THESIS

CAN COPPER-BASED SUBSTRATES BE USED TO PROTECT HATCHERIES FROM INVASION BY THE NEW ZEALAND MUDSNAIL?

Submitted by

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ABSTRACT

CAN COPPER-BASED SUBSTRATES BE USED TO PROTECT HATCHERIES FROM INVASION BY THE NEW ZEALAND MUDSNAIL?

Aquaculture facilities throughout North America are at risk of invasion by the New Zealand mudsnail (*Potamopyrgus antipodarum*). Mudsnails can enter facilities in several ways including by crawling through effluent pipes. There is evidence to suggest that lining the insides of these pipes with copper-based substrates to create a contact deterrent could reduce the risk of mudsnail invasion. However, before copper-based deterrents can be recommended for wide-scale use, it is important that we understand how these materials perform across the range of physicochemical conditions common to hatcheries. The goal of this project was to evaluate the relative ability of four types of copper-based materials (copper sheet; SC (0.323 mm, 99.9% pure), copper mesh; MC (6.3 opening/cm, 99% pure), copper-based ablative anti-fouling paint; AP (Vivid Anti-fouling Paint, 25% cuprous thiocyanate as the active ingredient), and copper-based non-ablative anti-fouling paint; NP (Sealife 1000, 39% cuprous oxide as the active ingredient)) to serve as effective mudsnail contact deterrents across a range of water temperatures (8, 12, 18, and 24° C), hardness (75, 125, 175, and 300 mg/L as $CaCO_3$), pH (6, 7, and 8.5), fouling (0, 6, and 10 weeks of exposure), and water velocities (0, 9, and 33 cm/s). Each of these factors was evaluated in a sequential set of separate experiments conducted at the Colorado State University Foothills Fisheries Laboratory during 2009-10. Mean crawling distance (MCD) of the mudsnails in the temperature, hardness, and pH experiments was significantly lower on the SC and MC surface treatment compared to the NP treatment ($p < 0.05$). Additionally, maximum observed crawling distance (CD_{max}) was also consistently lower on the SC (1139 mm), MC (672 mm), and AP (1509 mm) treatments versus the NP (1969 mm) treatment. The NP treatment was the only surface where MCD was significantly affected by all three physicochemical parameters ($p > 0.05$). In the fouling experiment, MCD increased significantly on the AP surface treatment after exposure to fouling from 353 ± 83 mm (mean ± SE) at week 0 to 1207 ± 196 at week 6; no significant increase in this parameter was found on either solid copper surface. Finally, in the water velocity experiment, overall MCD on the copper surfaces was significantly lower in the 0 cm/s velocity treatment (30 ± 6.3 mm) compared to either 9 cm/s (302 ± 47.4 mm) or 33 cm/s (278 ± 50.2 mm). Under flowing water conditions, MC was the most effective treatment for limiting the MCD and CD_{max} of the mudsnails. Finally, there was no evidence to suggest that at the levels tested, velocity alone could serve as a deterrent to mudsnails. Overall, MC and SC were the most effective surfaces in terms of limiting the locomotor activity of the mudsnail. We recommend that barriers constructed of either of these materials be a minimum of 250 cm long to provide a satisfactory level of protection against mudsnail invasion. Additional considerations including design and integration with other types of barriers are discussed.

ACKNOWLEDGEMENTS

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CHAPTER 1

EVALUATION OF THE EFFECTS OF WATER TEMPERATURE, HARDNESS, AND PH ON THE NEW ZEALAND MUDSNAILS' REPONSE TO COPPER-BASED COMPOUNDS

Introduction

Freshwater ecosystems and aquaculture facilities worldwide are at risk of invasion by the New Zealand mudsnail (*Potamopyrgus antipodarum*; hereby referred to as NZMS). Originally endemic to New Zealand and nearby islands (Winterbourn 1970), NZMS have successfully spread through unintentional introductions (Ponder 1988; Richards 2002; Zaranko et al. 1997) to 3 continents over the last 150 years (ANSTF 2006; Bowler 1991). Outside of its native range, populations can reach densities of up to 500,000 snails/m^2 (Hall et al. 2003), at times significantly altering ecosystem processes (Arango et al. 2009; Hall et al. 2003; Lysne and Koetsier 2008; Riley et al. 2008). Though it is not thought to be a disease vector (Beck 2004), valuable sportfish populations may still be at risk of decline when the main food sources for these fish, native benthos, are replaced by the nearly indigestible NZMS (Vinson and Baker 2008).

Concerns about the NZMS effect on native and naturalized communities have prompted management agencies to implement "slow the spread" strategies in several areas of the country. Over the last decade, officials in California, Idaho, and Colorado have been forced to close or otherwise restrict activities associated with recreational fisheries and aquaculture operations affected by this organism. Given the NZMS broad tolerances of water temperature (Hylleberg and Siegismund 1987; Winterbourn 1969), water chemistry (Alonso and Camargo 2003; Leppakoski and Olenin 2000; Richards 2002) and human disturbance (Gerard and Poullain 2005; Richards et al. 2001; Schreiber et al. 2003), coupled with its generalist dietary (Dorgelo and Leonards 2001; Haynes and Taylor 1984; Jensen et al. 2001) and habitat requirements (Heywood and Edwards 1962; van den Berg et al. 1997; Weatherhead and James 2001), and explosive asexual reproduction potential (Richards 2002; Zaranko et al. 1997), it is likely this organism will continue to expand its range unless effective strategies to control the NZMS spread are developed and tested.

The NZMS spreads to novel areas by multiple means, including passive entrainment on or in gear, transport water, and fish associated with routine stocking activities by infested fish hatcheries (ANSTF 2006). The incentive for natural resource agencies and aquaculture personnel to prevent hatchery infestation by NZMS is two-fold. First, cultured organisms may be transported (along with entrained snails) hundreds of miles prior to release, increasing the likelihood of rapid range expansion. Secondly, infested facilities stand to lose significant amounts of time and money attempting to eradicate NZMS from their operation, and in some cases, may never be allowed to resume production/deliveries of cultured organisms to their original markets.

Hatchery invasion occurs through three primary pathways. First, NZMS can be introduced from an outside source via waders, nets, or transport water. Second, if a facility relies on surface water supply (i.e. springs, streams, or lakes), or a groundwater supply that is exposed to the atmosphere prior to entering the facility, infestation of the source, as has happened in some Idaho salmonid hatcheries, results in NZMS entering the facility through the water supply. Finally, if a facility discharges effluent water into a NZMS-positive body of water, it is possible for NZMS to enter the hatchery from the receiving waters by crawling through the facility's effluent pipe. This is believed to be the pathway that led to the invasion of a small hatchery in Boulder, Colorado (K. Cline. Cline Trout Farms. pers. comm.).

Control methods have been proposed for the first two pathways, including proper disinfection of gear (Schisler et al. 2008), securing facility water supplies by switching from above-ground (springs, creeks, etc.) sources to ground water, or by removing mudsnails from the water supply through hydrocyclonic separation (Nielson 2006). However, invasion through effluent pipes remains without a well-tested solution, leaving hatcheries at risk until this final pathway can be accounted for.

Lining the inside of effluent pipes with copper-based substrate treatments may solve this problem by creating a contact deterrent to NZMS. Copper and copper-based materials are used in a variety of situations to control unwanted or invasive aquatic organisms including aquatic snails in aquaculture ponds (e.g., *Planorbella trivolvis*) (Wise et al. 2006). In the terrestrial environment, contact barriers composed of copper substrates can be used to protect ornamental plants from damage by snails and slugs by limiting the locomotor activity of these organisms (Schüder et al. 2003; Schüder et al. 2004). However, a review of the literature found no instances where contact deterrents were applied in aquatic environments. To address this issue, a pilot study for this project found that copper and copper-based materials did serve as NZMS deterrents by reducing the crawling distance under static (non-flowing) conditions (Myrick and Conlin In Press). These findings are supported by anecdotal evidence from the formerly infested aquaculture facility that has remained free of NZMS since installing copper sheet in their facility's effluent pipes (K. Cline. Cline Trout Farms. pers. comm.).

In spite of these encouraging results, *in situ* field tests of copper-based deterrents at locations in Utah, Colorado, and Idaho have shown conflicting levels of effectiveness. The crawling distance of NZMS over the copper deterrents were dramatically reduced at some locations whereas at other hatcheries, the organism's movements were unaffected.

These results may have been caused by several factors, perhaps most notably by differences in the physicochemical composition of each facility's effluent water.

Copper toxicity (and therefore presumably mudsnail deterrent efficiency) is affected by physical and chemical properties of water. In general, copper toxicity is expected to increase with water temperature (Gupta et al. 1981; Rao and Khan 2000; Rao et al. 1988), partially as the result of greater uptake caused by increased metabolic activity by the organism (Cairns et al. 1975). Alternatively, an increase in either pH or water hardness can decrease copper toxicity (Erickson et al. 1996; Rathore and Khangarot 2003; Sciera et al. 2004) through complexation of the copper ions and/or competition for biological binding sites (Di Toro et al. 2001). To what degree these factors affect the NZMS response to copper deterrents is uncertain. If copper deterrents are to be used in the future to help secure hatcheries against invasion, then it is essential to understand how the NZMS movements (mainly crawling distance) and behavior on copper-based materials are affected by these key physical and chemical water properties.

To address this question, a series of experiments were conducted in 2009 and 2010 to determine the relative ability of four types of readily available copper-based materials to serve as effective NZMS contact deterrents. This work was a continuation of the pilot study conducted by Myrick and Conlin (In press) and focused on how deterrent performance of the copper materials was affected by several key physicochemical variables. Three separate experiments were performed to examine the deterrent efficiency of the copper treatments at water temperatures between 8 and 24° C, water hardness levels between 75 and 300 mg/L as $CaCO_3$, and pH levels between 6 and 8.5. These ranges were chosen in order to include much of the NZMS reported temperature tolerance range, reported as approximately 0° C (Hylleberg and Siegismund 1987) to 32° C (Quinn et al. 1994), and to include the range of physicochemical conditions typical of water used to culture many fish species in the United States (Avault 1996).

Methods

Materials tested

Four materials were evaluated for their ability to limit the movements of the NZMS over a range of physicochemical conditions. The materials, the justification for their selection, and a cost comparison (prices as of Fall 2010), are given below.

1.) Copper sheeting; SC (0.323 mm, 99.9% pure). Sheets of this material could be installed in effluent pipes, culverts, effluent collection boxes, or in the receiving water directly below the effluent outfall. Alternately, a portion of the effluent system could be fitted with solid copper pipes. Cost per square meter: $75 USD.

2.) Copper mesh; MC (6.3 opening/cm, 99% pure). This material was chosen because, unlike copper sheeting, it can be more easily installed over irregular surfaces and may have comparatively lower copper leaching rates. Cost per square meter: $81 USD.

3.) Ablative anti-fouling paint[1]; AP (Vivid Anti-fouling Paint, 25% cuprous thiocyanate as the active ingredient). This material could be applied directly to effluent pipes or other surfaces in the effluent system. Unlike copper sheet or mesh, copper-based paints can be easily applied to irregular surfaces, but does require a water-free period during application. Cost per square meter (two coats): $11 USD.

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[1] Kop-Coat, Pettit Paint Division, Rockaway, NJ 07866

4.) Non-ablative anti-fouling paint[2]; NP (Sealife 1000, 39% cuprous oxide as the active ingredient). This material is designed to leach copper at a slower rate relative to the other materials thus lessening its deleterious impacts on non-target aquatic organisms. Cost per square meter (two coats): $26 USD.

Collection and acclimation

NZMS were collected by hand from Boulder Creek (Boulder County, CO UTM: 13 481800.032, 4432000.974) or the South Platte River (Park County, CO UTM: 13 460693.005, 4306588.032) and transported to the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado). While in the laboratory, the snails were held in temperature-controlled static containers holding approximately 7.4 liters of air-saturated lake water (College Lake, Fort Collins, Colorado) and were fed freeze-dried *Spirulina* algae. Water quality parameters (pH, nitrate, nitrite, hardness, and ammonia) were recorded every 48 hours; approximately 50% of the water in the static containers was replaced in the containers every 72 hours to prevent the buildup of nitrogenous wastes.

The NZMS were held in captivity for two weeks prior to the initiation of the experiments. During the first week, the physicochemical variables of the containment water were manipulated as described below, followed by the second week, when the snails were allowed to acclimate undisturbed to the specific treatment conditions. Initially, an acclimation period of approximately one month was tried but was altered after high levels of mortality and reduced activity were observed following 3 weeks of captivity.

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[2] Sealife Marine Products Inc., Culver City, CA 90230

Three separate experiments were conducted to examine a different physicochemical variable thought to influence the snails' responses to the copper substrates. The first experiment focused on the effects of water temperature (tested at 8, 12, 18, or 24° C). Acclimation was carried out by adjusting the temperature at 3° C per day until the treatment level had been reached. The second experiment examined the effects of water hardness on the NZMS response to the copper substrates. Calcium hardness was adjusted to four levels (75, 125, 175, or 300 mg/L $CaCO_3$) using a commercially available mixture[3] that raised calcium hardness without altering alkalinity. Finally, the third experiment tested the NZMS response to the copper materials at pH levels of 6.0, 7.0, and 8.5. The pH was either raised using sodium hydroxide or lowered using nitric acid (15.7 M) at 0.2 pH units per day until the desired treatment level had been reached. The hardness and pH experiments were run at 18° C after observing the highest level of locomotive activity at this temperature during the water temperature experiment.

Equipment and procedures

All three experiments were performed at the Colorado State University Foothills Fisheries Laboratory during 2009 and 2010. Each substrate × physicochemical parameter combination was replicated 12 times. Trials were conducted in 21.5 cm diameter × 3.0 cm deep PVC (polyvinyl chloride) test arenas filled to a depth of 2.5 cm with air-saturated water. In these arenas, one-half of the area was covered with a copper-based substrate; the other half remained bare PVC to serve as a control (Figure 1). Figure 2 shows the double containment system that was used during the experiments. During the 2-h trials, peristaltic pumps continually re-circulated water through the arenas at 5 ml/s.

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[3] Reef Calcium Advantage, Seachem Laboratories Inc., Madison, GA 30650

The re-circulated water was filtered through activated carbon and was also partially replaced during the trials to remove aqueous copper that had leached from the copper substrates. These steps were taken to ensure that any observed response was the result of *contact* with the copper, not aqueous exposure.

At the start of a trial, a single NZMS was placed near the center of the arena and its locomotor activity was then monitored using Unibrain Fire-i digital video cameras connected to a desktop computer running SecuritySpy Software (version 1.3.1). Timelapse videos were recorded at 1 frame every 30 seconds. Following the completion of a trial, the snail was monitored for 24 h to determine post-exposure mortality as recognized by loss of operculum control. At the end of the post-exposure observation period, the NZMS was then euthanized and preserved by prolonged freezing.

The data files were analyzed using NIH Image J 1.38I (Rasband 2007a), running Manual Tracking (Cordelie`res 2005) and Quicktime Capture (Rasband 2007b) software as described in Myrick (2009). Mean and maximum crawling distances were used as the primary measure of the effectiveness of each surface. Additionally, mean velocity, the percentage of snails that were immobilized (i.e., inactivity > 30 min), and the duration of time spent actively crawling on each surface were also evaluated. For each of the substrate × water parameter combinations, locomotor activity was compared on the treated versus untreated surfaces along with comparisons of activity between the various treatment groups. During video analysis, locomotor activity by the snails was quantified using Equation 1.

$$Distance = (((X2 - X1)2 + ((Y2 - Y1)2))0.5 \qquad (Equation\ 1)$$

Where:

$X1$ = Previous horizontal axis location
$X2$ = Current horizontal axis location
$Y1$ = Previous vertical axis location
$Y2$ = Current vertical axis location

Data analyses

One-way analysis of variance (ANOVA) tests were used to evaluate differences in mean crawling distance on the treated versus untreated (control) sides of the test arenas.

Two-way ANOVA tests followed by Tukey HSD *post-hoc* tests were conducted to examine the main effects of each variable and also to test for interactions between the copper surfaces and the physicochemical parameters. All data were analyzed at the $\alpha = 0.05$ significance level using JMP 8.0 statistical software (SAS 2008). Histograms, Shapiro-Wilk W-tests, and normal quantile plots were used to check assumptions of normality and distribution of errors. Data sets that were not normally distributed were log-normalized and reassessed. Assumptions of normality were met following log-transformation.

Results

Carapace length of the NZMS used in the three experiments ranged from 2.5 to 5.7 mm with a mean (± SE) of 4.4 ± 0.2 mm. No significant difference ($p < 0.05$) in size was detected between the three experiments or between the various treatments with a given experiment. Overall, mean crawling distance (MCD) tended to be lower on the copper treated side of the arenas compared to the untreated sides (Tables 2-4) though within-treatment trends were inconsistent. Finally, post-exposure survival ranged from 67 to 100% (mean = 94.9%) with no clear patterns in mortality observed within the copper surface types (for complete results, please see Appendix C).

Water temperature experiment

Analysis of the main effects caused by temperature and copper substrate type indicated MCD was affected by substrate type ($p < 0.0001$) but was not significantly altered by temperature (though a positive correlation between MCD and temperature was observed). *Post-hoc* analyses of the main effects caused by surface type indicated that MCD was significantly greater ($p < 0.05$) on the painted surfaces (i.e., AP and NP) versus the solid copper surface treatments (MC and SC). Regardless of water temperature, MCD was consistently greater on the painted surfaces compared to the solid surfaces (Figure 3, Panel A; Table 1). A significant ($p = 0.0014$) whole-model interaction term was also detected in the analysis. Within the four surface treatments, a significant surface × temperature interaction ($p < 0.05$) was detected only within the NP surface treatment; no temperature interactions were observed for the remaining surfaces. Finally, maximum observed crawling distance (CD_{max}) was up to 4 times greater on the painted surface treatments compared to either solid copper surface.

Water hardness experiment

In general, MCD tended to be greater on the paint-based treatments relative to the solid copper treatments across the entire experiment (Figure 3, Panel B). Analyses of the main effects in this experiment indicated that MCD was significantly greater on the painted copper surface treatments relative to the solid copper treatment ($p < 0.0001$), while for water hardness, MCD was greater in the 125 mg/L treatment compared to the 175 and 300 mg/L treatments ($p < 0.0001$). A significant surface × hardness interactions was found in the SC and NP surface treatment groups with MCD significantly greater in the 125 mg/L treatment compared to the 300 mg/L treatment (Figure 3, Panel B; Table

1). Overall, CD_{max} appeared to be unaffected by water hardness and was consistently greater on the NP surface treatment relative to the remaining surface treatments.

pH experiment

Mean crawling distance was significantly affected by both pH ($p < 0.0001$) and copper type ($p < 0.0001$). *Post-hoc* analyses indicated that for surface type, MCD was significantly greater on the NP surface treatment relative to the three remaining surfaces while the analysis of the effect caused by pH indicated MCD significantly increased with each pH level. In each of the four surface treatments, MCD was statistically lower in the pH 6 treatments versus the pH 8.5 treatment (Figure 3, Panel C; Table 1). Finally, in each surface treatment, CD_{max} decreased with pH and overall was consistently lower on the solid copper surfaces compared to the painted surfaces.

Discussion

In each experiment, copper sheet and copper mesh were the most effective surface treatments in limiting the crawling distance of the NZMS. Mean crawling distance was up to 8 times lower on the solid copper surfaces compared to those distances recorded on copper-based paint surfaces. Deterrent performance in terms of mean crawling velocity, duration of activity, and percent immobilization were also consistently greatest on SC and MC (for exact values, please see Appendices A and B). Generally, changes in temperature and hardness did not significantly affect MCD or CD_{max} on the SC and MC surfaces, suggesting these materials would function as effective contact deterrent to NZMS across the wide range of physicochemical conditions common to hatcheries including those facilities that experience wide annual changes in water temperature.

The NZMS did not appear to avoid contact with any of the copper surfaces and it was only after several minutes of exposure did the snails show any noticeable response to the treatments. This response was manifested as either slowing or completely ceasing crawling activity followed by retraction of the body into the carapace for the remainder of the trial. Across all three experiments, this behavioral response was recorded in 69% of trials involving the solid copper treatments but only 11% of trials on the painted treatments where the snails appeared to move freely between the treated and untreated sides showing no signs of adverse effects. This lack of a clear avoidance or immediate response to the copper treatments resulted in few instances where MCD on the control sides of the arena was significantly greater than on the copper treated sides. Also within a given treatment combination, crawling distance on both sides of the arenas ranged from 10 to nearly 2000 mm which led to inflated confidence intervals and loss of statistical power.

In the CS and NP surface treatments, crawling distance unexpectedly showed an inverse relationship to water hardness. Chronic and lethal responses of organisms exposed to elevated levels of aqueous copper have been negatively correlated with water hardness (e.g., Erickson et al. 1996; Sciera et al. 2004) suggesting that MCD in the low hardness treatments would be reduced. The mechanism responsible for this relationship is thought to be the cations associated with water hardness (primarily in the form of Mg and Ca) that attenuate the effects of copper through ion complexation and competitive exclusion for binding sites on the cell membrane (Pagenkopf 1983). This relationship formed the basis for the EPA’s hardness-dependent criteria for dissolved copper in aquatic environments (EPA 1985). However, it has also been reported that aquatic organisms exposed to elevated levels of heavy metals can show a temporary increase in activity during the period of initial exposure (Eissa et al. 2009). Therefore, the snails in the low hardness treatment groups may have shown temporarily elevated levels of activity following exposure to the copper treatments. To what degree this ultimately affects deterrent performance of the copper materials is unclear, however these results do suggest that deterrent length should be increased in cases where hatcheries are discharging effluent water with low hardness levels.

Levels of dissolved aqueous copper measured at the conclusion of the 2-h trials ranged from 4.5 to 8.5 ppb but did not exceed the hardness–adjusted criteria for our water supply of 8.9 ppb. Chronic effects to NZMS caused by aqueous exposure to copper were recorded at concentration levels much above those found in this experiment and after a much longer exposure period (48 and 96 hours) (Watton and Hawkes 1984). Copper can be absorbed through the epidermis of terrestrial gastropods (Bullock et al. 1992; Ryder and Bowen 1977) and an analogous response appears to be present in the NZMS. The NZMS reactions to a comparatively low concentration of dissolved copper in a short time-span (i.e., two hours) suggests that the slowing and cessation of movements stemmed at least partially from *contact* exposure to the copper surface treatments. Finally, post-exposure survival rates generally exceeded 90% (please see Appendix C) suggesting that the snails did not absorb a lethal amount of copper ions and the observed responses are likely to be short-term.

The maximum observed crawling distance on either copper sheet or copper mesh was 1139 mm suggesting that a deterrent composed of either material would need to be a minimum of approximately 1.2 m long in order to provide a satisfactory level of protection. On both surfaces, CD_{max} ranged from 28 to 1139 mm with less than 1% of snails exceeded 1000 mm during the 2 h trials. However, given the NZMS ability to reproduce parthenogenetically, its delayed response to the copper surfaces, and the relatively low cost of installing a deterrent longer than the bare minimum effective length, it is likely best to use a conservative estimate of effective deterrent length to achieve the desired level of protection. Designing copper sheet or copper mesh deterrents that are double the CD_{max} (i.e. approximately 2500 mm) should provide an adequate level of protection against NZMS invasion at a reasonable monetary cost. Retrofitting the inside of steel or PVC effluent pipe with a copper sleeve has been used by several hatcheries as a means of employing this type of design. Additionally, replacing a section of steel or PVC pipe with a length of copper pipe may also be feasible. Actual designs are likely to be unique to a particular facility based on the current design of their effluent system, water chemistry, and cost or logistic constraints. Regardless of design, the deterrents should be monitored during the first year after their installation to check for the response of snails under the local physicochemical conditions.

Compliance with current environmental standards for the discharge of aqueous copper must be a consideration when utilizing this type of deterrent design. In these experiments, SC and MC released copper at an average rate of 5.11×10^{-4} ppb/min/cm^2 and 4.94×10^{-4} ppb/min/cm^2, respectively. Facilities considering the use of solid copper deterrents can use these leaching rates, along with the EPA's equation for hardness adjusted criteria, to determine the maximum surface area of copper that can be used under a particular set of physicochemical conditions without violating environmental policy standards. However, this information will provide only a rough estimate of copper discharge; deterrents should be designed on a case-by-case basis and tested on a pilot scale to account for facility-unique differences in water chemistry and effluent discharge regime. Facility managers must consider that the release of aqueous copper from a solid surface is affected by water chemistry (Broo et al. 1997), water velocity (erosion corrosion) and the presence and actions of biofilms (Critchley et al. 2003; Dutkiewicz and Fallowfield 1998).

Coupling solid copper materials with other deterrent designs would provide multi-level protection against NZMS invasion. Electrified collars affixed inside of effluent pipes have been shown to function as NZMS deterrents (R. Oplinger. Utah DNR. pers. comm.) and, by employing both methods, a hatchery could effectively guard against invasion even in circumstances where power had been lost to the electrified collars. Modifying the design of a facility's effluent pipes would also be effective. A "free-fall" barrier created by raising the point of discharge out of the receiving water body would effectively eliminate the threat of invasion in most circumstances. However, a deterrent affixed inside of the effluent pipe would provide an additional level of cost-effective protection and would secure the facility in instances where the water level of the receiving waterbody rose to the level of the effluent pipe such as during spring run-off.

Hatcheries, especially those in the western United States, face a constant threat of invasion by aquatic nuisance species, including the NZMS. Securing these facilities will help to insure the uninterrupted production of fishes used to stock public waterways while also helping to limit the spread of this organism. When implemented as part of a larger facility protection plan, contact deterrents constructed of solid copper materials, whether used alone or in conjunction with other designs, should provide a satisfactory level of protection against invasion under the physicochemical conditions that were examined. However, the effect of two additional factors, water velocity and biofouling, on the NZMS deterrent efficiency of copper surfaces need to be addressed to fully understand the deterrent ability of these materials and are the focus of additional research related to this project.

Figure 1. An example of the 21.5 cm diameter PVC arena used to test the New Zealand mudsnails (*Potamopyrgus antipodarum*) response to four types of copper-based substrates under various physicochemical conditions. One-half of each arena was covered with a copper-based material; the other half remained bare PVC to serve as a control. Experiments were conducted from November 2009 to June 2010 at the Colorado State University Foothill Fisheries Laboratory (Fort Collins, Colorado).

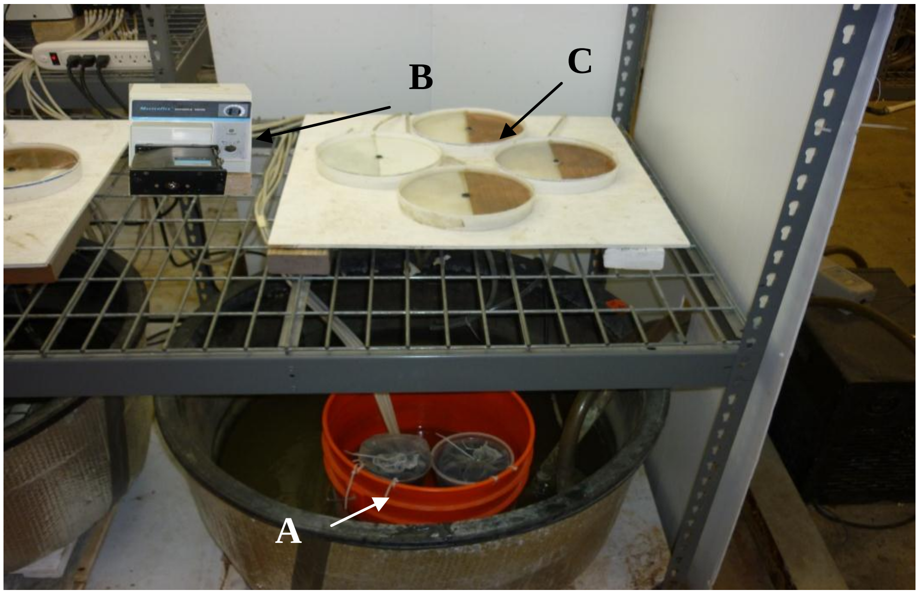


Figure 1. The equipment used to test the New Zealand mudsnails (*Potamopyrgus antipodarum*) response to four types of copper-based substrates at various water temperature, hardness, and pH levels. At the beginning of a trial, a single mudsnail was placed in the center of each arena and its movements were recorded by a digital motion tracking system. During each trial, temperature controlled water was drawn from the 19-L reservoir (A) at a rate of 5 ml/s by an 8-channel peristaltic pump (B) and circulated through the PVC arenas (C). Water exited the arenas through a center drain and was filtered through activated carbon filters before returning to the reservoir. Aqueous copper concentrations were held below the EPA's hardness adjusted criteria through absorption by the carbon filters and by partially replacing the reservoir water during the 2 h trials. Experiments were conducted between November 2009 and June 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

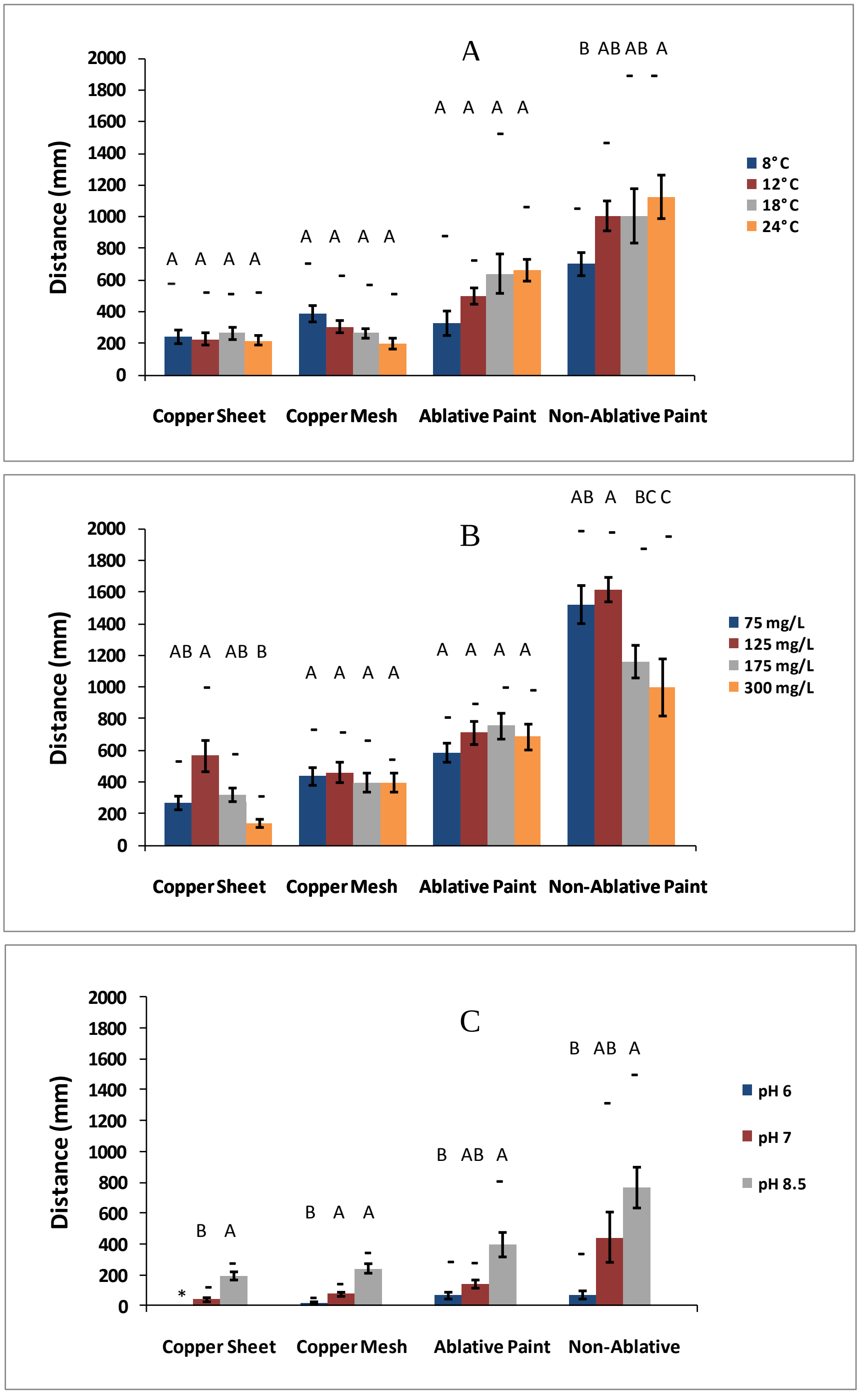


Figure 3. **Mean crawling distance (plus standard error bars) the New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to four types of copper-based surface treatments at various water temperature (panel A), water hardness (panel B), and pH (panel C) levels. Within a treatment combination, bars not connected by the same letter are considered statistically different ($p < 0.05$). Maximum observed crawling distance is expressed above each bar as a "–" symbol. No activity was observed on copper sheet at pH 6 and noted by * in the figure. Each treatment combination was replicated 12 times and the three experiments were conducted between November 2009 and June 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).**

Table 1. Results of Tukey HSD analyses showing the statistical differences in mean crawling distance (± SE) New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to four types of copper-based substrates in three separate experiments. Within an experiment, treatment combinations not connected by the same letter are considered statistically different ($p < 0.05$). In each experiment, all treatment combinations were replicated 12 times. Trials lasted for 2 hours and were conducted between November 2009 and June 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

Temperture Experiment

Temp. (°C)	Surface	Mean Crawling Distance (mm)							
24	Non-Ablative Paint	1125 (137.0)	A						
18	Non-Ablative Paint	1007 (170.0)	A	B					
12	Non-Ablative Paint	1002 (94.2)	A	B	C				
8	Non-Ablative Paint	702 (74.3)		B	C	D			
24	Ablative Paint	662 (65.0)			C	D	E		
18	Ablative Paint	637 (123.7)			C	D	E	F	
12	Ablative Paint	500 (52.8)				D	E	F	G
8	Copper Mesh	391 (51.7)				D	E	F	G
8	Ablative Paint	328 (73.4)					E	F	G
12	Copper Mesh	303 (37.6)					E	F	G
18	Copper Mesh	263 (34.0)					E	F	G
8	Copper Sheet	243 (43.0)						F	G
18	Copper Sheet	265 (42.0)							G
12	Copper Sheet	226 (39.3)							G
24	Copper Sheet	219 (30.4)							G
24	Copper Mesh	200 (34.3)							G

Water Hardness Experiment

Hardness (mg/L $CaCO_3$)	Surface	Mean Crawling Distance (mm)								
125	Non-Ablative Paint	1616 (79.2)	A							
75	Non-Ablative Paint	1521 (121.1)	A	B						
175	Non-Ablative Paint	1157 (102.6)		B	C					
300	Non-Ablative Paint	999 (178.8)			C	D				
175	Ablative Paint	754 (79.9)			C	D	E			
125	Ablative Paint	712 (72.1)				D	E			
300	Ablative Paint	687 (82.3)				D	E	F		
75	Ablative Paint	583 (61.1)					E	F	G	
125	Copper Sheet	564 (102.1)					E	F	G	
125	Copper Mesh	458 (63.1)					E	F	G	H
75	Copper Mesh	436 (56.4)					E	F	G	H
175	Copper Mesh	395 (63.8)					E	F	G	H
175	Copper Sheet	317 (45.8)						F	G	H
300	Copper Mesh	395 (43.4)						F	G	H
75	Copper Sheet	267 (44.3)							G	H
300	Copper Sheet	139 (25.3)								H

Table 1 (continued)

pH Experiment

pH	Surface	Mean Crawling Distance (mm)					
8.5	Non-Ablative Paint	766 (130.4)	A				
8.5	Ablative Paint	397 (78.2)	A	B			
8.5	Copper Sheet	192 (27.0)	A	B	C		
7	Non-Ablative Paint	443 (159.5)	A	B	C	D	
8.5	Copper Mesh	228 (38.4)	A	B	C	D	
7	Ablative Paint	141 (28.5)		B	C	D	
7	Copper Mesh	76 (12.8)		B	C	D	
6	Ablative Paint	67 (20.5)			C	D	
6	Non-Ablative Paint	68 (28.8)			C	D	
7	Copper Sheet	40 (12.7)				D	
6	Copper Mesh	4 (2.4)					E
6	Copper Sheet	--					

Table 2. Mean crawling distance in mm (plus standard error in parenthesis) of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to various copper-based surface treatments at four water temperatures. Trials were conducted in 21.5 cm diameter arenas that were 50% covered with a copper surface treatment; the other half remained bare PVC to serve as a control. All copper surface × water temperature combinations were replicated 12 times. Asterisks denote significant differences ($p < 0.05$) between treated and control sides of the arenas. Trials lasted for 2 hours in and were conducted in November 2009 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado). See Table 1 for the relationships between treatments.

	Temperature							
	8° C		12° C		18° C		24° C	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Copper Sheet	264 (69)	391 (52)	890 (167)	303 (77)	503 (176)	265 (31)	531 (204)	199 (34)
Copper Mesh	589 (105)	243 (42)	660 (283)	226 (39)	828 (139)	265 (39)	449 (159)	219 (30)
Ablative Paint	570 (88)	328 (74)	1162 (157)*	500 (53)	1303 (217)*	638 (124)	1046 (211)	662 (65)
Non-Ablative Paint	700 (79)	702 (74)	897 (81)	1002 (94)	1244 (193)	1067 (170)	1001 (130)	1125 (137)

Table 3. Mean crawling distance in mm (plus standard error in parenthesis) of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to various copper-based surface treatments at four water hardness levels. Trials were conducted in 21.5 cm diameter arenas that were 50% covered with a copper surface treatment; the other half remained bare PVC to serve as a control. All copper surface × water hardness combinations were replicated 12 times at a water temperature of 18° C. Asterisks denote significant differences ($p < 0.05$) between treated and control sides of the arenas. Trials lasted for 2 hours and were conducted in January 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado). See Table 1 for the relationships between treatments.

	Water Hardness							
	75 mg/L $CaCO_3$		125 mg/L $CaCO_3$		175 mg/L $CaCO_3$		300 mg/L $CaCO_3$	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Copper Sheet	762 (181)	267 (44)	374 (128)	564 (102)	1281 (213)*	317 (41)	235 (120)	139 (25)
Copper Mesh	448 (130)	436 (56)	111 (39)	458 (63)	592 (92)	395 (60)	557 (330)	395 (57)
Ablative Paint	1500 (182)*	583 (61)	1241 (204)	712 (72)	929 (119)	754 (80)	1531 (126)*	687 (82)
Non-Ablative Paint	1305 (106)	1521 (121)	1427 (118)	1617 (79)	1421 (104)	1158 (102)	904 (138)	999 (179)

Table 4. Mean crawling distance in mm (plus standard error in parenthesis) of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to various copper-based surface treatments at three pH levels. Trials were conducted in 21.5 cm diameter arenas that were 50% covered with a copper surface treatment; the other half remained bare PVC to serve as a control. All copper surface × pH combinations were replicated 12 times at a water temperature of 18° C. Asterisks denote significant differences ($p < 0.05$) between treated and control sides of the arenas. No movement was recorded on copper sheet at pH 6. Trials lasted for 2 hours and were conducted in June 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

pH

	pH 6		pH 7		pH 8.5	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Copper Sheet	31 --	-- --	166 (90)	40 (13)	363 (129)	192 (27)
Copper Mesh	31 --	20 (8)	92 (27)	76 (13)	350 (165)	239 (33)
Ablative Paint	499 (132)	67 (21)	1350 (159)*	141 (29)	1300 (237)*	397 (78)
Non-Ablative Paint	185 (111)	68 (29)	988 (209)	443 (160)	511 (101)	765 (130)

CHAPTER 2

DOES FOULING OR WATER VELOCITY AFFECT THE NEW ZEALAND MUDSNAILS' RESPONSE TO COPPER-BASED COMPOUNDS?

Introduction

Freshwater ecosystems and aquaculture facilities worldwide are at risk of invasion by the New Zealand mudsnail (*Potamopyrgus antipodarum*; hereby referred to as NZMS). Originally endemic to New Zealand and nearby islands (Gangloff 1998; Winterbourn 1970), the NZMS has successfully spread to three continents over the last 150 years (ANSTF 2006; Bowler 1991) through unintentional introductions (Ponder 1988; Richards 2002; Zaranko et al. 1997). Outside of its native range, populations can reach densities of up to 500,000 snails/m^2 (Richards et al. 2001) at times altering ecosystem processes (Arango et al. 2009; Hall et al. 2003; Lysne and Koetsier 2008; Riley et al. 2008). Though not a vector for disease (Beck 2004), valuable sportfish populations may still be at risk of decline when the main food sources for these fish, native benthos, are replaced by the nearly indigestible NZMS (Vinson and Baker 2008).

Concerns about the NZMS effect on native and naturalized communities have prompted management agencies to implement “slow the spread” strategies in several areas of the country. Over the last decade, officials in California, Idaho, and Colorado have been forced to close or otherwise restrict activities associated with recreational fisheries and aquaculture operations affected by this organism. Given the NZMS broad tolerances of water temperature (Hylleberg and Siegismund 1987; Winterbourn 1969), water chemistry (Alonso and Camargo 2003; Leppakoski and Olenin 2000; Richards 2002), and human disturbance (Gerard and Poullain 2005; Richards et al. 2001; Schreiber et al. 2003) coupled with its generalist dietary (Dorgelo and Leonards 2001; Haynes and Taylor 1984; Jensen et al. 2001) and habitat requirements (Heywood and Edwards 1962; van den Berg et al. 1997; Weatherhead and James 2001), and explosive asexual reproduction potential (Richards 2002; Zaranko et al. 1997), it is likely that the NZMS will continue to expand its range unless effective control measures to limit the NZMS spread are developed and tested.

The NZMS spreads to novel areas by multiple means, including passive entrainment on or in gear, transport water, and fish associated with routine stocking activities by infested fish hatcheries (ANSTF 2006). The incentive for natural resource agencies and aquaculture personnel to prevent NZMS from invading their facilities is two-fold. First, cultured organisms may be transported (along with entrained snails) hundreds of miles prior to release, increasing the likelihood of rapid range expansion. Secondly, facilities found to harbor NZMS stand to lose significant amounts of time and money attempting to eradicate snails from their operation, and, in some cases, may never be allowed to resume production or deliveries of cultured organisms to their original markets. Protecting these facilities must be a priority if we are to slow the spread of the NZMS while continuing to enjoy the recreational benefits associated with the stocking of popular sportfish species.

Invasion of hatcheries occurs through three primary pathways. First, snails can be introduced from an outside source via waders, nets, or transport water. Second, if a facility relies on a surface water supply (i.e. springs, streams, or lakes), or a groundwater supply that is exposed to the atmosphere prior to entering a facility, infestation of this source, as has happened in some Idaho salmonid hatcheries, results in NZMS entering the facility through the water supply. Finally, if a facility discharges its effluent water into a NZMS-positive body of water, it is possible for the snails to enter the hatchery from the receiving waters, by crawling through the effluent pipe. This is believed to be the pathway that led to the invasion of a small hatchery in Boulder, Colorado (K. Cline, Cline Trout Farms, pers. comm.).

Methods that have been proposed to begin to control the first two pathways include proper disinfection of gear (Schisler et al. 2008), securing facility water supplies by switching from above ground sources to ground water, or by filtering snails from an incoming water supply through hydrocyclonic separation (Nielson 2006). However, invasion through effluent pipes remains without a well-tested solution, with perhaps the exception of physically separating the outlet from the receiving water (Myrick and Conlin In Press), leaving hatcheries at risk until this final pathway can be controlled.

Lining the inside of effluent pipes with copper-based substrates may solve this problem by creating a contact deterrent to NZMS. Copper and copper-based materials are used in a variety of situations to control unwanted or invasive aquatic organisms including zebra mussels (*Dreissena polymorpha*) (Dormon et al. 1996) and aquatic snails (e.g., *Planorbella trivolvis*) (Wise et al. 2006). In the terrestrial environment, copper substrates have been found to act as contact deterrents to snails and slugs by reducing or eliminating locomotor activity (Schüder et al. 2003; Schüder et al. 2004). However, a review of the literature found no instances where contact deterrents were applied in an aquatic environment to deter snails. A preliminary study that laid the groundwork for this project concluded that copper and copper-based materials did serve as NZMS deterrents by reducing the crawling distance under static (non-flowing) conditions (Myrick 2007). These findings are supported by anecdotal evidence from the formerly infested aquaculture facility that has remained free of NZMS since installing copper sheeting in their facility’s effluent system (K. Cline, Cline Trout Farms, pers. comm.).

Myrick and Conlin (In Press) identified several key questions that needed to be addressed before copper-based NZMS deterrents can be effectively used by hatcheries. Water temperature, pH, and hardness all affect copper toxicity in aquatic environments, and therefore presumably alter the deterrent potential of copper-based materials. These parameters were the focus of a related portion of this project (see Chapter 1). However, two additional variables, biofouling and water velocity, are also likely to affect the NZMS' response to copper surfaces. Organic particles (i.e., biofouling) affect copper toxicity in aquatic environments (Meador 1991) and effluent pipes typically convey water that has high concentrations of organic particles (e.g., sloughed algal and bacterial mats, feces, uneaten feed, etc.) that may accumulate on the copper surfaces, potentially limiting the effectiveness of contact designs. In addition, erosion of the copper surfaces by moving water may also reduce the effectiveness of the deterrents with time. Second, the velocity of water discharged through effluent pipes is highly variable based on the organism(s) that are being cultured and the volume of production at the facility. Water velocity may in itself serve as a deterrent to NZMS or it may alter the organism's response to the copper surface. An additive or synergistic interaction between velocity and copper-coated substrates may reduce the length of deterrent necessary to protect against invasion. This interaction between water velocity and deterrent performance is likely to play a role in determining the optimal deterrent length that will provide a satisfactory level of protection against NZMS invasion at a reasonable cost.

To address these questions, two experiments were conducted at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado) to determine the relative ability of several copper-based materials to serve as effective contact deterrents for NZMS. During the summer of 2010, an experiment was conducted that examined the effect of particle fouling on the NZMS deterrent efficiency of three readily available copper materials. A second experiment took place in September 2010 that focused on the NZMS response to copper materials at three water velocity levels.

Methods

Materials tested

The first experiment focused on the NZMS response to three types of copper-based materials at various levels of fouling buildup. The second experiment, examining the effect of water velocity on the snails' response to copper surfaces, only focused on two materials, copper sheet and copper mesh. The ablative paint treatment was not tested in the velocity experiment due to its ineffectiveness during the fouling experiment (please see Results and Discussion). The materials, the justification for their selection, and a cost comparison (prices as of Fall 2010) are listed below.

1.) Copper sheeting; SC (0.323 mm, 99.9% pure). Sheets of this material could be installed in effluent pipes, culverts, effluent collection boxes, or in the receiving water directly below the effluent outfall. Alternately, a portion of the effluent system could be fitted with solid copper pipes. Cost per square meter: $75 USD.

2.) Copper mesh; MC (6.3 opening/cm, 99% pure). This material was chosen because, unlike copper sheeting, it can be easily installed over irregular surfaces and may have reduced copper leaching rates compared to solid copper. Cost per square meter: $81 USD.

3.) Ablative anti-fouling paint; AP (25% cuprous thiocyanate as the active ingredient). This material could be applied directly to effluent pipes or other surfaces in the effluent system. Unlike copper sheet or mesh, copper-based paints can be easily applied to irregular surfaces, but does require a water-free period during application. Cost per square meter (two coats): $11 USD.

Collection and acclimation

NZMS were collected by hand from Boulder Creek (Boulder County, CO. UTM: 13 481800.032, 4432000.974) and transported to the Colorado State University Foothills Fisheries Laboratory. While in the laboratory, the snails were held in temperature-controlled containers holding approximately 7.4 liters of air-saturated lake water (College Lake, Fort Collins, Colorado) and fed freeze-dried *Spirulina* algae. Water quality parameters (pH, nitrate, nitrite, hardness, and ammonia) were recorded every 48 hours; approximately 50% of the water was replaced in the containers every 72 hours to reduce the concentration of nitrogenous wastes.

The NZMS were held in captivity for two weeks prior to the initiation of the experiments. During the first week, the temperature of the holding water was adjusted at 3° C/day until a temperature of 18° C had been reached. This temperature was chosen because a previous experiment for this project found that NZMS activity (crawling distance and velocity) was greatest at 18° C (Chapter 1). After the target water temperature was reached, the snails were allowed to acclimate for one week prior to the initiation of the experiments. Initially, an acclimation period of approximately one month was attempted but was altered after high levels of mortality and reduced activity were observed after 3 weeks of captivity.

Surface fouling experiment

The first experiment examined the effect of fouling on the deterrent efficiency of SC, MC, and AP surface treatments. To test this, a copper surface treatment was applied to one-half of the surface of a 20.5 cm diameter PVC disk; the other half of the disk remained bare PVC to serve as a control (Figure 1, Panel A). The NZMS response to each surface treatment was tested after 0, 6, and 10 weeks of exposure to fouling. Each surface × exposure period combination was replicated 8 times. Disks from the two fouling treatments (6 and 10 weeks exposure) were placed in a nearby irrigation canal to allow natural fouling to occur (Figure 1, panel B); the remaining exposure treatment (0 weeks) served as a pre-fouling baseline of the deterrent ability of each material. In the canal, the disks were mounted parallel to the direction of flow 6 to 16 cm above the substrate on threaded steel rods. Water could flow freely over the disk; however, the treatment surface was not exposed to direct sunlight in an effort to limit photosynthetic activity that would not be present in an enclosed effluent pipe.

At the end of the exposure period, the disks were removed from the canal and transported to the Foothills Fisheries Laboratory. The testing of the NZMS response to the copper-based surface treatments was carried out in a temperature-controlled double containment unit designed specifically for this project (Figure 2). Inside the containment unit, the disks were fitted inside 21.2 cm (dia.) × 3.0 cm (height) circular PVC units that had been filled to a depth of 2.0 cm with 18°C water. During a trial, the water was continually re-circulated through the PVC units at approximately 5 ml/s. To reduce the concentration of aqueous copper that had leached from the substrates, the re-circulated water was filtered through activated carbon and was also partially replaced during the trials. These steps were taken to ensure that any effect caused by the treatment surfaces was the result of *contact* with the copper, not aqueous exposure from the leached copper ions.

At the beginning of a trial, a single NZMS was placed near the center of the disk and its locomotor activity was monitored for 2 by Unibrain Fire-i digital video cameras connected to a desktop computer running SecuritySpy Software (version 1.3.1). Timelapse videos were recorded at 1 frame every 30 seconds.

After the initial test had been completed, the disk was removed from the circular PVC unit and a 12.9 cm^2 area of the copper surface was sampled to quantify the amount of fouling that had accumulated while in the canal. Sample collection and dry-weight analysis were conducted using standard procedures (APHA 1995). After sampling, each disk was then scrubbed with a stiff-bristle brush to determine if removing the fouling materials would restore the deterrent efficiency of the copper substrates. Scrubbing was carried out in a standardized manner by making 20 complete passes over each disk with the brush. After the cleaning treatment had been applied, the disk was re-tested following the same procedures outlined above using a different snail.

The data files collected during each trial were analyzed with the program NIH Image J 1.38I (Rasband 2007a), running Manual Tracking (Cordelie`res 2005) and Quicktime Capture (Rasband 2007b) software as described in Myrick (2009). Mean and maximum crawling distances were used as the primary measure of each substrate’s effectiveness. In addition, mean velocity and duration of activity on each surface were also quantified. Equation 1 shows the formula used to calculate crawling distance during video analysis.

$$Distance\ =\ (((X2-X1)2+((Y2-Y1)2))0.5 \qquad (Equation\ 1)$$

Where:

X1 = Previous horizontal axis location
X2 = Current horizontal axis location

Y1 = Previous vertical axis location
Y2 = Current vertical axis location

Water velocity experiment

The second experiment focused on the effect of water velocity on the NZMS response to copper sheet and copper mesh surfaces. A third surface treatment, bare PVC, was also tested to serve as a baseline reference. Trials were conducted in Loligo Model 90 swimming flumes at 0, 9, and 33 cm/s (as measured 1.5 cm above the substrate using a Marsh-McBirney 2-D velocity meter). At the beginning of a trial, a single NZMS was placed at the downstream end of a 30 × 12 cm treatment surface and the flume was started after the snail had attached to the substrate. The locomotor activities (mean and maximum crawling distance, velocity) of the NZMS were recorded using the same methods described in the first experiment. The trial continued until the snail was either dislodged from the substrate or two hours had elapsed. Each velocity × substrate combination was replicated 8 times. In this and the previous experiment, snails were euthanized and preserved after the completion of the trials by prolonged freezing.

Data analyses

One-way analyses of variance (ANOVA) were used to compare mean crawling distance on the treated versus untreated (control) sides of the test arenas in the fouling experiment.

Two-way ANOVA tests followed by Tukey HSD *post-hoc* tests were performed to identify differences in locomotor activities and interactions among the surface treatments within both experiments. Finally, a three-way ANOVA (surface × exposure period × cleaning treatment) was conducted using the Week 6 and Week 10 data from the fouling experiment to determine if cleaning significantly altered mean crawling distance (MCD) in each surface treatment.

All data were analyzed at $\alpha = 0.05$ significance level using JMP 8.0 statistical software (SAS 2008). Histograms, Shapiro-Wilk W-tests, and normal quantile plots were used to check assumptions of normality and distribution of errors. Data sets that were not normally distributed were log-normalized and reassessed. Assumptions of normality were met following log-transformation.

Results

Fouling experiment

Carapace length of the NZMS ranged from 3.7 to 5.9 mm with a mean (± SE) of 4.8 ± 0.03 mm with no significant differences detected between the various treatment groups ($p < 0.05$). The concentration of total dissolved copper (TDCu) measured at the end of the trials ranged from 5.8 to 7.6 ppb but did not exceed the EPA's hardness-adjusted criteria for our water supply of 8.9 ppb.

Mean crawling distance was consistently lower on the two solid copper surfaces compared to the AP treatment ($p < 0.0001$) (Figure 3 panel A, Table 1). On the AP surface, MCD was significantly greater ($p < 0.05$) after 6 weeks of exposure to fouling relative to either 0 or 10 weeks; fouling did not affect MCD on either SC or MC. Maximum observed crawling distance (CD_{max}) was consistently lower on the solid copper surfaces compared to the AP treatment. Finally, though MCD tended to be greater on the control side of the arenas, statistical differences between the two sides of the arenas were inconsistent (Appendix D, Table 2).

Overall, accumulation of materials after 6 weeks of exposure was significantly greater ($p < 0.0001$) than those values recorded after 10 weeks. Between the three surface treatments, MC accumulated significantly more surface matter ($p < 0.0001$) (Table 2) relative to the others. Finally, cleaning did not significantly alter MCD of the NZMS on any of the treatment surfaces ($p = 0.831$), though a decrease in this variable was noted on the AP treatment following cleaning.

Water velocity experiment

Carapace length ranged from 4.1 to 6.0 mm with a mean (± SE) of 5.1 ± 0.03 mm with no significant differences ($p < 0.05$) detected between the various surface × velocity treatment groups. The maximum concentration of TDCu measured at the end of the trials was 8.1 ppb in the non-static treatments; TDCu measured in the static treatments ranged from 21.4 to 25.3 ppb.

On the control surface, no difference in MCD was detected between the three velocity treatments (Figure 4 panel B, Table 1) though a negative correlation was noted. On both copper treated surfaces, MCD was significantly lower in the static treatments ($p < 0.05$) versus the non-static treatments. In the non-static treatments, MCD on the copper mesh was significantly lower relative to the control surface ($p < 0.05$); no difference in MCD was detected between copper sheet and control. In the flowing water treatments, CD_{max} was 42-86% lower on the treated surfaces compared to control. Finally, only a small percentage of NZMS (21%) were dislodged during the trials (Table 3) with a greater proportion of dislodgment observed on the copper surfaces in the 33 cm/s velocity treatment.

Discussion

In the surface fouling experiment, SC and MC were the most effective surface treatments in limiting locomotor activity of the NZMS regardless of exposure duration. Mean crawling distance was up to 14 times greater on the anti-fouling paint treatment compared to either solid copper surface while CD_{max} ranged from 32 to 96% lower on the solid copper treatments. Mean crawling velocity and the duration of activity also followed a similar pattern (for exact values, please see Appendix D, Table 1). In the velocity experiment, the overall lowest MCD and CD_{max} values were observed in the static water treatments on the copper treated surfaces. Relative to control, the copper mesh surface was more effective than copper sheet in limiting MCD under non-static conditions. Finally, there was no evidence from this experiment to suggest that water velocity alone, in the range tested, could serve as an effective deterrent against NZMS.

Copper is absorbed through the epidermis of terrestrial gastropods (Bullock et al. 1992; Ryder and Bowen 1977) and an analogous response appears to be present in the NZMS. The acute reactions (in terms limited locomotor activity) to a comparatively low concentration of TDCu in a short time-span (i.e., two hours) suggests that the effects observed in this experiment stemmed at least partially from *contact* exposure to the copper surface treatments. Chronic effects to benthic invertebrates caused by aqueous copper are typically reported at concentration levels above those levels measured in this experiment and are usually observed after an extended exposure period (i.e. 96 hours). For example, Watton and Hawkes (1984) examined the chronic response of NZMS to copper and reported 48 and 96 h EC_{50} values ranging from 48 to 89 ppb. Furthermore, except in the static treatments of the water velocity experiment, levels of TDCu measured at the conclusion of the 2-h trials did not exceed the EPA water hardness adjusted criteria.

While an examination of the AP surfaces found no outward signs of wear, the deterrent efficiency of this surface treatment decreased significantly after only six-weeks of exposure to fouling. Dry-weight analysis found that only a small amount of fouling matter had accumulated on the AP treatments, suggesting that the effectiveness of this material may be significantly compromised by buildup. Accumulation decreased between week 6 and week 10 on all surfaces treatments (presumably as the result of high-flow events between the two time periods) and may have caused the reduction in both MCD and CD_{max} observed on the AP surface treatment. Fouling did not affect MCD and CD_{max} on either solid copper surface, suggesting these materials would provide a robust NZMS deterrent even in situations where the accumulation of fouling matter is a concern.

Only small amounts (< 0.1 g/cm^2) of fouling accumulation were recorded on each of the three surface treatments. No effort was taken to quantitatively identify the composition of the accumulated matter, though inorganic sediments and small particles of vascularized plant material were commonly observed. Algal activity did not appear to be present which was expected given copper's strong biocidal properties (Borkow and Gabbay 2005). Fouling matter dry-weight was significantly greater on the MCU surface compared to the other two surfaces and was likely due to inorganic particle impingement and entrainment in the spaces between the wire mesh. However, MCD by the NZMS on the fouled mesh surfaces were significantly different from the baseline levels indicating that accumulation did not hinder the deterrent ability of this material.

The NZMS lack of a strong avoidance to copper differs from the responses of terrestrial slugs and snails observed in other studies (i.e., Schüder 2003; Schüder 2004). Mean crawling distance did not differ significantly on the copper treated versus untreated (control) sides of the test arenas in the majority of the treatments as was reported for terrestrial gastropods. The NZMS were not repelled by initial contact with the copper treatments and readily crawled onto these surfaces, suggesting a slow response of the organism to the copper treatments.

Water velocity alone did not significantly affect locomotor activity of the NZMS. On the control surface, MCD and CD_{max} displayed a negative correlation to water velocity, however, complete cessation of movement or dislodgment was rarely observed. The surface characteristics of materials typical of effluent pipes may prevent velocity (or at least those velocities common to hatcheries) from serving as a deterrent. Though not directly measured in this experiment, the boundary layer (or viscous sub-layer) formed near the substrate/water interface may create an area of reduced turbulence for the NZMS. The extent of the boundary layer is predicted to be greater in instances involving smooth versus rough textured surfaces (Allan 1995). Since effluent pipes are typically constructed of smooth materials such as PVC or steel, it seems unlikely that velocity in the range tested could be used to deter NZMS without in some way first reducing the boundary layer possibly by incorporating a coarse or irregular substrate inside of the effluent pipe, by increasing water velocity, or by both.

On the copper treated surfaces, MCD under static conditions were considerably lower than those values recorded under flowing water conditions. This response is likely the result of difference in the levels of dissolved copper between the flowing and static treatments. Total dissolved copper was nearly 3 times higher in the static treatments and this may have significantly reduced the NZMS activity as is suggested by research that has linked elevated levels of dissolved heavy metals to reduced locomotor activity of aquatic invertebrates (Mogren and Trumble 2010). The results from these trials suggest copper strips could function as very effective NZMS deterrents under static conditions. However for this type of deterrent design to be completely effective, behaviors that allow the snails to avoid contact with the copper substrates (such as floating on aquatic macrophytes), must be accounted for.

Under non-static conditions, MCD on the MC surface was significantly lower relative to the control surface; there was no detectable difference in MCD between SC and control. Though the two copper surfaces are composed of the same material, the unique flow dynamics created by the texture of the copper mesh may have increased its effectiveness. The rough texture of the mesh surface is predicted to reduce boundary layer thickness and thereby possibly expose the snails to greater amounts of turbulent hydraulic forces. Several studies have noted that flow dynamics affect the behavior and locomotor activity of NZMS (Holomuzki and Biggs 1999; Holomuzki and Biggs 2000) possibly explaining why both MCD and dislodgement were greatest on the MC surface. Though speculative, it is also possible that the behavior of the snails was affected by dissolved aqueous copper that may have accumulated in the static areas created by the openings on the mesh surface.

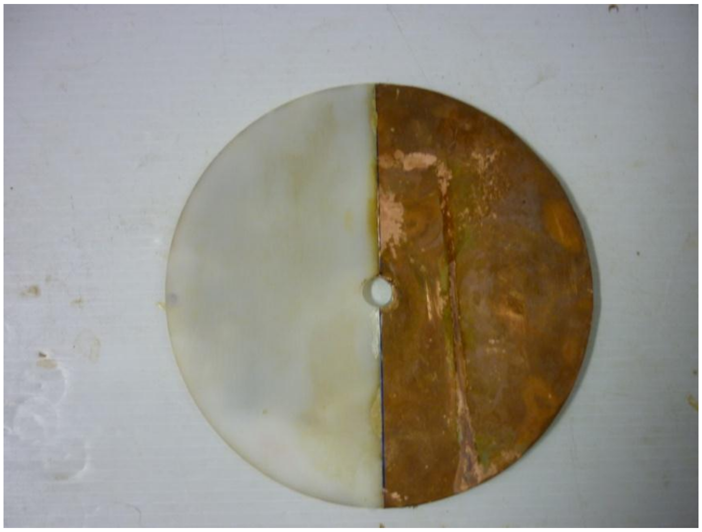
This project consisted of five separate experiments (please see Chapter 1), each testing a different physicochemical variable thought to influence the NZMS response to copper-based deterrents. Across these experiments, the absolute maximum crawling distance by a NZMS on either copper sheet or copper mesh was 1139 mm suggesting that a deterrent composed of either material would need to be a minimum of approximately 1.2 m in order to provide a satisfactory level of protection against invasion. Overall,

CD_{max} ranged from 28 to 1139 mm with less than 1% of snails exceeded 1000 mm during the 2 h trials. However, given the NZMS ability to reproduce parthenogenetically, its delayed response to copper substrates, and the relatively low cost of installing a deterrent longer than the bare minimum effective length, it is likely best to use a conservative estimate of effective deterrent length to achieve the desired level of protection. Designing copper sheet or copper mesh deterrents that are double the CD_{max} (i.e. approximately 2500 mm) should provide an adequate level of protection against invasion at a reasonable monetary cost. Retrofitting the inside of steel or PVC effluent pipe with a copper sleeve has been used by several hatcheries as a means of employing this deterrent design. Replacing a section of steel or PVC pipe with a length of copper pipe may also be feasible. Actual designs are likely to be unique to a particular facility based on the current design of their effluent system, water chemistry, and cost or logistic constraints. Regardless of design, the deterrents should be regularly monitored after their installation to check for the response of NZMS under the local physicochemical and biofouling conditions.

Multi-level protection could be achieved by coupling solid copper materials with other deterrent designs. Electrified collars affixed inside of effluent pipes have been shown to function as NZMS deterrents (R. Oplinger 2010. Utah DNR. pers. comm.). and by employing both methods, a hatchery could effectively guard against invasion even in circumstances where the power supply to the electrified collars was interrupted. Modifying the design of effluent discharges would also be effective. As Myrick and Conlin (In Press) noted, a “free-fall” deterrent created by raising the point of discharge out of the receiving water body would effectively eliminate the threat of invasion in most circumstances. However, a deterrent affixed inside of the effluent pipe would provide an additional level of cost-effective protection and would secure the pipe in instances where the water level of the receiving water body rose to the level of the effluent pipe such as during spring run-off.

Hatcheries, especially those in the western United States, face a constant threat of invasion by aquatic nuisance species, including the NZMS. Securing these facilities will help to insure the uninterrupted production of fishes used to stock public waterways while also helping to limit the spread of this organism. When implemented as part of a larger facility protection plan, contact deterrents constructed of solid copper materials, whether used alone or in conjunction with other designs, should provide a satisfactory level of protection against NZMS invasion across the range of physicochemical conditions typical of most hatcheries. Additional research focused specifically on determining the absolute maximum crawling distance capable of NZMS on either copper sheet or mesh would provide an additional level of assurance that facilities utilizing this style of deterrent are satisfactorily protected.

A.



B.

Figure 2. The effect of surface fouling on the New Zealand mudsnails (*Potamopyrgus antipodarum*) response to copper-based substrates was tested on 20.5 cm (dia.) PVC disks that had been exposed to 0, 6, or 10 weeks of fouling. One-half of the surface of each disk was treated with one of several copper-based surface treatments; the other half remained bare to serve as a control (panel A). Disks in the 6 and 10 week exposure treatments were affixed to steel rods 6 – 16 cm above the substrate of an irrigation canal (panel B) to allow for fouling to occur; disks in the 0 week group were not exposed to fouling and served as a baseline estimate of the mudsnails' response to each treatment surface. Each substrate x exposure combination was replicated 8 times. This experiment was conducted between June and August of 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

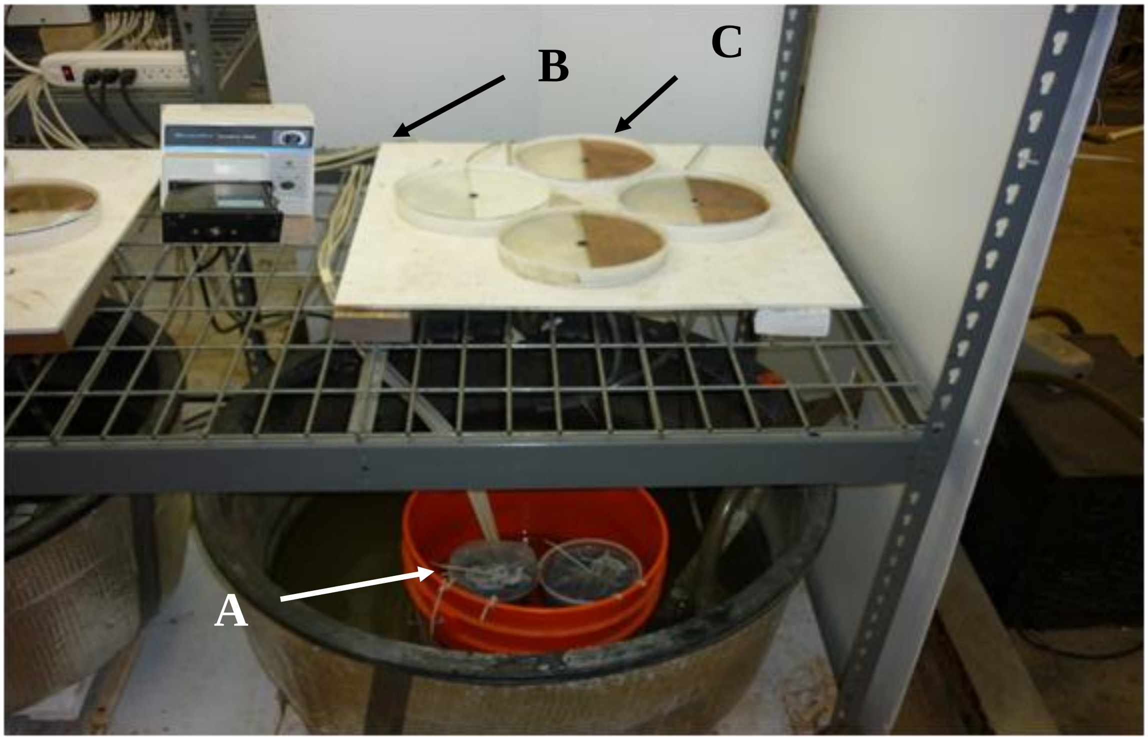


Figure 3. The equipment used to test the New Zealand mudsnails (*Potamopyrgus antipodarum*) response to three types of copper-based substrates at various levels of surface fouling. At the beginning of a trial, a single mudsnail was placed in the center of each arena and its movements were recorded by digital motion tracking system. During each trial, temperature controlled water (18° C) was drawn from the 19-L reservoir (A) at a rate of 5 ml/s by an 8-channel peristaltic pump (B) and circulated through four 21.5 cm diameter PVC arenas (C). Water exited the arenas through a center drain and was filtered through activated carbon filters before returning to the reservoir. Aqueous copper concentrations were held below regulatory standards through absorption of copper ions by the carbon filters and by partially replacing the reservoir water during the 2 h trials. Experiments were conducted June through August 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

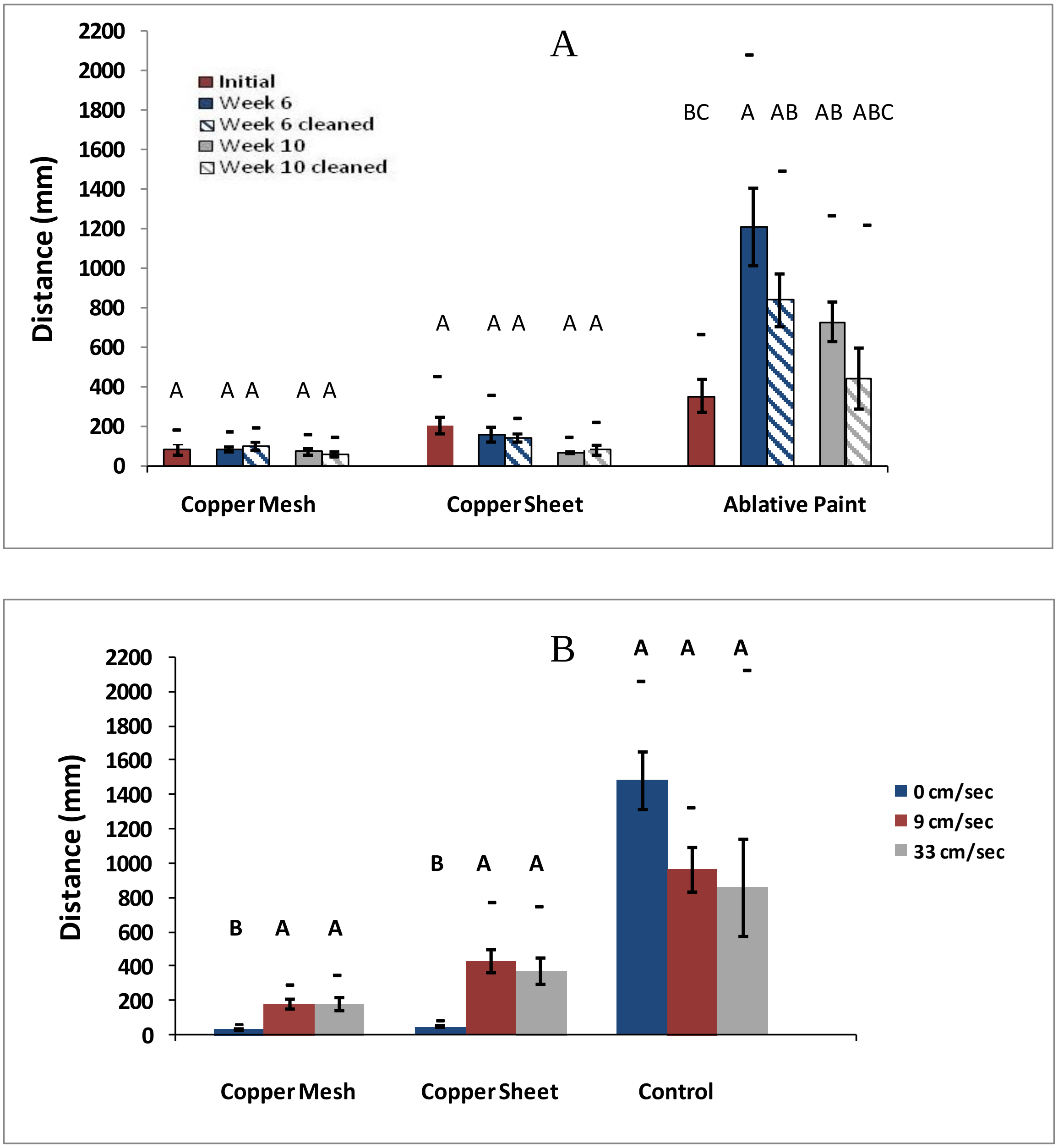


Figure 3. Mean crawling distance (plus standard error bars) of the New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to copper-based surface treatments at 3 durations of exposure to fouling (panel A) and at 3 water velocity levels (panel B). Within a treatment combination, bars not connected by the same letter are considered statistically different ($p < 0.05$). Maximum observed crawling distance within each treatment combination is expressed above each bar as a " –" symbol. Both experiments were conducted at a water temperature of 18° C. Each treatment combination was replicated 8 times and the two experiments were conducted from June through September 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

Table 2. Results of Tukey HSD analyses showing the statistical differences in crawling distances of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to copper-based substrates in two separate experiments. Mean values with standard errors are shown. Within an experiment, treatment combinations not connected by the same letter are considered statistically different ($p < 0.05$). Each experiment was conducted at a water temperature of 18° C and all treatment combinations were replicated 8 times. Both experiments were conducted between June and September 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

Fouling Experiment

Exposure Time (weeks)	Surface	Cleaning Treatment	Mean Carapace Length (mm)	Mean Crawling Distance (mm)					
6	Ablative Paint	Uncleaned	4.6 (0.11)	1207 (196.0)	A				
6	Ablative Paint	Cleaned	4.6 (0.13)	840 (135.9)	A	B			
10	Ablative Paint	Uncleaned	4.4 (0.18)	728 (103.3)	A	B			
10	Ablative Paint	Cleaned	4.2 (0.25)	444 (155.9)	A	B	C		
0	Ablative Paint	--	4.3 (0.12)	353 (82.8)		B	C	D	
0	Copper Sheet	--	3.8 (0.19)	204 (44.4)		B	C	D	E
6	Copper Sheet	Uncleaned	4.4 (0.16)	157 (37.5)			C	D	E
6	Copper Sheet	Cleaned	4.4 (0.08)	142 (22.5)			C	D	E
6	Copper Mesh	Cleaned	4.7 (0.06)	100 (24.4)			C	D	E
6	Copper Mesh	Uncleaned	4.9 (0.11)	81 (13.8)			C	D	E
0	Copper Mesh	--	4.0 (0.17)	83 (27.5)				D	E
10	Copper Sheet	Uncleaned	4.2 (0.27)	63 (6.4)				D	E
10	Copper Sheet	Cleaned	4.5 (0.21)	81 (25.8)					E
10	Copper Mesh	Uncleaned	4.4 (0.18)	70 (18.8)					E
10	Copper Mesh	Cleaned	4.2 (0.27)	57 (12.3)					E

Water Velocity Experiment

Velocity (cm/sec)	Surface	Mean Carapace Length (mm)	Mean Crawling Distance (mm)					
0	Control	5.1 (0.08)	1480 (164.5)	A				
9	Control	5.1 (0.08)	963 (129.8)	A	B			
33	Control	5.1 (0.11)	859 (282.5)	A	B	C		
9	Copper Sheet	5.2 (0.11)	425 (67.4)		B	C	D	
33	Copper Sheet	5.2 (0.13)	372 (79.8)			C	D	
9	Copper Mesh	5.1 (0.11)	180 (27.9)				D	
33	Copper Mesh	5.1 (0.05)	180 (38.9)				D	
0	Copper Sheet	4.9 (0.15)	48 (5.6)					E
0	Copper Mesh	5.2 (0.15)	34 (5.0)					E

Table 2. Mean dry-weight (± SE) of fouling accumulation sampled from three copper surfaces using standard collection and analysis procedures. Each surface × time combination was replicated 8 times. Fouling occurred over a ten week period from June through August 2010 in an irrigation canal located near the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Time	
	Week 6	**Week 10**
	Dry Weight (g)	**Dry Weight (g)**
Copper Mesh	0.030 (0.005)	0.021 (0.003)
Copper Sheet	0.013 (0.002)	0.004 (0.001)
Ablative Paint	0.011 (0.002)	0.005 (0.001)

Table 3. Percent and mean time of dislodgment (± SE) of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to two types of copper surface treatments (plus a bare PVC control surface) at two water velocity levels. Trials were conducted in Model 90 Loligo swimming flumes at a water temperature of 18° C. Each surface × velocity combination was replicated 8 times. This experiment took place in September 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Velocity			
	9 cm/sec		**33 cm/sec**	
	% Dislodged	Mean Time of Dislodgement (min)	% Dislodged	Mean Time of Dislodgement (min)
Control	0	--	25	42 (22.8)
Copper Mesh	12.5	77	50	51 (14.1)
Copper Sheet	0	--	37.5	68 (15.8)

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Appendices

Appendix A

Table 1. Mean crawling velocity and duration of time spent actively crawling by the New Zealand mudsnail (*Potamopyrgus antipodarum)* on various types of copper-based substrates at four water temperatures. Mean values are shown with standard errors. Each surface × water temperature combination was replicated 12 times. All trials were 2 hrs. in length and were conducted in January 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Temperature							
	8° C		**12° C**		**18° C**		**24° C**	
	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)
Copper Mesh	0.13 (0.006)	47 (5.7)	0.17 (0.011)	29 (3.3)	0.20 (0.014)	22 (3.1)	0.21 (0.011)	16 (2.5)
Copper Sheet	0.13 (0.010)	27 (4.9)	0.16 (0.007)	24 (3.8)	0.23 (0.021)	17 (3.1	0.20 (0.011)	18 (2.3)
Ablative Paint	0.15 (0.009)	35 (7.5)	0.24 (0.012)	36 (4.8)	0.31 (0.019)	33 (6.1)	0.25 (0.018)	45 (4.8)
Non-Ablative Paint	0.21 (0.007)	57 (5.8)	0.28 (0.009)	59 (5.4)	0.37 (0.013)	48 (7.3)	0.30 (0.023)	61 (5.2)

Table 2. Mean crawling velocity and duration of time spent actively crawling by the New Zealand mudsnail (*Potamopyrgus antipodarum)* on various types of copper-based substrates at four water hardness levels. Mean values are shown with standard errors. Each surface × water hardness combination was replicated 12 times. All trials were 2 hrs. in length and were conducted in January 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Hardness (mg/L $CaCO_3$)							
	75 mg/L		**125 mg/L**		**175 mg/L**		**300 mg/L**	
	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)
Copper Mesh	0.29 (0.012)	26 (3.5)	0.22 (0.015)	34 (4.0)	0.23 (0.011)	24 (4.3)	0.17 (0.008)	27 (4.0)
Copper Sheet	0.29 (0.020)	16 (2.5)	0.24 (0.011)	38 (6.0)	0.24 (0.015)	19 (2.6)	0.18 (0.007)	12 (2.1)
Ablative Paint	0.29 (0.011)	34 (4.3)	0.29 (0.013)	43 (5.4)	0.25 (0.014)	49 (4.3)	0.32 (0.024)	34 (4.1)
Non-Ablative Paint	0.40 (0.018)	64 (4.9)	0.41 (0.012)	66 (3.6)	0.36 (0.015)	55 (4.1)	0.32 (0.020)	50 (7.1)

Appendix A (continued)

Table 3. Mean crawling velocity and duration of time spent actively crawling by the New Zealand mudsnail (*Potamopyrgus antipodarum*) on various types of copper-based substrates at four pH levels. Mean values are shown with standard errors. Each surface × pH combination was replicated 12 times. All trials were 2 hrs. in length and were conducted in January 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	pH					
	6		**7**		**8.5**	
	Mean Crawling Velocity (mm/sec)	Time Active (min)	Mean Crawling Velocity (mm/sec)	Time Active (min)	Mean Crawling Velocity (mm/sec)	Time Active (min)
Copper Mesh	0.08 (0.010)	3 (1.6)	0.11 (.007)	9 (1.9)	0.15 (0.011)	26 (4.3)
Copper Sheet	--	--	0.11 (0.011)	4 (1.6)	0.15 (0.013)	19 (3.0)
Ablative Paint	0.13 (0.018)	8 (2.4)	0.23 (0.022)	11 (1.7)	0.26 (0.020)	26 (5.7)
Non-Ablative Paint	0.09 (0.009)	10 (2.5)	0.23 (0.030)	24 (7.9)	0.23 (0.020)	46 (7.4)

Appendix B

Table 1. Percentage and mean time of immobilization (with standard error) of New Zealand mudsnails (*Potamopyrgus antipodarum*) that were exposed to various types of copper-based surface treatments at four water temperatures. Immobilization was defined as cessation of locomotor activity for > 30 min. following contact with the copper surface treatment. Each surface × water temperature combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in November 2009 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Temperature							
	8° C		12° C		18° C		24° C	
	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)
Copper Mesh	42	57 (9.9)	42	65 (6.0)	90	54 (5.7)	92	38 (6.3)
Copper Sheet	42	79 (2.6)	75	53 (7.1)	67	65 (6.9)	92	43 (6.6)
Ablative Paint	0	--	0	--	8	37 --	8	89 --
Non-Ablative Paint	0	--	0	--	0	--	0	--

Table 2. Percentage and mean time of immobilization (with standard error) of New Zealand mudsnails (*Potamopyrgus antipodarum*) that were exposed to various types of copper-based surface treatments at four water hardness levels. Immobilization was defined as cessation of locomotor activity for > 30 min. following contact with the copper surface treatment. Trials were conducted at 18° C and each surface × water hardness combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in January 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Water Hardness (mg/L $CaCO_3$)							
	75 mg/L		125 mg/L		175 mg/L		300 mg/L	
	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)	Percent Immobilized	Time of Immobilization (min)
Copper Mesh	42	53 (6.1)	100	44 (4.9)	83	56 (9.3)	75	44 (5.4)
Copper Sheet	67	56 (5.3)	75	47 (6.3)	50	47 (11.1)	92	29 (3.9)
Ablative Paint	0	--	0	--	16	62 (11.5)	0	0 --
Non-Ablative Paint	0	--	0	--	0	--	25	73 (7.5)

Appendix B (continued)

Table 3. Percentage and mean time of immobilization (with standard error) of New Zealand mudsnails (*Potamopyrgus antipodarum*) that were exposed to various types of copper-based surface treatments at three pH levels. Immobilization was defined as cessation of locomotor activity for > 30 min. following contact with the copper surface treatment. Trials were conducted at 18° C and each surface × water hardness combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in June 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	pH					
	6		7		8.5	
	Percent Immobilized	**Time of Immobilization (min)**	**Percent Immobilized**	**Time of Immobilization (min)**	**Percent Immobilized**	**Time of Immobilization (min)**
Copper Mesh	100	25 (3.9)	83	29 (4.1)	58	58 (8.3)
Copper Sheet	--	--	75	37 (5.6)	50	50 (9.1)
Ablative Paint	45	64 (7.5)	25	72 (16.8)	16	17 (9.5)
Non-Ablative Paint	90	37 (6.6)	25	72 (12.6)	8	8 --

Appendix C

Table 1. The percentage of New Zealand mudsnails (*Potamopyrgus antipodarum*) surviving after 24 hrs. following exposure to copper-based surface treatments at four water temperatures. Survival was assessed as the ability of the snail to close its operculum. Mean carapace length with standard error is also shown. Each surface treatment × temperature combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in November 2009 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Temperature							
	8° C		12° C		18° C		24° C	
	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival
Copper Mesh	4.5 (0.10)	100	4.5 (0.06)	100	4.3 (0.06)	100	4.4 (0.11)	92
Copper Sheet	4.3 (0.10)	100	4.4 (0.14)	100	4.5 (0.12)	100	4.1 (0.10)	92
Ablative Paint	4.4 (0.08)	100	4.2 (0.13)	100	4.2 (0.19)	100	4.2 (0.14)	100
Non-Ablative Paint	4.3 (0.17)	100	4.3 (0.14)	100	4.2 (0.14)	100	4.3 (0.19)	83

Table 2. The percentage of New Zealand mudsnails (*Potamopyrgus antipodarum*) surviving after 24 hrs. following exposure to copper-based surface treatments at four water hardness levels (expressed as mg/L $CaCO_3$). Survival was assessed as the ability of the snail to close its operculum. Mean carapace length with standard error is also shown. Each surface treatment *x* hardness combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in January 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Water Hardness							
	75 mg/L		125 mg/L		175 mg/L		300 mg/L	
	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival
Copper Mesh	4.3 (0.13)	100	4.4 (0.09)	100	4.3 (0.08)	100	4.4 (0.09)	83
Copper Sheet	4.5 (0.07)	100	4.4 (0.13)	100	4.4 (0.09)	100	4.5 (0.12)	92
Ablative Paint	4.5 (0.11)	100	4.4 (0.10)	100	4.5 (0.08)	100	4.5 (0.12)	92
Non-Ablative Paint	4.5 (0.13)	100	4.5 (0.11)	83	4.3 (0.09)	100	4.3 (0.06)	92

Appendix C (continued)

Table 3. The percentage of New Zealand mudsnails (*Potamopyrgus antipodarum*) surviving after 24 hrs. following exposure to copper-based surface treatments at three pH levels. Survival was assessed as the ability of the snail to close its operculum. Mean carapace length with standard error is also shown. Each surface treatment × pH combination was replicated 12 times. Trials were 2 hrs. in length and were conducted in June 2010 at the Colorado State University Foothills Fisheries Laboratory (Fort Collins, Colorado).

	pH					
	6		7		8.5	
	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival	Carapace Length (mm)	Percent Survival
Copper Mesh	4.6 (0.16)	67	4.5 (0.13)	92	4.7 (0.14)	100
Copper Sheet	4.4 (0.19)	75	4.6 (0.12)	92	4.5 (0.18)	100
Ablative Paint	4.4 (0.20)	67	4.0 (0.22)	100	4.2 (0.18)	100
Non-Ablative Paint	4.3 (0.20)	75	4.4 (0.13)	100	4.4 (0.17)	100

Appendix D

Table 1. Mean crawling velocity and duration of time spent actively crawling by the New Zealand mudsnail (*Potamopyrgus antipodarum*) on three types of copper-based substrates after various lengths of exposure to fouling. Mean values are shown with standard errors. Each surface × exposure duration combination was replicated 8 times. All trials were conducted at a water temperature of 18 C. and took place June through August 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

Time

	Initial		**Week 6**				**Week 10**			
	Mean Velocity (mm/sec)	Time Active (Min)	Mean Velocity (mm/sec)		Time Active (Min)		Mean Velocity (mm/sec)		Time Active (Min)	
			Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Copper Mesh	0.16 (0.014)	7.5 (2.8)	0.16 (0.020)	0.17 (0.028)	6.6 (1.9)	4.8 (1.9)	0.20 (0.037)	0.14 (0.009)	5.9 (1.7)	6.1 (1.1)
Copper Sheet	0.16 (0.013)	18.3 (4.9)	0.23 (0.024)	0.20 (0.014)	12.8 (3.4)	5.5 (2.0)	0.14 (0.009)	0.17 (0.028)	6.8 (1.3)	6.9 (2.1)
Ablative Paint	0.21 (0.023)	25.6 (6.0)	0.41 (0.024)	0.29 (0.013)	49.8 (7.2)	48.1 (8.3)	0.30 (0.030)	0.23 (0.032)	41.1 (5.1)	24.1 (6.0)

Table 4. Mean crawling distance (mm) plus standard error of New Zealand mudsnails (*Potamopyrgus antipodarum*) exposed to various copper-based surface treatments. Each material was exposed to three durations of surface fouling: 0, 6, and 10 weeks. Trials were conducted on 20.5 cm diameter PVC disks that were 50% covered with a copper surface treatment; the other half remained bare PVC to serve as a control. Asterisks' indicate control/treatment combinations found to vary significantly from one another ($p < 0.05$). Each copper surface × exposure duration combinations were replicated 8 times at a water temperature of 18° C. All trials were conducted from June through August 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

Time

	Initial		**Week 6**		**Week 10**	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Copper Mesh	471 (223)	83 (27)	774 (207)*	82 (14)	218 (76)	70 (19)
Copper Sheet	214 (124)	204 (44)	905 (207)*	157 (37)	390 (142)	64 (6)
Ablative Paint	1074 (120)*	353 (83)	1130 (194)	1207 (196)	687 (111)	729 (103)

Appendix E

Table 1. Mean crawling velocity and duration of time spent actively crawling by the New Zealand mudsnail (*Potamopyrgus antipodarum*) on three substrates at three water velocity levels. Mean values are shown with standard errors. Each surface × velocity combination was replicated 8 times. All trials were conducted at a water temperature of 18° C in Model 90 Loligo swimming flumes. This experiment was conducted from June through August 2010 at the Colorado State Foothills Fisheries Laboratory (Fort Collins, Colorado).

	Velocity					
	0 cm/sec		**9 cm/sec**		**33 cm/sec**	
	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)	Mean Crawling Velocity (mm/sec)	Time Active (Min)
Control	0.32 (0.031)	77 (6.3)	0.23 (0.010)	73 (7.4)	0.13 (0.018)	56 (11.2)
Copper Sheet	0.10 (0.008)	11 (0.7)	0.11 (0.009)	66 (8.9)	0.10 (0.009)	57 (8.4)
Copper Mesh	0.07 (0.003)	7 (0.3)	0.12 (0.007)	26 (4.3)	0.10 (0.007)	31 (6.4)