	Certainty weight
Very high	>0.6
High	0.6-0.4
Medium	0.4-0.2
Low	0.2-.01
Very low	<.01

Table 4.10. Summary of information regarding control measures obtained from literature that was peer-reviewed (PR), thesis, articles, and personal communications (PC). Details of the tests were evaluated and rated for completeness and effectiveness. When studies reported and monitored the weight of evidence was ranked higher using Equation 4.3. NT= not tested; NA=not applicable

Control measure		Percent effective	Calculated /provided	Neonates	Lab/ field	Data type	Source	Certainty weight
Filtration	Drum	80	Calculated	Yes	Lab	Theoretical	Summerfelt & Penne 2005	0.240
	Hydrocyclone	100	Provided	Yes	Field	Thesis	Nielson 2008	0.648
	Sieves	80	Calculated	No	Field	Article	Richards. 2008	0.202
	Screens	100	Provided	Yes	Field	PC	Eileen Ryce 2009	0.486
Freezing	Temps	98	Provided	No	Field	Thesis	Cheng and Le Clair 2011;	0.357
Heat	Boiling water	100	Provided	Yes	Lab	PR	Dwyer et al. 2003	0.610
	Fire	100	Provided	Yes	Field	PC	Kline 2008	0.486
Ultra violet light		50	Calculated	No	Lab	PR	Wong and Wagner 1956	0.135
Ozone		0	Provided	Yes	Lab	Article	Capaul 2006	0.005
Depuration strategies		100	Provided	Yes	Lab	PR	Bruce and Moffitt 2010	0.608
Mixed cell		80	Calculated	No	Field	Thesis	Stockton this thesis	0.230
Barriers	Copper	35	Provided	No	Lab	PR	Oplinger and Wagner 2009b	0.118
		100	Provided	No	Lab	Thesis	Hoyer 2011	0.270
	Electrical	100	Provided	No	Field	Article	Oplinger 2008; VanDyke 2010	0.236
	Velocity	80	Provided	No	Field	Article	Oplinger 2010	0.227
Removal	Aeration	NT	NA	No	Field	PC	Dalton 2010	0.003
	Suction dredge	80	Provided	No	Field	PC	Sepluveda 2011	0.162
	Disturbance	NT	NA	No	Field	PC	Kennworthy 2011	0.003
Chemicals	KMNO4	3	Provided	No	Field	PR	Oplinger and Wagner 2010	0.011
	Bayluscide	98	Provided	No	Field	Article	McMillin and Trumbo 2009	0.278
	H_2O_2	98	Provided	No	Field	PC	Oplinger 2010	0.238
	NaCl	60	Provided	No	Lab	PR	Oplinger et al.2010	0.182
	Carbon dioxide	100	Provided	Yes	Lab	Thesis	Nielson 2008	0.54
Decontamination station		Determined by the method of decontamination used						

Table 4.11. The certainty ranking of control measure evaluated in Table 4.10. Certainty ranking from weights was determined using ranking rules (Table 4.9).The certainty ranking is used in supplemental document 1 in risk analysis form 5.

Control measure	**Certainty ranking**
Hydrocyclone	Very high
Boiling water	Very high
Depuration strategies	Very high
Carbon dioxide	High
Screens	High
Fire	High
Drum filter	Medium
Sieves	Medium
Freezing temps	Medium
Mixed cell raceway	Medium
Copper barrier	Medium
Electrical barrier	Medium
Velocity barrier	Medium
Bayluscide	Medium
Hydrogen peroxide	Medium
Ultra Violet light	Low
Suction dredge	Low
Sodium chloride	Low
Ozone	Very low
Aeration barrier	Very low
Disturbance	Very low
Potassium permanganate	Very low
Decontamination station	Determined by method used

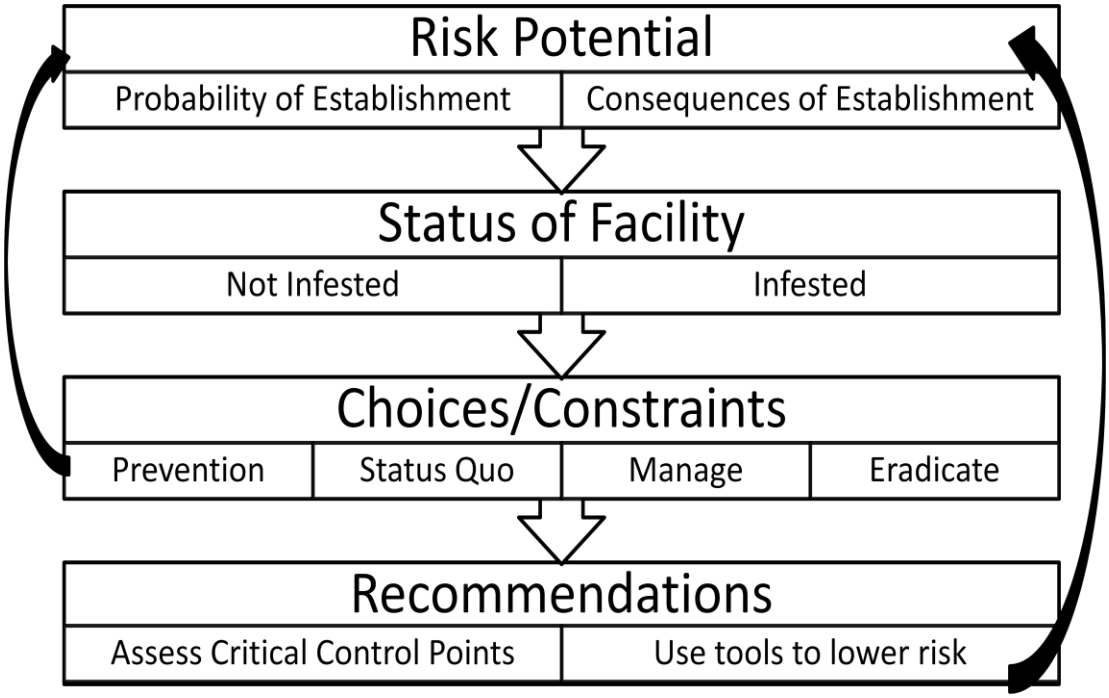

Figure 4.1. Framework for the adaptive management process of a risk analysis for an unwanted organism at a facility, such as a fish hatchery.

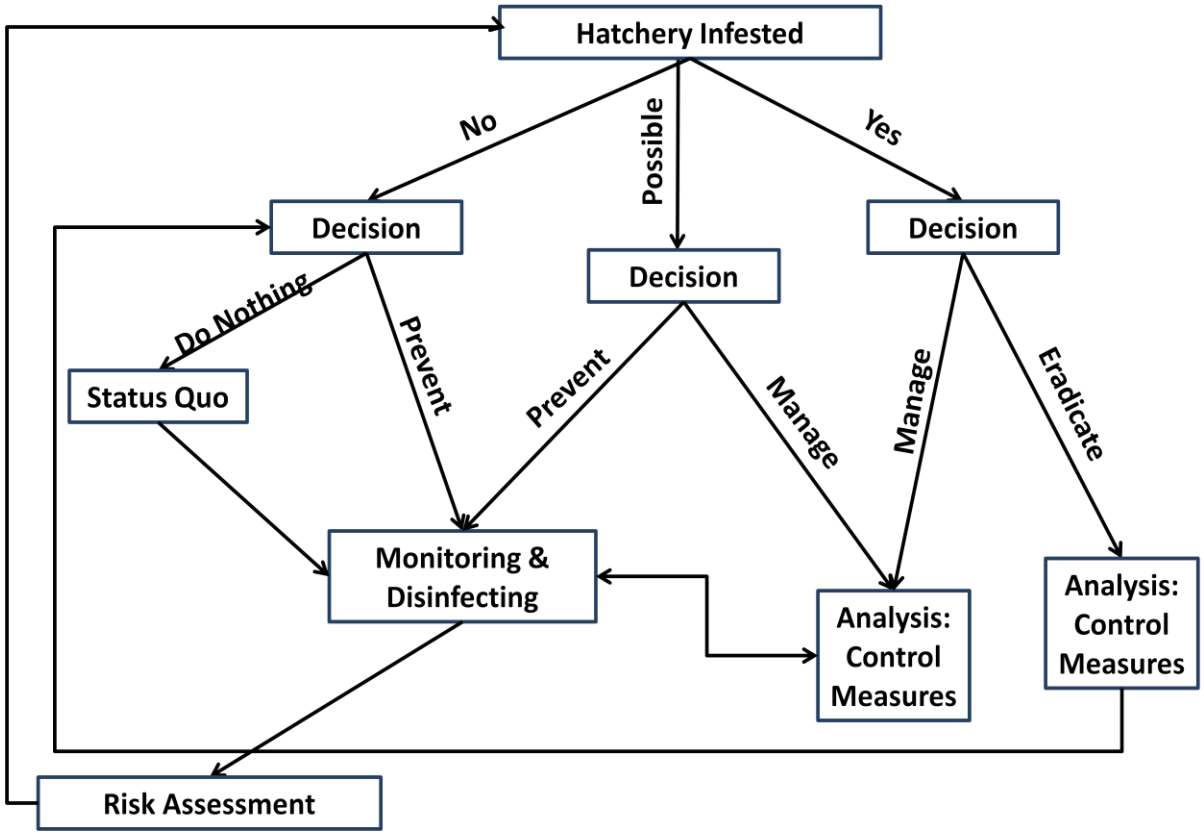

Figure 4.2. Decision tree to evaluate infestation and the choices that lead to actions such as monitoring and disinfection, risk analysis and control measures, and risk assessment.

Control measure ranking	Pathway ranking: Very low	Low	Medium	High	Very high
Very high	LS	LS	LS	LS	OK
High	LS	LS	LS	OK	UR
Medium	LS	LS	OK	UR	UR
Low	LS	OK	UR	UR	UR
Very low	OK	UR	UR	UR	UR

Figure 4.3. The matrix scores suggested to evaluate how control measure certainty ranking affects pathway ranking. LS= lower risk significantly, OK= lower risk, UR= undetermined risk or no effect.