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Impact of copper mine tailings (stamp sand) on survival and development of aquatic organisms near Gay, Michigan

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IMPACT OF COPPER MINE TAILINGS (STAMP SAND) ON SURVIVAL AND DEVELOPMENT OF AQUATIC ORGANISMS NEAR GAY, MICHIGAN

By

Danielle M. Haak

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

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This thesis, “Impact of Copper Mine Tailings (Stamp Sand) on Survival and Development of Aquatic Organisms near Gay, Michigan,” is hereby approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN BIOLOGICAL SCIENCES.

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Abstract

Heavy metal-rich copper mine tailings, called stamp sands, were dumped by mining companies directly into streams and along the Lake Superior shoreline, degrading Keweenaw Peninsula waterways. One of the largest disposal sites is near Gay, Michigan, where tailings have been moved along the shoreline by currents since mining ceased. As a result, the smallest sand particles have been washed into deeper water and are filling the interstitial spaces of Buffalo Reef, a critical lake trout spawning site. This research is the first to investigate if stamp sand is detrimental to survival and early development of eggs and larvae of lake sturgeon, lake trout, and Northern leopard frogs, and also examines if the presence of stamp sands influences substrate selection of earthworms. This study found that stamp sand had significantly larger mean particle sizes and irregular shapes compared to natural sand, and earthworms show a strong preference for natural substrate over any combination that included stamp sand. Additionally, copper analysis (Cu^{2+}) of surface water over stamp sand and natural sand showed concentrations were significantly higher in stamp sand surface water (100 µg/L) compared to natural sand surface water (10 µg/L). Frog embryos had similar hatch success over both types of sand, but tadpoles reared over natural sand grew faster and had higher survival rates. Eggs of lake sturgeon showed similar hatch success and development over natural vs. stamp sand over 17 days, while lake trout eggs hatched earlier and developed faster when incubated over stamp sand, yet showed similar development over a 163 day period. Copper from stamp sand appears to impact amphibians more than fish species in this study. These results will help

determine what impact stamp sand has on organisms found throughout the Keweenaw Peninsula which encounter the material at some point in their life history.

Introduction

Copper mining was a thriving industry in Upper Michigan's Keweenaw Peninsula for almost 150 years (Rasmussen et al. 2002). During this time, approximately 5 billion kilograms of refined native (elemental) copper was extracted from beneath the Keweenaw (Kerfoot et al. 1994; Rasmussen et al. 2002). Copper mining in the region peaked between 1900 and 1930 (Babcock and Spiroff 1970), and this production generated approximately 500 million tons of mine tailings, known as stamp sands, that were then dumped in waterways throughout the Keweenaw and along the shoreline of Lake Superior (Jeong et al. 1999). The Wolverine and Mohawk companies focused on the Keweenaw Greenstone Belt deposit, transferring their ore to the Gay, Michigan stamp mills for processing (Kerfoot et al. 1994). Stamp mills, such as the ones at Gay, were used to crush ore into smaller pieces, and the addition of water allowed the copper to be more easily extracted. The remaining waste rock was then deposited via conveyor belt to the Lake Superior shoreline (Rasmussen et al. 2002) and has been constantly reworked by wind and water energy over time. It is estimated that the Gay shoreline received 25 billion kilograms of stamp sand before use of the stamp mill ceased in 1933 (Rasmussen et al. 2002).

This stamp sand contains elevated concentrations of trace metals and is physically and chemically different than natural Lake Superior sediments (Kerfoot et al. 1994). Stamp sand is composed of irregular shaped particles of varying sizes, while natural sand is more homogeneous in size and shape (Kerfoot et al. 1994). Of specific interest to this study are the elevated concentrations of heavy metals found in stamp sand, including copper, cadmium, chromium, arsenic, and lead (Jeong 1997). However, there have been conflicting reports (Lopez Diaz 1973; Leddy 1973; Malueg et al. 1984; Helmer and Beltman 1990) on the impact stamp sands have on fish and other aquatic organisms.

To formulate hypotheses about the effects stamp sand may have on aquatic organisms, I looked to a number of studies examining toxicity in fish and amphibians. Numerous studies (McKim et al. 1978; Nelson et al. 1986; Carreau and Pyle 2005; Linbo et al. 2006; McIntyre et al. 2008; Garcia-Munoz et al. 2009; Craig et al. 2010; Green et al. 2010) have investigated copper toxicity in different fish species and found that embryos are less susceptible to adverse effects from copper than exposed larval fish and copper toxicity results in a number of physiological responses. Though copper is a necessary micronutrient for most organisms, toxic levels of copper can cause oxidative damage as well as disrupted ionregulation and endocrine processes (Craig et al. 2010). Additionally, fish gills are sensitive to heavy metals, and copper concentrations greater than 5 μg/L can reduce olfactory sensitivity and impair behavioral responses in individuals (Green et al. 2010). Increased copper concentrations can disrupt osmoregulation by inhibiting sodium uptake in the gills which can cause death at high exposure concentrations (McIntyre et al. 2008). Previous copper toxicity tests on lake trout (*Salvelinus namaycush*) show that early juveniles are more sensitive to copper than embryos, and lake trout were significantly reduced in numbers when copper reached 31-43 μg/L (McKim et al. 1978). Carreau and Pyle (2005) studied the effect of copper on chemosensory function of juvenile fathead minnows (*Pimephales promelas*) and concluded that fish exposed to copper during embryological development had long-term effects on their chemosensory function; however, fish exposed later in life showed short-term effects but eventually recovered. Fish exposed to copper failed to respond to alarm cues that would typically suggest the presence of a predator.

An additional study (Linbo et al. 2006) on larval zebrafish (*Danio rerio*) tested pulses of copper exposure on fish. Each exposure lasted less than 12 hours and fish were then given a chance to recover. The study concluded that cell death occurs in the olfactory epithelium after exposure for four hours at a concentration higher than 25 ppb. At concentrations lower than 20 ppb, copper diminishes neurophysiological responsiveness of a fish's sensory neurons to odorants found in the surrounding environment. This means that the olfactory system of a fish is impaired at lower copper concentrations than those that cause cell death. Additionally, lateral line regeneration occurred after acute exposure to copper, but not after chronic exposure.

While copper has two oxidation states, Cu^{1+} and Cu^{2+}, Cu^{2+} is the most toxic form to aquatic life (Garcia-Munoz et al. 2009), and toxicity varies with other water quality parameters, such as pH, hardness, and suspended clays (Nelson et al. 1986). For example, the dominant copper species changes from Cu^{2+} to $CuCO_3$ and $Cu(OH)_2$ when the surrounding water pH level rises to 8 (Tao et al. 2002), and copper bioavailability decreases as water hardness values increase (Green et al. 2010). Finally, suspended clays provide ideal bonding sites for free copper ions, so an increase in total suspended solids decreases the concentration of bioavailable copper in a system (Nelson et al. 1986). At a water hardness of 45 mg/L, the chronic copper LC_{50} for lake trout is between 22.0-43.5 µg/L (McKim et al. 1978). In this study, juvenile lake trout were exposed to copper for 66 days post-hatch with water hardness between 44-50 ppm $CaCO_3$. As water hardness increases, calcium begins competing with copper for binding sites on the fish, decreasing the impact of excess copper (Buchwalter et al. 1996). In a similar study using juvenile coho salmon (*Oncorhynchus kisutch*), detrimental effects on the olfactory system were partially reversed by increasing organic matter to the system. This is because copper has a higher affinity for organic matter than for the biotic ligand found in a fish gill (McIntyre et al. 2008).

Some stamp sand remediation has been carried out in the Keweenaw Peninsula. Torch Lake, Michigan was listed as a Superfund site due to the large volume of stamp sand deposited both in the lake and along the shoreline. Lopez Diaz (1973) concluded that fish and aquatic plants were able to successfully live in Torch Lake, despite the high copper concentrations. However, Leddy (1973) found copper concentrations to be highly toxic to the aquatic life in Torch Lake. Later, studies on zooplankter *Daphnia magna* and invertebrate *Hexagenia limbata* showed that the mine tailing sediments were highly toxic to both species (Malueg et al. 1984).

As previously mentioned, many fine particles were included in the stamp sand deposits. These fine particles are especially susceptible to being transported by wind and waves. The Keweenaw current flows around the Peninsula and has been slowly moving the stamp sand down the shoreline, farther and farther from the original site, depositing the particles both along shore and onto offshore reefs used as spawning sites by Great Lakes fishes (Kerfoot et al. 1994). Lake trout typically spawn in late October through mid-November on large cobble containing interstitial spaces (Marsden and Krueger 1991). Cobble is defined as 26-100 cm in diameter, and ideal interstitial depth is between

10-50 cm (Kelso et al. 1995, Edsall and Kennedy 1995, Corradin and Hansen 2008). Previous research has shown that as natural reefs become filled with silt, lake trout are forced to spawn in less desirable habitats (Sly 1988; Marsden et al. 1995). Egg survival is far less successful when eggs are deposited on other substrate types; for example, Marsden et al. (1995), found only 9% of eggs deposited on sandy substrates survived, compared with 38-61% of eggs on cobble.

In addition to dispersing into Lake Superior, the stamp sand beach at the Gay site is continuously reworked, causing the pile to shift. Ephemeral ponds form in the sand and are used by terrestrial organisms including frogs, birds, and snakes (Jarvie 2005). The Northern leopard frog (*Rana pipiens*) has specifically been observed using these ponds for reproductive purposes (Jarvie 2005). These ponds are mostly devoid of vegetation (personal observation) and are used by local biota, including frogs, birds, and reptiles (Jarvie 2005). Pond water is rich in organics and the water levels are higher than Lake Superior (Wang 1999). Previous research shows that *Daphnia* did not survive longer than 11 days in the stamp sand ponds (Wang 1999).

Similarly to copper toxicity to fish, there are conflicting reports regarding copper toxicity to amphibian embryos and tadpoles (Eisler 1985; Herkovits and Helguero 1998; McDaniel et al. 2004; Chen et al. 2007; Stolyar et al. 2008) emphasizing the need for further research, particularly on tadpole development. Amphibians are an excellent indicator species, their disappearance often signaling an ecosystem is in peril, and they are more susceptible to environmental contamination than fish due to their porous skin (Bridges et al. 2002). During the embryo and larval stage, they are aquatic before metamorphosing to a terrestrial adult phase (Garcia-Munoz et al. 2009). During this aquatic phase, they are more susceptible to toxic substances that can absorb through the epithelium, making them excellent research species (Vitt et al. 1990). Responses to these toxic substances can be either sublethal or lethal. Sublethal effects include decreased growth and development, a delay in metamorphosis, and even behavioral changes (Garcia-Munoz et al. 2009).

A previous chronic copper exposure study on *Rana pipiens* (Chen et al. 2007) found that during developmental stages GS19-GS25 (Gosner 1960) survival was high across all concentrations. (The Gosner (GS) scale assigns numbers to the developmental stages of frogs.) However, survival dropped after 60 days, especially at the highest copper concentrations (100 ppb). The authors speculated that short-term studies may not sufficiently address the effects copper exposure has on tadpoles. Additionally, decreased growth rates were found in tadpoles exposed to copper when compared to the control groups. Finally, Chen et al. (2007) found that a smaller percentage of exposed tadpoles made it to metamorphosis than their control counterparts and those that did had higher occurrence of malformations.

While Chen et al. (2007) found that tadpoles exposed to copper took longer to reach metamorphosis, McDaniel et al. (2004) concluded that copper exposure caused metamorphosis to occur earlier than the Northern leopard tadpoles in water with normal environmental copper concentrations (approximately 5 ppb). Additionally, exposed tadpoles reached metamorphosis at approximately the same time, while the control group's timing was spread out over a longer window, independent of temperature variations (McDaniel et al. 2004). A similar study of leopard frog embryo incubation over contaminated sediment (Gillan et al. 1998) discovered that exposure to heavy metals caused cytotoxicity (cell death) of certain cell lines. Many of the mechanisms described for fish apply to the aquatic phase of amphibians as well, as tadpoles rely on gills to breathe during this life stage (Buchwalter et al. 1996). Using these prior research results, we formed our objectives and hypotheses for our study.

Objectives and Hypotheses

The objectives of this study were to 1) determine how stamp sand and natural Lake Superior sand vary physically and chemically, including measuring some leached metal concentrations in surface waters, 2) assess the effect stamp sand has on fish and frog embryo and larvae survival and development, and 3) determine if structure of stamp sand and natural sand influences substrate selection of earthworms.

The stamp sands found in the Gay/Keweenaw area are known to increase copper concentrations in surrounding surface waters (Jeong 1997), and metals such as Cu^{2+} are known to impact development of embryos and larvae of both frogs and fish. This research compares the survival and early development of lake sturgeon (*Acipenser fulvescens*), lake trout (*Salvelinus namaycush*), and Northern leopard frog (*Rana pipiens*) eggs and young by laboratory rearing on both stamp sand and natural Lake Superior sand. Particularly, I am investigating the effects copper has on these populations. Additionally, earthworms (nightcrawler *Lumbricus terrestris*) are used to test organism preference between stamp sand and natural sand. I hypothesize that copper concentrations will be higher in the surface waters above stamp sand than in the surface waters above natural sand. Additionally, I hypothesize that both the fish and amphibian embryo survival will decrease when incubated over stamp sand, possibly due to elevated heavy metal concentrations, and that larval development will be slower or stunted with possible larval developmental malformations when compared to those on natural sand. Finally, I hypothesize that, given a choice, earthworms will choose substrate with natural sand rather than stamp sand due to the smooth shape and size of natural sand and the possible chemistry of stamp sand.

Experimental Methods

Study Site

Gay, Michigan (47° 13′ 39″ N, 88° 9′ 49″ W) is located on the eastern shore of the Keweenaw Peninsula of Upper Michigan, along Lake Superior. Stamp sand was collected from both the Gay beach near the town and from an area known as Coal Dock (Figure 1). Stamp sand was collected from Lake Superior at the Coal Dock site. Natural sand was collected from Bete Gris beach (47° 22′ 27″ N, 87° 58′ 0″ W), near Lac La Belle, Michigan (Figure 1). Uncontaminated natural sand substrate was collected from Bete Gris, located northeast of Gay along Lake Superior.

Substrate Collection

On April 17, 2010, approximately 930 cm^3 of stamp sand substrate was collected in 0.5 m of water using a plastic cylinder (13 cm diameter), open on both ends to reduce mixing and retain as much pore water as possible. Combined water and sand was transported to a larger, clear cylinder (18 cm diameter). Three cylinders were filled in this manner with washed stamp sand from Coal Dock, and three cylinders were filled with natural sand from Bete Gris. Washed stamp sand refers to stamp sand that was collected from Lake Superior, thus have been "washed" by wave action, removing many of the fine particles. Unwashed stamp sand from higher on the beach was also collected to mimic the original deposits which included fine particles. Unwashed stamp sand refers to sand collected from the beach that has not been washed by wave action. A 0.3 m hole was dug and the same 13 cm cylinders were used to collect approximately 1300 cm^3 of sand. Lake Superior water was added and three additional cylinders were filled in this manner. Upon arrival in the lab, all 9 cylinders were placed in a Conviron incubator and kept at 12°C, with continuous aeration. Cylinders sat in the incubator for 12 days before lake sturgeon eggs were added on May 4, 2010. Additional dry, unwashed stamp sand for the lake trout, frog, and earthworms experiments was collected near the town of Gay's beachfront park.

Water Sample Analysis

Initial dissolved copper and mercury concentrations were analyzed by White Water Associates, Inc. (Amasa, MI) using surface water from the lake sturgeon embryo incubation experiment. Water was filtered through 0.45µm filters before being sent for analysis.

In an experiment using lake trout, additional dissolved metal concentrations (Cd, Cr, As, Pb, and Cu) were determined through the use of Perkin Elmer Optima 7000DV Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) through the Forestry Analytical Laboratory at Michigan Technological University. Three samples were taken from each of the two lake trout Frigid Units and from the three prepared $CuSO_4$ stock solutions (5, 25, and 100 µg/L) used as control. Samples were filtered through 0.45µm filters and preserved with HNO_3 until analysis. An ICP-OES heavy metal standard was acquired through Inorganic Ventures, Inc. (Christiansburg, VA).

Substrate Particle Size Comparison

U.S. standard sieves were used to separate particle sizes of each type of sand substrate. Three 1000 gram samples of either natural sand, dry/unwashed stamp sand, and submerged/washed stamp sand were sieved through mesh sizes of 0.063 mm, 0.125 mm, 0.250 mm, 0.500 mm, and 2.00 mm. Microscope photography and SigmaScan Pro were used to assess structural differences of particles by calculating perimeter and shape factor (a shape factor = 1 indicates a perfect circle) by photographing the substrate at 100x magnification and uploading the image into the SigmaScan Pro software. After calibration, outlining each individual particle produced an output with both shape factor and perimeter, measuring to the nearest 0.01 mm.

Earthworm Substrate Selection

Using 15 gallon glass aquaria, soil and sand combinations were mixed and placed into each of three compartments, divided by clear Plexiglas partitions. Three initial tanks were set up. All contained three compartments with one of the following substrate mixtures: 50% potting soil/50% natural sand, 50% potting soil/25% stamp sand/25% natural sand, and 50% potting soil/50% stamp sand. The order of compartments was mixed in each of the three tanks to avoid influence by outside laboratory stimuli and to test different combinations (See Appendix A). All possible combinations were used during the experiment. Two earthworms were added to the center of each compartment (six earthworms per tank), and approximately 0.5 L of water was added until the soil moisture level was two in each compartment (General Model No. GLMM200). Finally, each tank was covered with Plexiglas and left for 72 hours. At the end of the trial period, the number of worms in each compartment was counted and recorded. Earthworms were removed and not used more than once in selection trials. Each of the three tank arrangements was tested using five different sets of worms (30 worms each).

Frog Experiment

Approximately 600 Northern leopard frog embryos were ordered from Carolina Biological Supply (Burlington, NC). Upon arrival in the lab on March 24, 2011, eggs were pooled and sorted into 150mL Petri dishes containing various substrate types and solutions. Three replicates were used for both the controls (lake water) and each of seven experimental trials, which included 100 mL of lake water and 50 g of either stamp sand, natural sand, autoclaved stamp sand, autoclaved natural sand, and 100 mL of either 5µg/L $CuSO_4$ solution, 25µg/L $CuSO_4$, and 100 µg/L $CuSO_4$ with no substrate, for a total of 24 dishes. Autoclaved sand was used to eliminate any life organisms present in the samples. Lake water was pumped in to the laboratory from the Portage Canal and is transported through copper-free piping. Twenty-five embryos were added to each container, and water was replaced daily to ensure adequate dissolved oxygen.

On April 4, 2011, after hatching was complete, tadpoles were moved into larger containers with aerators. A 50% water exchange occurred every other day, and tadpoles were fed goldfish and rabbit food (Chen et al. 2007) until completion of the experiment.

Dissolved oxygen (Table 1) and temperature were monitored to ensure adequate conditions for tadpole growth. Dead tadpoles were removed daily and preserved in 10% Formalin/Borat solution. At the end of the experiment (May 25, 2011) tadpoles were euthanized before total length was measured and each was assessed for malformations. Microscope photography was used to determine development among experimental groups.

Lake Sturgeon Experiments

The first fish experiments were conducted using lake sturgeon embryos. I had access to lake sturgeon gametes and was able to streamline my experimental design before rearing lake trout embryos, which require a longer incubation period for development. Lake sturgeon gametes were collected from the Sturgeon River (Houghton County, MI) on May 3, 2010. The spawning population in the Sturgeon River was utilized for gamete collection for the Ontonagon Streamside Rearing Facility in spring 2010. Gametes were immediately transported to the Ontonagon Streamside Rearing Facility following collection for fertilization. Fertilized eggs (1:1 male:female ratio from 13 males and 3 females and then pooled together) were then transported to Michigan Technological University, and fifty eggs were placed in lake water in each of the nine Plexiglas cylinders described previously. Eggs were kept in the dark unless being checked and dead eggs were removed daily. During the first two days, new live eggs replaced the dead eggs to keep the egg count at fifty to account for unfertilized eggs or eggs damaged during transport.

Lake sturgeon eggs take between 8-10 days to hatch (Auer and Baker 2002). In this study, the number of dead eggs removed from each cylinder and development of fungus over the egg masses was recorded. At the time of total hatch, all organic material, including both live and dead fish, was preserved in a 10% formalin solution for future analysis, including total length (TL) measurements. The experiment was terminated May 21, 2010 due to the fungal infection in many of the cylinders.

Lake Trout Experiments

Lake trout gametes were collected in Big Traverse Bay, Lake Superior on the morning of November 1, 2010 by the Keweenaw Bay Indian Community (KBIC) Department of Natural Resources. The KBIC maintains a lake trout hatchery and eggs and milt for this project were obtained from this effort.

Lake trout eggs hatch in approximately 110 days at 4-6°C (Allen et al. 2005). Fertilized (1:1 male:female ratio from five individual pairings) and water hardened eggs were refrigerated in Lake Superior water for approximately 1 hour during transport. Upon arrival at MTU on November 1, 2010, all eggs were pooled together and randomly placed in one of two LS-700 Frigid Unit streams (530 L, 84"L x 24"W x 22"D), each with either unwashed Gay stamp sand or natural beach sand and held between 4-6°C. Each stream was divided into 3 compartments and 200 eggs were placed into each division, held in 53 μm net incubators. These divisions were labeled A, B, and C, with A located closest to the circulator, B in the middle, and C farthest from the circulator. Dead eggs were removed daily until hatching and an original volume of 0.5 m^3 water was circulated until levels evaporated to 0.4 m^3. At this time, more lake water was added, bringing the volume back to 0.5 m^3. We added new lake water three times over the study which was terminated on April 12, 2011, after 163 days. No carbon filtration was used in this experiment, but a foam filter was used to remove large particles. Flows through each tank averaged 0.05 m/s. Total hardness, pH, carbon dioxide, and dissolved oxygen were monitored weekly.

Once eggs hatched, floats were added to suspend each net incubator to prevent the fish from swimming into the tank. Initial total lengths were recorded using a caliper to the nearest millimeter for each hatched fish in both tanks. Final total length measurements were also taken from every dead fish removed from the stream units. Dead fish were preserved in a 10% formalin/Borat solution and stored for future analysis. All of the research involving frogs, lake sturgeon, and lake trout was approved by the MTU Office of Research Integrity and Compliance (Approval Number L0221).

Statistical Analysis

Hatch success values, substrate preference, and heavy metal concentrations were analyzed using single-factor ANOVA and the post-hoc Tukey test to identify any differences between the stamp sand and natural sand trials. Particle size was analyzed using a two-way t-test. Water quality parameters in each Frigid Unit during lake trout incubation were compared using a two-way T-test. Frog embryo hatch success and tadpole total length was compared using single-factor ANOVA and Tukey tests. An alpha value of 0.05 was used for all statistical analyses.

Results

Water Sample Analysis

Initial heavy metal concentration was analyzed on May 23, 2010 (after 34 days) using water from each Plexiglas cylinder used in the lake sturgeon experiment. No significant mercury concentration was detected in any of the samples, but copper was detected. Water from the washed stamp sand had a mean copper concentration of 62 µg/L, with a standard deviation of ±14.9 µg/L; the unwashed sand from the Gay beach had a mean copper concentration of 100 µg/L (±15.9); and water over the natural sand had a mean copper concentration of 10.3 µg/L (±0.6) (Figure 2). A single-factor ANOVA yielded a p-value < 0.001 (df=2), and a Tukey test confirmed each substrate's surface water copper concentration is significantly different from the other for the lake sturgeon trial.

A second heavy metal analysis was conducted following the lake trout experiment. Water from each Frigid Unit was filtered using a 0.45 µm filter and analyzed for copper, chromium, cadmium, arsenic, and lead. The water from the stream with stamp sand had a mean copper concentration of 19.0 µg/L (±0.2), and water from the natural sand stream had a mean copper concentration of 13.7 µg/L (±0.3) (Figure 3). No chromium, cadmium, arsenic, or lead was detected in this analysis.

In the lake sturgeon water, there was 500 g of substrate per 2 L of water; however, in the lake trout water, there was 3000 g of substrate per 530 L of water, meaning the lake trout water had approximately 44 times the volume of water than if we had used the same ratio found in the lake sturgeon water, so we would expect lower copper concentrations in the lake trout experiment.

Substrate Particle Size Comparison

Stamp sand particles had a mean perimeter (±1 SD) of 2.59 mm (±1.20) and a mean shape factor of 0.72 (±0.08) (Figure 4 (a)). A shape factor of 1 denotes a perfect circle. The maximum and minimum perimeters measured were 7.24 mm and 1.13, respectively. The maximum and minimum shape factors were 0.86 and 0.45, respectively (Figure 4b).

Natural sand particles had a mean perimeter of 0.88 mm (±0.22) and a mean shape factor of 0.79 (±0.06) (Figure 4 (a)). The maximum and minimum perimeters measured were 1.62 and 0.39, respectively. The maximum and minimum shape factors were 0.89 and 0.61, respectively (Figure 4 (b)).

A two-way t-test analysis of mean perimeter values yielded a p-value $\ll 0.05$, and a two-way t-test analysis of mean shape factor values yielded a p-value also $\ll 0.05$, showing that both particle sizes and roundness significantly varies between stamp and natural sands.

Particle sizes were also measured using U.S. Standard Sieves. For unwashed stamp sand, 72% were between 0.5 and 2 mm (size 35). For natural sand, 91% were between 0.25 and 0.5 mm (size 60) (Table 3). See Figure 5 for microscope pictures of stamp sand and natural sand.

Earthworm Substrate Selection Experiments

Five preliminary trials resulted in a strong preference for the natural sand-potting soil mixture over the other two substrates (Figure 6). A single-factor ANOVA produced a p-value of 0.0002, indicating that the earthworms display a clear preference. A Tukey test confirmed that the number of earthworms choosing the natural sand-potting soil mixture was significantly different than the number choosing either of the other two types. However, no significant difference was found between either mixture containing stamp sand.

Additional trials were conducted, marking each earthworm to track individual movements. The two types of marking employed, food coloring insertion and shallow sutures, were both unsuccessful. Worms often detached the marked parts of their bodies, regenerating the tail. These data, as well as an outline of each tank arrangement, is located in Appendix A.

Frog Experiment

The mean number of frog embryos hatched over eight different substrate types is outlined in Table 4. The highest mean number of embryos (24/25) hatched in the 5 µg/L copper solution, and the lowest mean number of embryos (19/25) hatched in the 100 µg/L copper solution. The mean number of hatched embryos in both the stamp sand and natural sand dishes was the same (22/25).

Total lengths of tadpoles were recorded (Table 5) at the end of the experiment on May 25, 2011 (or once all tadpoles in a container were dead, whichever occurred first).

The smallest tadpoles grew in lake water without substrate, with a mean total length of 14.5 mm (±2.1 SD). The largest tadpoles were found in the natural sand containers with lake water, and had a mean total length of 22.1 mm (±2.0). A single-factor ANOVA of the stamp sand, natural sand, autoclaved stamp sand, and autoclaved natural sand produced a p-value $<<0.05$ (dF=85, F=46). A Tukey test showed that the tadpole mean total lengths were significantly higher in the natural sand containers (Figure 7). Graphing the total lengths of the tadpoles in each of the copper sulfate solutions depicts that there was not a trend of decreasing size with increased copper. In fact, the total lengths increased as the copper concentration increased (Figure 8), and the final R-squared value was 0.10, meaning there was a very weak correlation among the copper sulfate solutions and total length of tadpoles.

Once hatched, the number of tadpoles that made it to day 51 of the study was tallied (Table 4). Tadpoles in the natural sand containers fared best; 59% of tadpoles survived to day 51. Tadpoles in the stamp sand containers as well as the 100 µg/L Cu water fared worst; the last individuals in each category died by day 51 of the study, May 25, 2011. The tadpoles in lake water died off gradually and reached their peak total length at the end of the study (Figure 9 (a)). The tadpoles in the 5µg/L copper sulfate solution had an initial die-off in the first 20 days of the experiment, and the growth and number of survivor curves cross at about day 59 (Figure 9 (b)). Tadpoles in the 25 µg/L solution had the highest survival rate among the tadpoles in copper sulfate solutions (Figure 9 (c)), and the tadpoles in 100 µg/L solution all died by the end of the study, but the final three individuals grew to about the same mean total length as the other categories' tadpoles (Figure 9 (d)).

While making total length measurements, developmental abnormalities were noted. The most common abnormality was a crooked backbone, though this was found in tadpoles from all experimental groups. Another common malformation involved the tadpoles' intestines. In healthy tadpoles, the intestines were neatly coiled in the abdomen; however, a small number of tadpoles in the control (9 tadpoles), 5 µg/L (5 tadpoles), and the stamp sand (3 tadpoles) groups had bulging, twisted, or external intestines. None of the tadpoles surviving to the end of the study had any of these abnormalities, nor did tadpoles from any other of the experimental groups.

Lake Sturgeon Experiments

Lake sturgeon eggs incubated between 10-17 days at 12^{o}C. An average of 36 fish (±6.1 SD) hatched per washed stamp sand cylinder. An average of 28 fish (±13.1) hatched in each unwashed stamp sand cylinder and an average of 25 fish (±18.2) hatched in each natural sand cylinder (Figure 10). A single-factor ANOVA (dF=9, F=0.45) yielded a p-value of 0.57.

A white fungus was observed growing on embryos in the natural sand cylinders after 8 days of incubation. A smaller amount of fungus began growing in one of the unwashed stamp sand cylinders on incubation day 9. No fungus appeared in the washed stamp sand containers.

The mean total length (±1 SD) for lake sturgeon at hatch from the washed stamp sand cylinders was 13.2 (±1.2) mm. This was slightly larger than the sturgeon from the unwashed stamp sand, which had a mean length of 12.7 (±0.23) mm; however, the natural sand lake sturgeon had the largest mean total length of 13.3 (±0.5) mm (Figure

11). A single-factor ANOVA yields a p-value of 0.63, denoting that no significant difference among or between the initial lengths of lake sturgeon incubated over each substrate type. There were no abnormalities observed in any of the lake sturgeon.

Lake Trout Experiments

The total hardness in each stream varied between 36-60 ppm $CaCO_3$ in the natural sand stream and between 54-64 ppm $CaCO_3$ in the stamp sand stream. The dissolved oxygen in both tanks was consistently between 9.0-11.0 mg/L, and the dissolved carbon dioxide was between 2-3 ppm during the entire study. The pH was 7.0 for both streams throughout the entire experiment. Surface water temperature was measured daily. The temperature in the natural sand stream varied and was moderately lower than the temperature in the stamp sand stream. The natural stream temperature averaged 4.9 ^{o}C (range 3.5-6.5 ^{o}C), while the stamp sand stream temperature averaged 5.9 ^{o}C (range 5-8 ^{o}C).

Lake trout embryos were placed in streams on November 1, 2010 and started hatching at day 86 of incubation, on January 25, 2011 (Figure 12). All of the embryos over stamp sand hatched within the first 7 days, while the embryos over natural sand took 21 days to complete hatching.

A total of 490 embryos hatched (out of 600) in the stamp sand stream, while 402 embryos hatched (out of 600) in the natural sand stream (Figure 13 (a)). A paired t-test yields a p-value of 0.06. Using the predetermined alpha value of 0.05, the number of hatched fish was not statistically significant. While the lake trout over stamp sand initially had higher hatch success, the total number of live fish began to converge as time went on (Figure 13 (b)). No fungus was observed growing on the lake trout embryos in this experiment.

Final total lengths were measured for each intact fish, and total lengths were not significantly different between tanks (using a single-factor ANOVA). The mean total length (±1 SD) increase between hatching and the end of the experiment for the fish over stamp sand was 13.25 mm (±2.0), while the mean total length increase of the lake trout over natural sand was 14.45 mm (±1.7) (Figure 14). The number and mean total length of hatched lake trout per stream compartment (A, B, C) can be seen in Appendix A.

Some abnormalities were noted but were found in both natural sand and stamp sand groups. A bent vertebra was the most common malformation, and some fish were unable to straighten their tails. Of preserved fish, six in the stamp sand tank and four from the natural sand had notable bends, with some fish in a horseshoe shape. One of these individuals from the natural sand tank was coiled up completely and could not swim. Additionally, yolk sacs varied in a number of individuals. Approximately 10 preserved fish from each tank had oddly shaped or discolored yolk sacs. Finally, a set of joined fish sharing one yolk sac was found in the stamp sand tank and survived until the yolk sac was almost absorbed (Figure 15).

Discussion

Water Sample Analysis

The mean dissolved copper concentrations were higher in the lake sturgeon experiment stamp sand samples than in the lake trout experiment stamp sand samples. This may be as a result of varying volumes of water used in each experiment. The small cylinders held approximately 2 liters of water, and the large Frigid Units held approximately 530 liters, so the sand to water ratio was more equal in the small cylinders that housed the lake sturgeon. As a result, the volume of water in the lake sturgeon tanks was approximately 44x what the value would be had the ratio stayed the same as in the lake sturgeon water. When viewed in this manner, the lower copper concentrations found in the lake trout water makes more sense. We also added fresh lake water three times during the course of the lake trout experiment, filling 20% of the stream. This, in turn, diluted trace metal concentrations, while water from the collection site was used during the lake sturgeon experiment.

The detectable copper measured from the lake trout streams was much lower than previously reported in stamp sand research (Jeong 1997). A more thorough approach would be to analyze the amount of bioavailable copper present in the surface water, as this is the copper that would be taken up by the surrounding organisms (Garcia-Munoz et al. 2009). The dissolved copper measured by White Water Associates, Inc. depicted the total copper present in the water; copper species was not specified, so it is possible that Cu^{2+} was not the dominant form of copper present. Bioavailable copper binds to the gills of fish, eventually working in to the bloodstream and accumulating in the liver and gonads (McKim et al. 1978; McIntyre et al. 2008; Green et al. 2010), so knowing this concentration in each tank would allow us to better predict the toxicity of each treatment.

Dissolved copper concentrations did significantly vary between surface water from washed and unwashed stamp sand, suggesting that the eroding stamp sand pile will contribute more copper to the lake than the wave-washed stamp sand. This, in turn, has the potential to harm fish species, particularly those using Buffalo Reef as a spawning site. A recent study (McDonald et al. 2010) of the Keweenaw's Torch Lake discovered that the existing stamp sand in the lake is continuing to leach copper into the surface water, while the remediated shoreline contributes very little additional copper to the system. Soil and vegetation now cover most of the shoreline tailings. Combined with our results, these findings support the need for shoreline remediation at the Gay stamp sand site.

Water samples were also analyzed for chromium, cadmium, arsenic, and lead; however, none of these metals were detected using ICP-OES, despite previous reports (Jeong 1997) that these metals were present at toxic levels. Future research should focus on the bioavailability of all of these metals using a more sensitive analysis technique, such as an acid digestion and ICP trace analysis (a more sensitive form of ICP-OES analysis), as well as comparing concentrations in Lake Superior to those in beach ephemeral ponds.

Substrate Particle Size Comparison

We found that the mean perimeter and mean shape factor values vary significantly between natural sand and stamp sand. Natural sand has a significantly higher shape factor, indicating the typical shape of a sand particle more resembles a circle than that of stamp sand. As a result, natural sand has fewer irregularities in shape, meaning fewer sharp edges. Stamp sand particles are also larger in size, with a greater mean perimeter. Stamp sand also has a greater variance in perimeter (±1.4) compared to the variance of natural sand perimeter values (±0.04). This supports the hypothesis that natural sand particles are more homogenous in shape and size and have fewer sharp edges when compared to stamp sand. Therefore, stamp sand may irritate the skin of life forms and discourage habitation of these areas.

These results are supported by the U.S. Standard Sieve analysis. Ninety percent of natural sand particles had a maximum diameter between 0.25 and 0.5 mm. In contrast, stamp sand sizes were distributed over a wider range: 72% of particles were larger than 0.5 mm in maximum diameter, 24% were between 0.25 and 0.5 mm, and 3.69% had a maximum diameter smaller than 0.25 mm.

These differences in size and shape may alter the aquatic community in tailing areas. Organisms that prefer the natural sand will be displaced as the tailings continue to move along the shoreline. Little research has been done linking the physical properties of stamp sand with any potential impacts on biota despite its prevalence in the Keweenaw region.

Earthworm Substrate Selection Experiments

The earthworm selection trials (n=88 surviving earthworms) indicated that earthworms displayed a clear preference when selecting substrate type. Specifically, they are choosing the natural sand/potting soil combination over either combination including stamp sand. This indicates a clear distinction which we attribute to the sand particle shape and possibly chemical composition. As previously discussed, natural sand generally has smoother edges, making it easier to move through for soft-bodied worms. No previous research was found on organism substrate selection and stamp sand.

Earthworms have been used in remediation in regions with mining-contaminated soil and have decreased the concentrations of water soluble copper (Sizmur et al. 2011). They are able to do this by altering the pH and soluble organic carbon in the system. However, our results suggest earthworms will avoid the mine tailings.

Frog Experiment

Frog embryos developed rapidly (less than 4 days upon arrival) and substrate type (natural or stamp sand) did not influence the number of frog embryos that hatched. The mean number of hatched embryos was 22 out of 25 in both natural and stamp sand substrate types. This suggests the amount of copper that dissolves in surrounding surface water is either not enough to harm the organisms during egg incubation or is not bioavailable. It is possible the copper is complexing with organic material found in the sand (Jeong 1997). An analysis of the amount of organic matter present in the stamp sand would be useful information when trying to determine why copper is not available.

Embryos hatched best in the 5 μg/L copper sulfate solution. At this relatively low concentration, the available copper may be high enough to hold off the growth of fungus or bacteria (a concentration of 235 ppm is the average copper concentration in copper fungicides, but ranges between 25-500 ppm) that could harm the embryo, yet low enough so as not to overwhelm the embryo (OMRI 2001).

As expected, the fewest embryos hatched in the 100 μg/L copper sulfate solution, with a mean hatch success of 76%. With no organic matter to bind to, this solution is likely to have a higher percentage of Cu^{2+} ions which harm the developing embryo (Garcia Munoz et al. 2009).

Final total length measurements of the tadpoles were also made, and the results were unexpected. Tadpoles were smallest in the lake water control. This may be due to overcrowding in the larger container tadpoles were moved to after hatching (Rugh 1934). In contrast, the longest tadpoles were found in the natural sand containers. These tadpoles had a mean total length of 22.1 mm (±2.0) and were distributed among three larger aquaria. The natural sand tadpoles were larger than the stamp sand tadpoles by the end of the study. Each had three large aquaria and the same mean number of hatched embryos, so space was not a factor influencing size. As a result, the difference in growth must be due to a factor in the sand itself, such as bioavailable trace metals (Chen et al. 2007).

Finally, we looked at total surviving tadpoles at the end of the study. Again, the greatest number of tadpoles survived in the natural sand containers, while a majority of the tadpoles in the 100 μg/L container were lost in the first two weeks post-hatch and had only three survive for the full 51 day study.

The mean total lengths of the tadpoles in the copper sulfate solutions showed that as the copper concentrations increased, the tadpoles actually grew longer. This study should be redone using more containers to avoid the problem of overcrowding. As a result, no formal conclusions can be made regarding the tadpoles in the copper sulfate solutions.

These results support the hypothesis that decreased survival and stunted growth occurs for Northern leopard frogs incubated over stamp sand. Additionally, tadpoles seem to be more susceptible to trace metals than embryos, as the number of embryos surviving to hatch was equal in both natural and stamp sand containers. While some deformities were noted, they were also present in the control group which only had lake water; as a result, they cannot be attributed to stamp sand or presence of copper.

Lake Sturgeon Experiments

No significant difference was found among lake sturgeon hatch success values of the three lake substrate groups: natural sand, washed stamp sand, and unwashed stamp sand. However, an interesting observation was made after eight days of incubation: a white fungus began growing on the eggs in the natural sand containers, but the fungus was less prevalent in the stamp sand containers, allowing more embryos to hatch. When the fungus starts to grow, it encompasses the embryos in a mass, killing them. Because we used Lake Superior water in this experiment, microbes were present and thrived in the incubator environment.

Total lengths of larvae did not vary significantly among containers either. The short incubation period of lake sturgeon embryos may not be long enough for trace metals to harm the fish. Additionally, all fish were euthanized before complete yolk sac absorption, so fish were not fed during this experiment, eliminating one source of bioaccumulation (Gewurtz et al. 2009). These results did not support the hypothesis that organisms will show decreased survival or developmental differences when incubated on stamp sand. There were no abnormalities noted in any of the lake sturgeon.

Lake Trout Experiments

The number of lake trout embryos that hatched varied between stamp sand and natural sand, with almost 20% more fish hatching over stamp sand. The timing of the stamp sand embryo hatch was also earlier and faster than the natural sand embryos. The stamp sand stream was approximately 1^{o}C warmer during the beginning of incubation, and this may have given the embryos a chance to developmentally advance. Allen et al. (2005) found that a 2-degree Celsius increase from 4 to 6 degrees can cause lake trout to hatch an average of 30 days earlier. However, greater success of stamp sand embryos may be due to the effects of copper. While no fungus was observed in either tank, it is possible that the fungicide/algaecide properties of copper hindered the growth of harmful fungus or bacteria in the stamp sand embryos, allowing a larger number to successfully survive until hatching. While the p-value comparing hatch success is not significant at p=0.06, it is extremely close to the pre-determined alpha value of 0.05.

Timing of hatch may also be due to copper being an endocrine disruptor in fish (Schantz and Widholm 2001; Handy 2003). At sub-lethal concentrations, copper alters both neurological and endocrine control systems and changes the biochemical and cellular makeup in the body (Handy 2003). Combined with temperature variations, one result may be expedited embryo development, as seen in our stamp sand lake trout and frogs (McDaniel et al. 2004). A long-term survival study would clarify this relationship and allow a more thorough examination of copper effects on life stages in fish and amphibian species.

Additionally, total length was measured both after hatch and at the end of the experiment, and no significant difference was found between the two tanks. The stamp sand lake trout grew an average (±1 SD) of 13.25 mm (±2.0), while the average growth of the natural sand lake trout was 14.45 mm (±1.7). While the natural sand lake trout grew slightly longer during the study, despite hatching later, the growth differences were not statistically significant. Again, a long-term growth study would chronicle the long-term effects of stamp sand exposure on lake trout; however, larval fish are considered more sensitive to copper toxicity than adult fish (McKim et al. 1978) and young would be expected to move off the stamp sand-filled reef after swim-up.

Lake trout showed a trend similar to the amphibians in our experiments. Initially, both stamp sand and natural sand embryos had strong hatch success, but as days post-hatch increased, the number of surviving individuals in stamp sand or copper sulfate solutions decreased, and the natural sand fish and amphibians had a higher long-term survival rate, indicating that stamp sand is detrimental to long-term survival of lake trout and Northern leopard frogs. Again, long-term growth experiments should be repeated to clarify this trend, but this initial research supports the need for stamp sand remediation at Gay.

Conclusion

In this research, the stamp sand and natural sand particles were shown to be physically different from one another, with statistically different sizes and shapes and copper analysis of surface waters varied significantly between each type, with higher copper concentrations found in stamp sand surface waters.

Earthworms did display a clear selection preference, tending to prefer the natural sand/potting soil combination over either combination including stamp sand. The lake sturgeon and lake trout experiments, however, did not support the hypothesis that survival and early development would be hindered by stamp sand presence. Hatch success and total length were not significantly different among the different substrate types. A short incubation period and limited opportunity for bioaccumulation could explain this, or perhaps the copper detected with the ICP-OES was simply not in a bioavailable form. A long-term experiment is needed to further evaluate any impacts stamp sand may have on reef-spawning lake trout.

Lake trout may not be negatively impacted by chemical changes due to stamp sand at Buffalo Reef; however, they are more likely to be negatively impacted by the disappearance of crucial interstitial spaces where embryos safely develop.

The Northern leopard frogs displayed reduced survival and growth over stamp sand, so remediation efforts should focus first on the Gay beach, rather than on stamp sand already in the water. This would also prevent more stamp sand from entering the lake, reducing the amount of sand filling in Buffalo Reef. Amphibians may be more susceptible to trace amounts of copper in the surrounding water than either fish species, and the tadpoles were more susceptible than frog embryos, but more research should be done in this area with a more carefully thought-out experimental design.

Future research should focus on measuring the bioavailability of trace metals. It is possible that the leaching metals are complexing with organic matter found in the different sand types, making the Cu^{2+} concentrations low enough that they don't harm the exposed fish. It would also be useful to measure the copper concentrations of the fish carcasses as well, to see if there is a difference in the amount of copper accumulating in the tissues and where in the fish the copper (or other trace metals) are accumulating.

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Figures

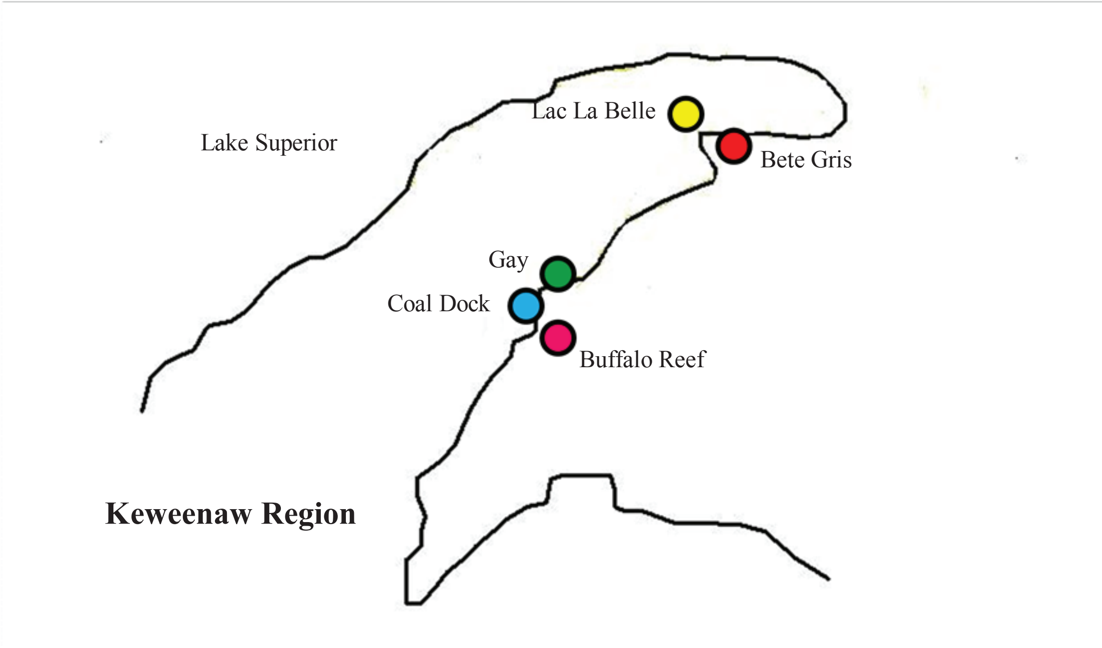


Figure 1: Location of substrate sampling sites, Gay, Coal Dock, and Bete Gris, and lake trout spawning site, Buffalo Reef. Lac La Belle is noted for reference purposes.

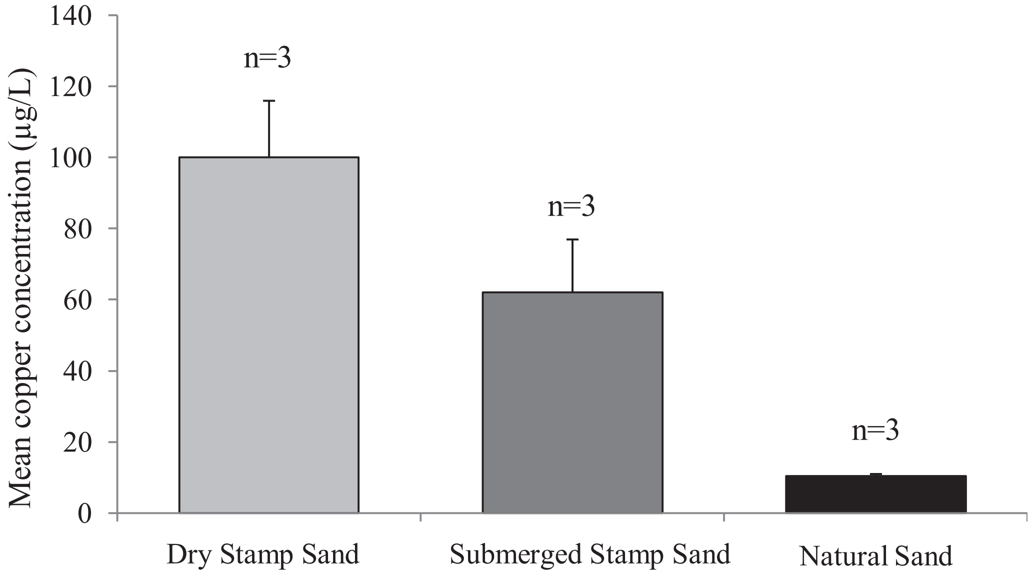


Figure 2. Mean surface water copper concentration (±1 SD) among the three substrate types on May 23, 2010 (after 34 days of contact).

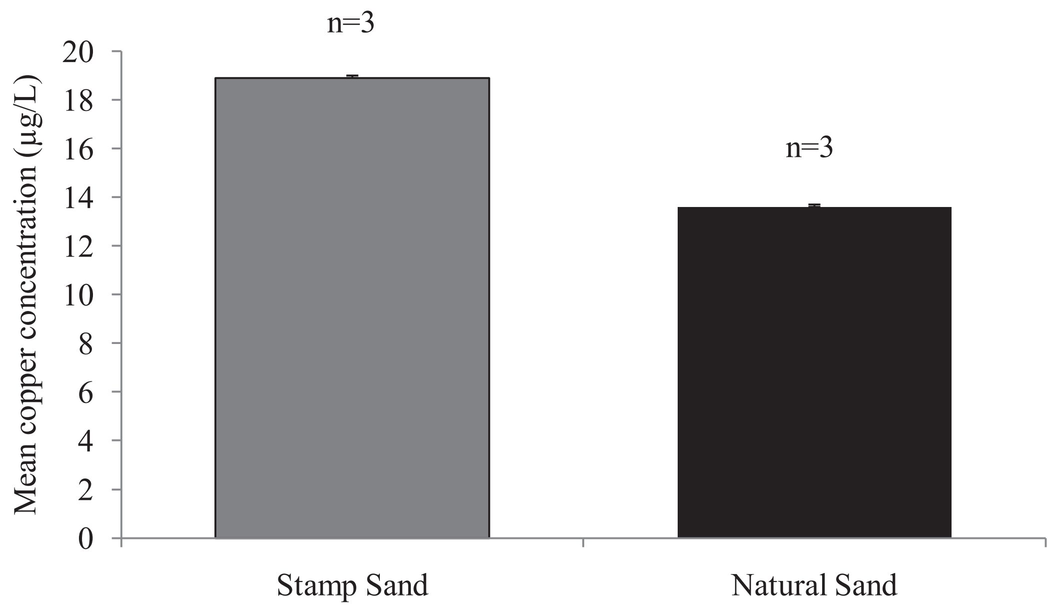
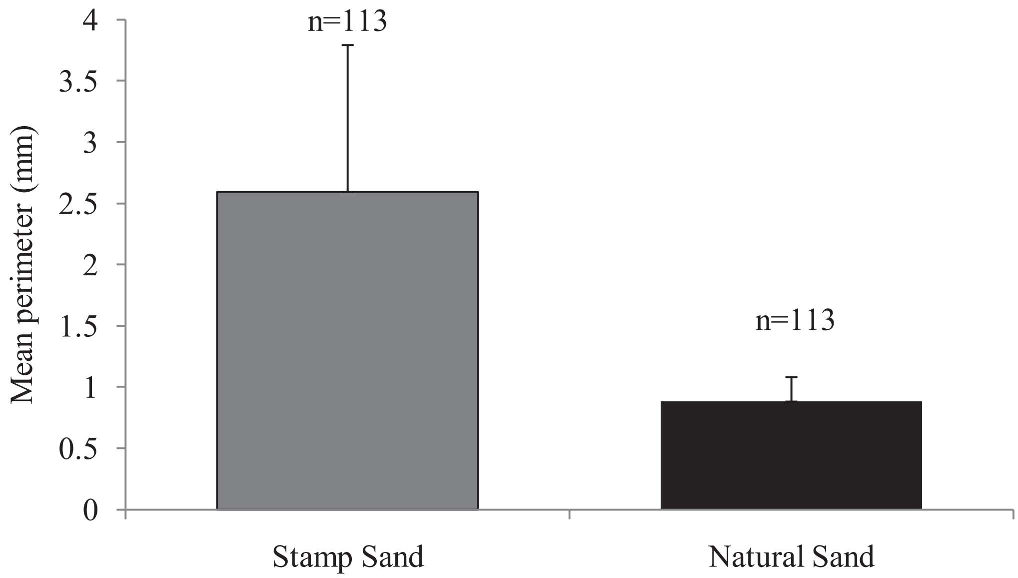


Figure 3. Mean copper concentration (µg/L) (±1 SD) in natural vs. stamp sand in the lake trout experiment, analysis on May 24, 2011.

a.



b.

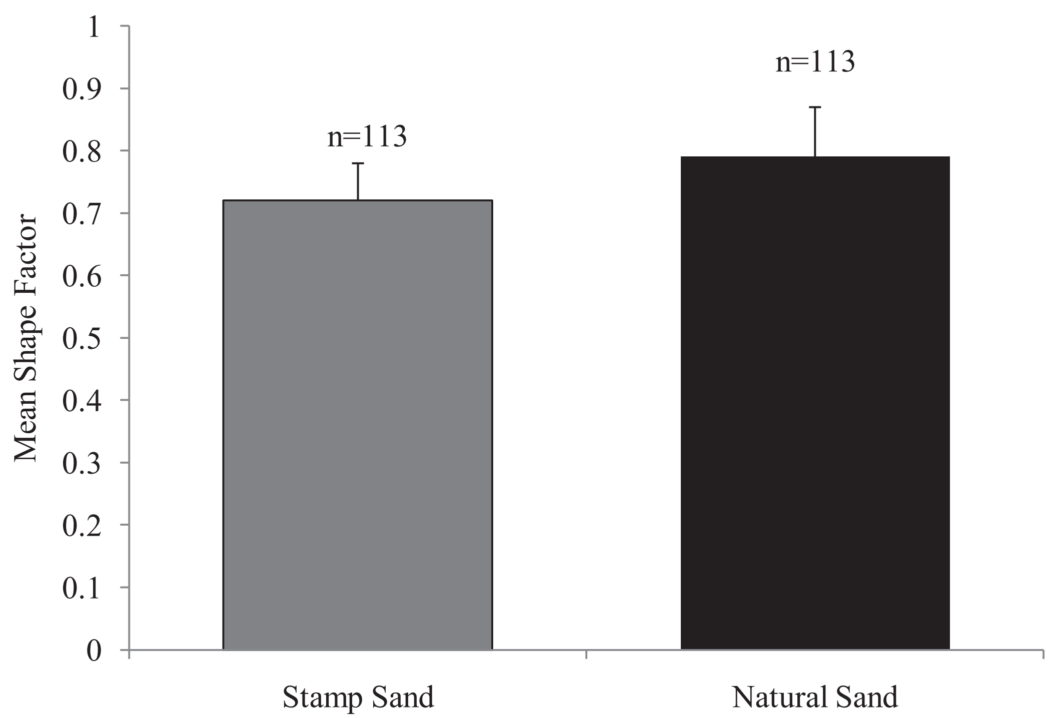
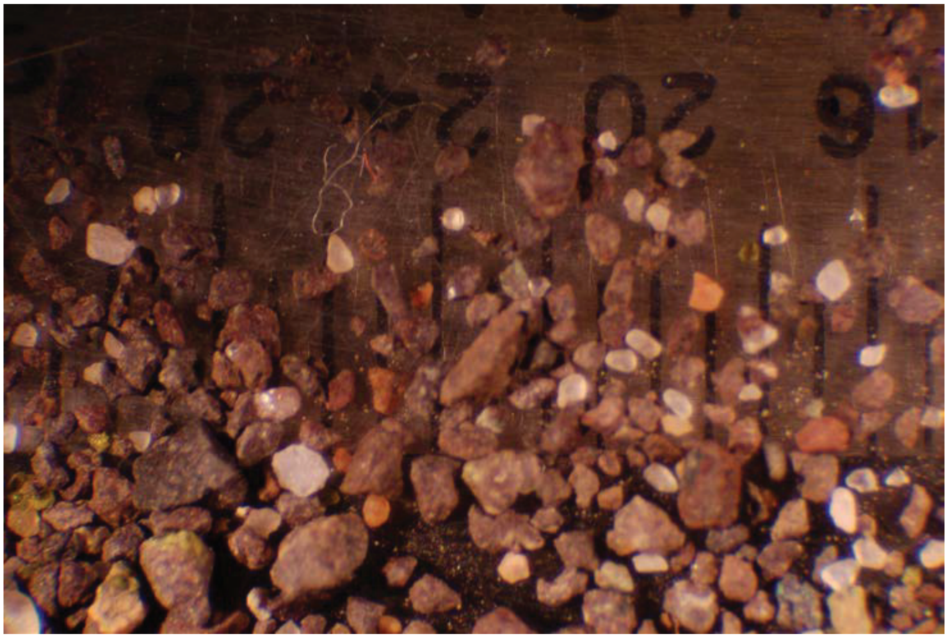


Figure 4. Mean particle perimeter (mm) (a) and mean shape factor (b) for both stamp and natural sand (± 1 SD, n=113 each) using SigmaScan Pro software.

a.



b.

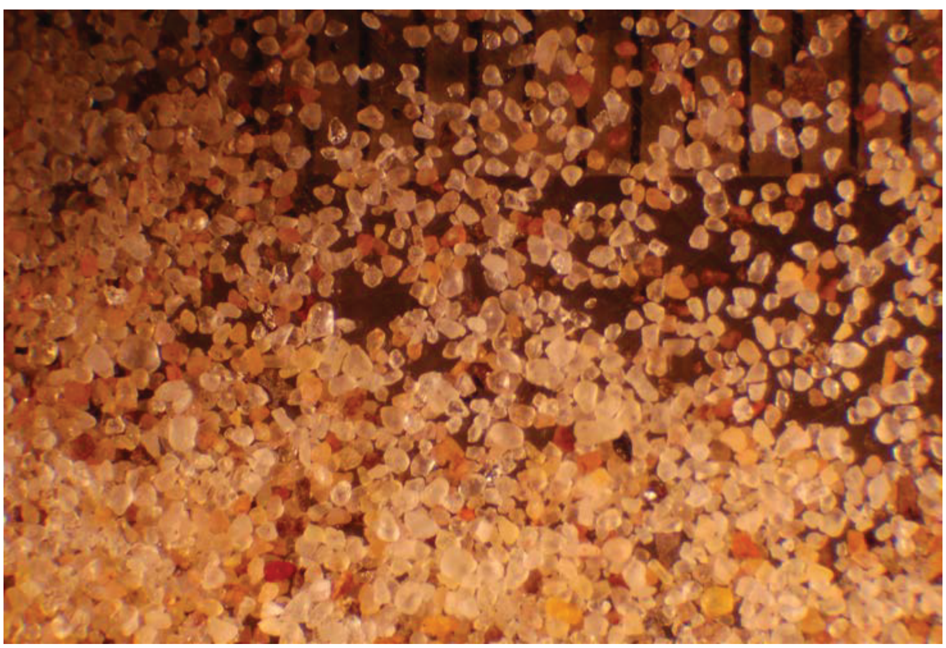


Figure 5. Stamp sand (a) and natural sand (b) as seen at a magnification of 100. The area between hatch marks is 0.05 mm.

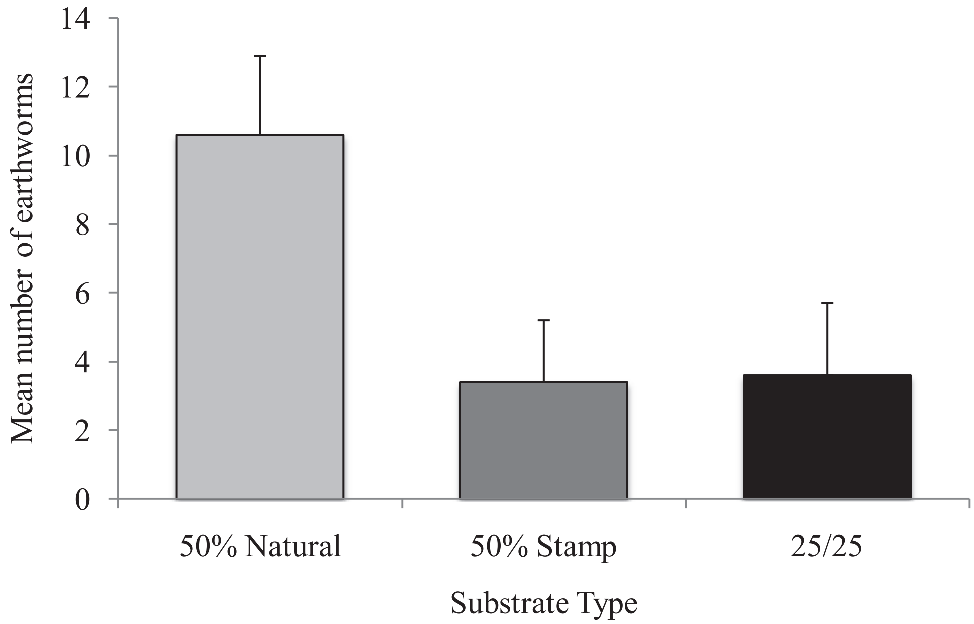


Figure 6. Mean number of earthworms found in each substrate type after 72 hours (±1 SD) over five trials, n=18, indicating a preference for the natural sand-potting soil mixture (p=0.0002 using single-factor ANOVA).

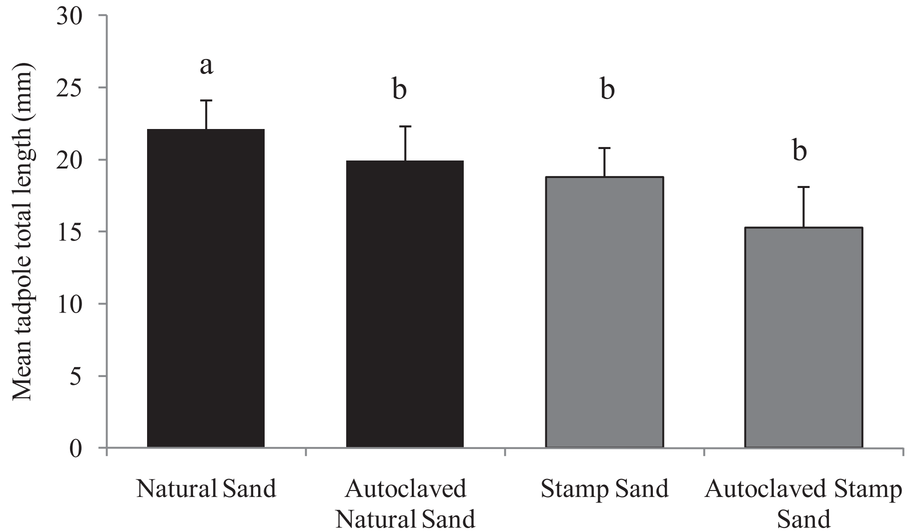


Figure 7. Mean total lengths (mm) (±1 SD) of tadpoles in the natural sand and stamp sand containers. A single-factor ANOVA and Tukey test show that the natural sand tadpoles were significantly longer than the other groups, as indicated by the letters above each bar.

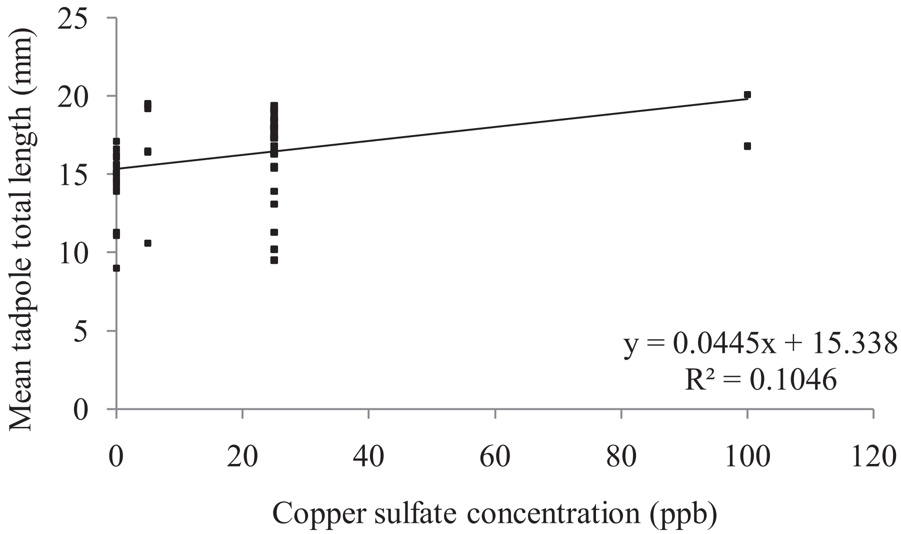


Figure 8. Mean tadpole total lengths (mm) in each of the copper sulfate solutions.

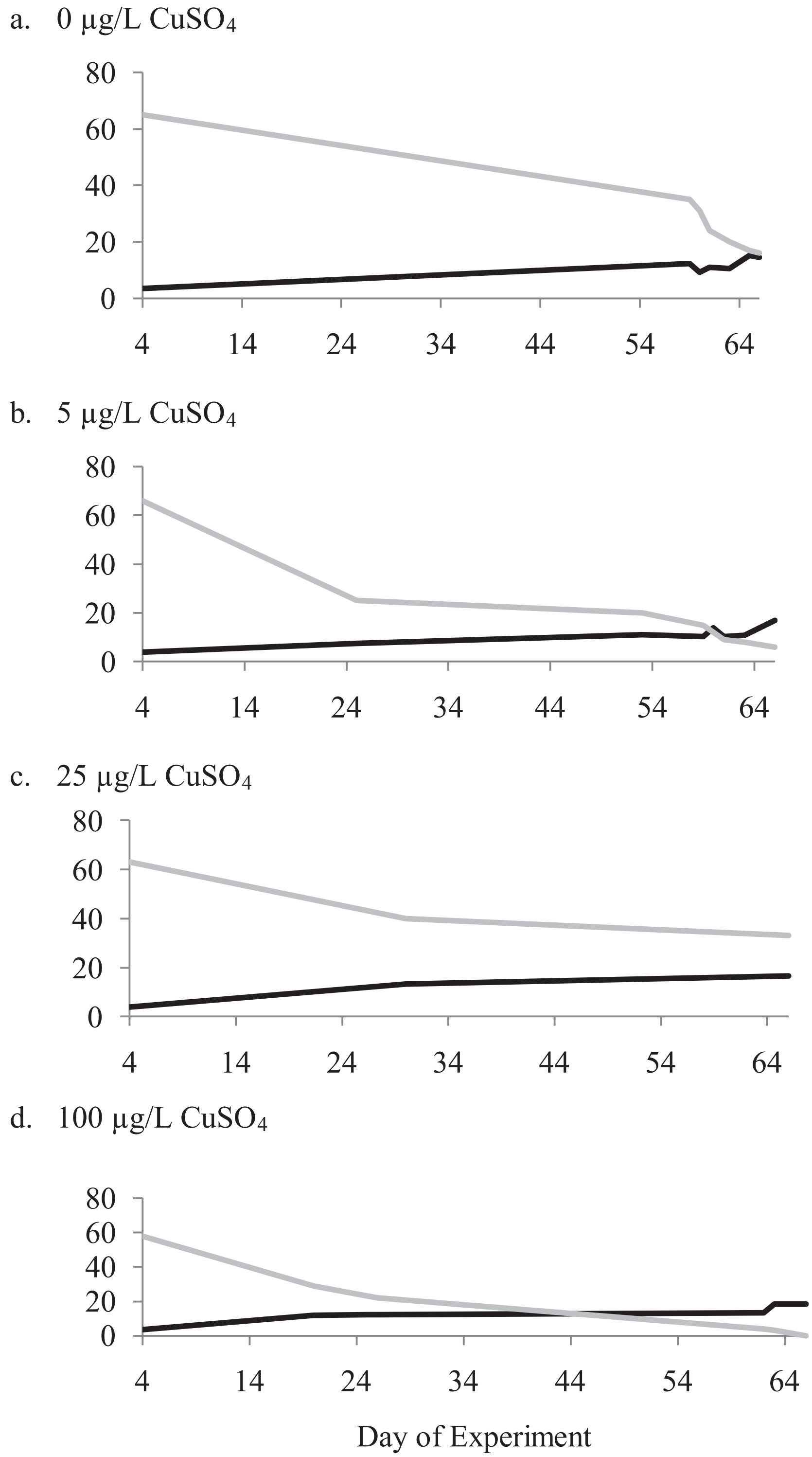


Figure 9. Mean total length (black) and number of tadpoles alive per day (gray) of experiment in 0 µg/L $CuSO_4$ (a), 5 µg/L $CuSO_4$ (b), 25 µg/L $CuSO_4$ (c), and 100 µg/L $CuSO_4$ (d).

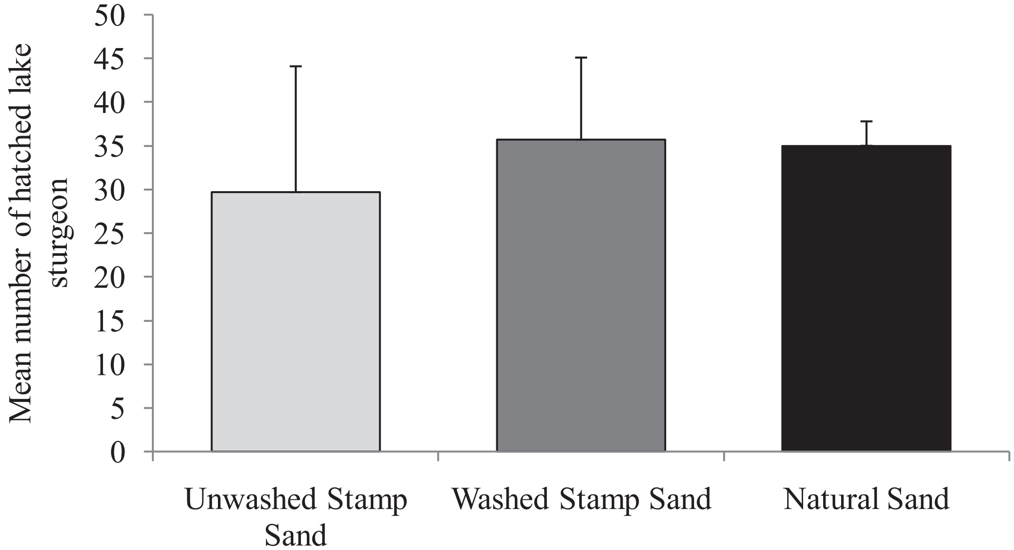


Figure 10. Mean number of lake sturgeon eggs (±1 SD) that hatched over unwashed stamp sand, washed stamp sand, and natural sand (n=50 eggs/cylinder, or 150 eggs/substrate type).

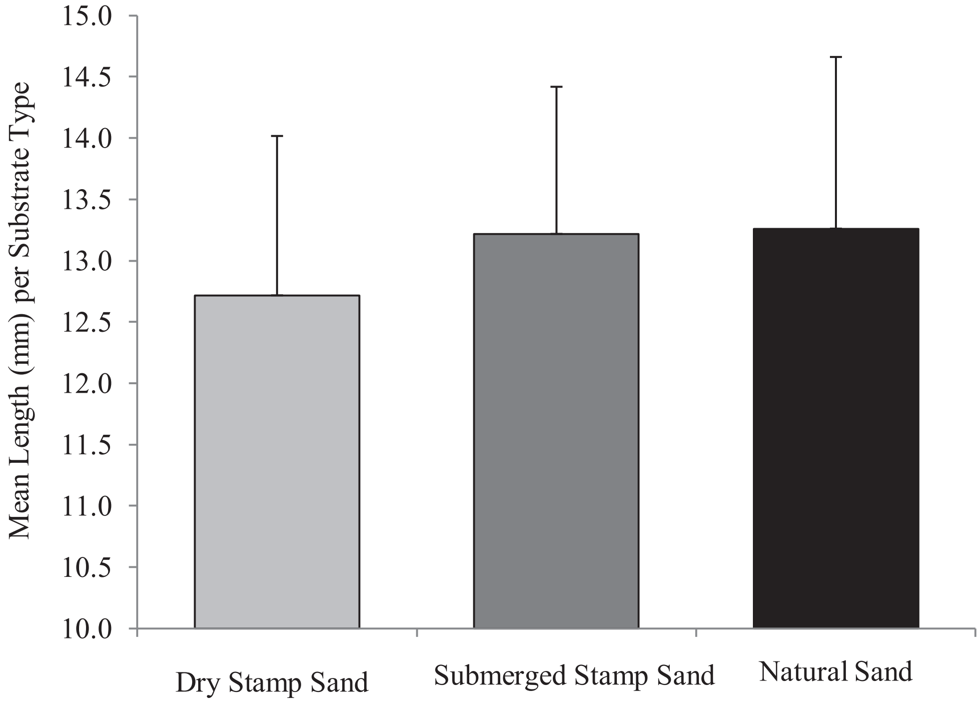


Figure 11. Mean total length (mm) (±1 SD) of lake sturgeon after hatching in both the unwashed and washed stamp sand cylinders (n=3 for each substrate type), as well as the natural sand cylinders.

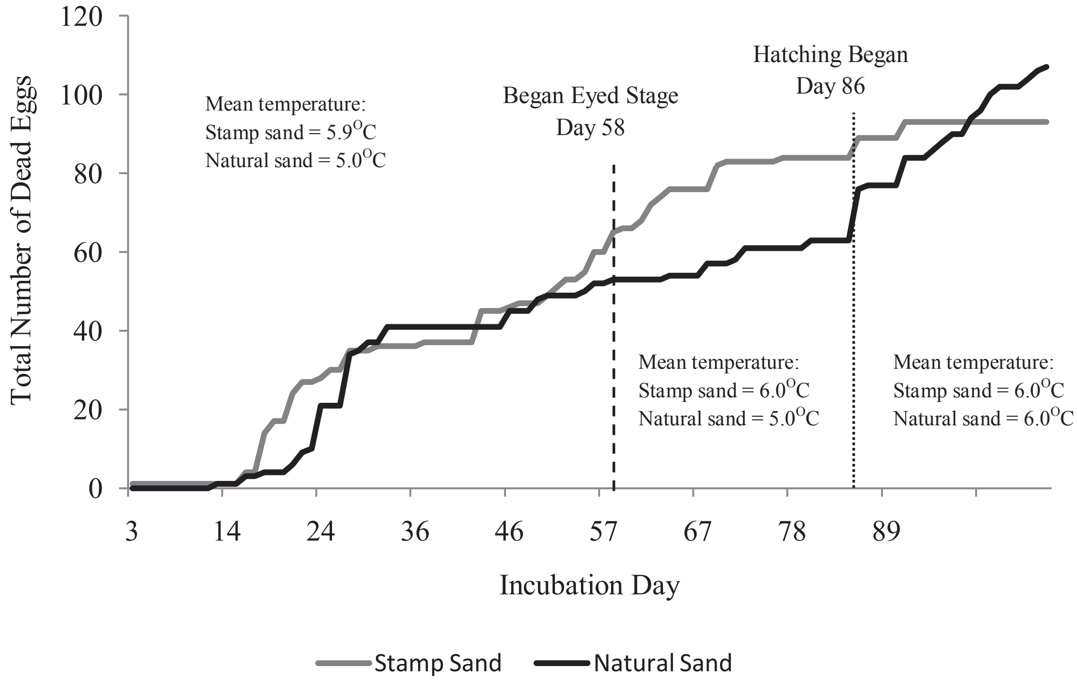
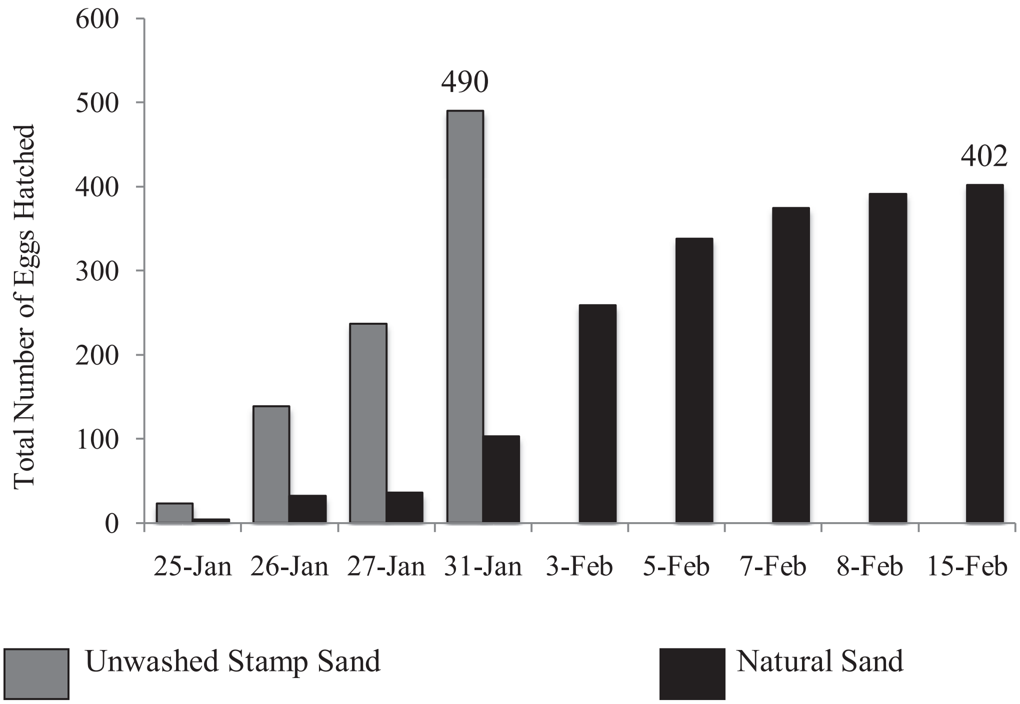


Figure 12. Total number of dead lake trout eggs removed from each stream. Beginning of eyed stage and beginning of hatching are marked with dashed/dotted lines, respectively.

a.



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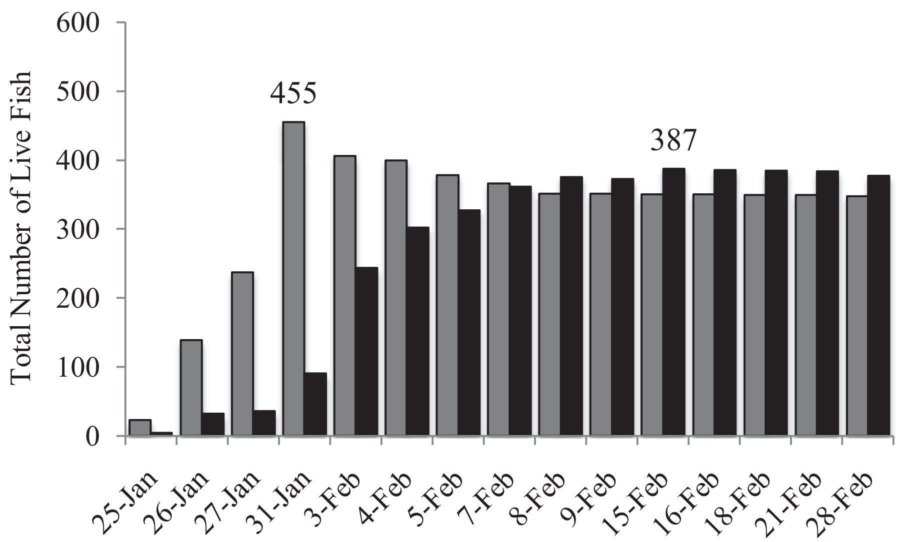

Figure 13.Total number of lake trout hatched (n=600) in each living stream as of February 11, 2011 (a) and daily total number of live lake trout in each stream after hatching started (b). Maximum number of fish is noted above each category.

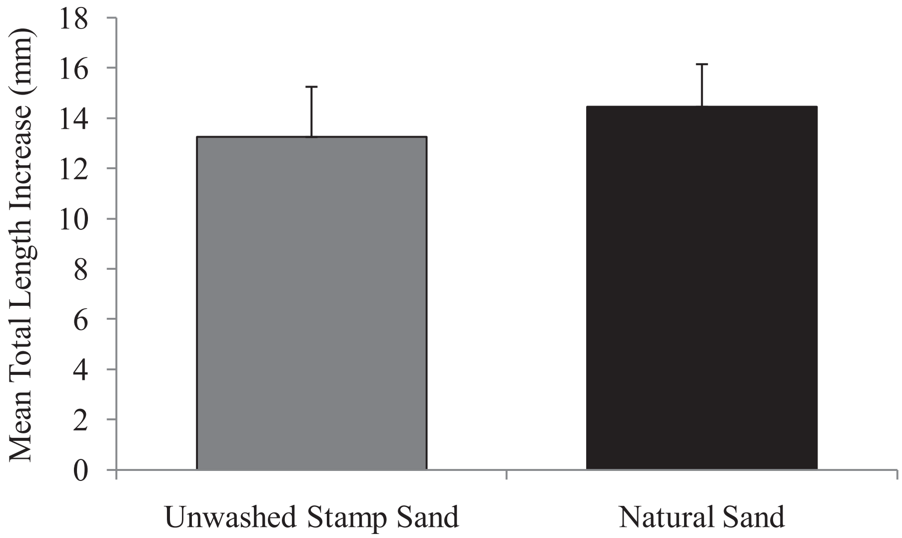

Figure 14. The mean total length increase (±1 SD) of lake trout over stamp sand and natural sand from initial hatch length to final total length at the end of the experiment (day 163).

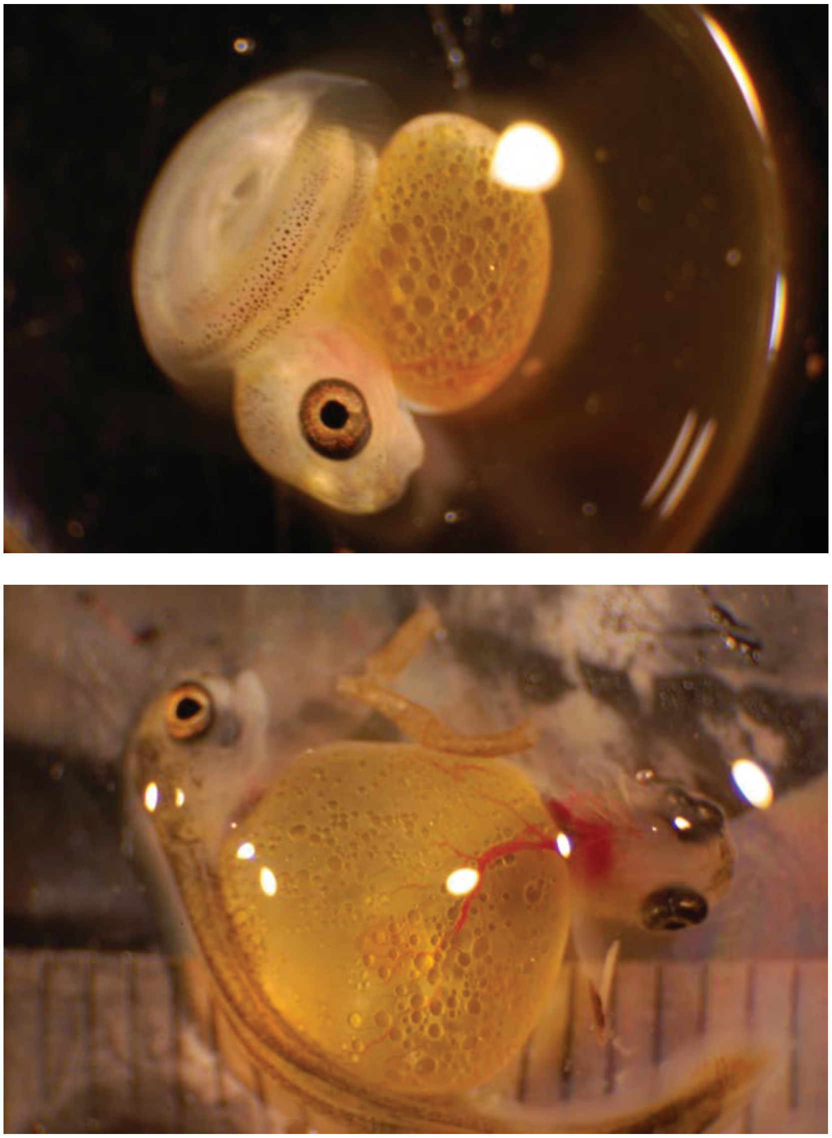

Figure 15. Two types of lake trout malformations observed during the study.

Tables

Table 1.

Mean dissolved oxygen concentration (mg/L) in each tadpole tank.

Tank ID	Mean Dissolved Oxygen (mg/L)
Stamp Sand 1	8.63
Stamp Sand 2	8.83
Stamp Sand 3	8.97
Natural Sand 1	8.97
Natural Sand 2	8.68
Natural Sand 3	8.22
Autoclaved Stamp Sand 1	9.05
Autoclaved Stamp Sand 2	8.98
Autoclaved Natural Sand 1	8.86
0 µg/L	9.04
5 µg/L	9.17
25 µg/L	8.61
100 µg/L	9.09

Table 2.

Percent particle size distribution for both stamp and natural sand using U.S. Standard Sieve analysis.

	<0.063 mm	<0.125 mm	0.125<x<0.25 Mm	0.25<x<0.5 mm	0.5<x<2 mm	>2 mm
Stamp Sand	0.0	0.1	3.6	24.0	72.0	0.3
Natural Sand	0.0	0.0	8.7	91.0	0.35	0.0

Table 3.

Mean number of tadpoles hatched (n=25) over each experimental group as well as mean total length (mm) ±1 SD of tadpoles at the end of the experiment. Eggs were received March 24, 2011, hatching was complete on April 4, 2011, and the final total length measurements were made May 25, 2011.

	Mean Number Hatched	Standard Deviation	Mean Total Length (mm)	Standard Deviation
Stamp Sand	22	3.5	18.8	2
Autoclaved Stamp Sand	20	1.5	15.3	2.8
Natural Sand	22	1.5	22.1	2
Autoclaved Natural Sand	22	3.2	19.9	2.4
Lake Water: 0 ug/L	22	0.6	14.5	2.1
5 ug/L	24	1.5	16.9	3.4
25 ug/L	22	2.5	16.7	2.5
100 ug/L	19	3.0	18.4	2.3

Table 4.

Number of tadpoles moved into larger aquaria on April 4, 2011 and number of tadpoles surviving at the end of the experiment, May 25, 2011. The far right column displays the mean surviving percentage of tadpoles in each category.

	4/4/2011 Total moved into tanks	5/25/2011 Total at end of study	% Remaining	Mean
Natural Sand 1	21	10	47.6	58.9
Natural Sand 2	20	18	90.0	
Natural Sand 3	23	9	39.1	
Autoclaved Natural Sand 1	32	0	0.0	10.6
Autoclaved Natural Sand 2	33	7	21.2	
100 µg/L	58	0	0.0	
25 µg/L	63	33	52.4	
5 µg/L	66	6	9.1	
0 µg/L	65	16	24.6	
Stamp Sand 1	23	0	0.0	0.0
Stamp Sand 2	18	0	0.0	
Stamp Sand 3	22	0	0.0	
Autoclaved Stamp Sand 1	30	4	13.3	6.7
Autoclaved Stamp Sand 2	29	0	0.0	

Appendix A

Literature Review

History of Copper Mining in the Keweenaw Peninsula

Copper mining in the Keweenaw Peninsula started around 1844 and ceased in 1968 once mining became unprofitable (Rasmussen et al. 2002). Almost five billion kilograms of elemental (or "native") copper was exported during this period (Rasmussen et al. 2002), with the peak of copper mining taking place between 1890 and 1930 (Kerfoot et al. 1994). The stretch of copper that runs beneath the Keweenaw was known as the Greenstone Belt, and more than 140 mines were extracting during the peak of this industry (Kerfoot et al. 1994). The earliest types of copper removed included float, vein, and mass copper (Lankton 1991), but once these were depleted, the companies started extracting copper from the amygdaloid and conglomerate ore present alongside the Belt (Kerfoot et al. 1994). This shift in copper types meant the process of acquiring copper had to change. As a result, stamp mills were built, and during the copper mining peak, over 40 stamp mills were processing this ore to remove the desired copper.

Stamp mills appeared throughout the Keweenaw and required a water source to function. The purpose of the stamp mills was to crush the amygdaloid and conglomerate ore down into sand particle size (hence, the name stamp sand) using steam powered machinery (Kerfoot et al. 1999). Water and chemicals were added to this new sand to extract the native copper (Rasmussen et al. 2002). The leftover material, known as stamp sand, was deposited along the nearest waterway. The coarse material was deposited on the shoreline while the finer particles, water, and chemicals were sent along a conveyor belt into the waterway (Lankton and Hyde 1982). This slurry of fine particles was called "slime clay" because it was a more homogenous mixture than the particles left behind onshore (Kerfoot et al. 2004).

Two stamp mills were present at the Gay, Michigan site and were operated by the Mohawk and Wolverine mining companies (Kerfoot et al. 1999). These mills produced over 25 billion kilograms of stamp sand that was deposited along the Gay, Michigan shoreline (Babcock and Spiroff 1970; Kolak et al. 1999) before the mills closed in 1932 (Kerfoot et al. 1999). These tailings have elevated concentrations of a number of heavy metals, including copper, arsenic, cadmium, chromium, and lead (Jeong 1997).

These initial mine tailings created fans that were subjected to reworking by wave and wind action (Kerfoot et al. 1994). As a result, coarse particles have been moved down shore, staying closer to shore, and finer particles have found their way into deeper waters (Lankton and Hyde 1982; Kerfoot et al. 1994). The Gay site has been the focus of research by a number of groups. The dispersing stamp sand has been studied (Kerfoot et al. 1999; Rasmussen et al. 2002), along with the impact on terrestrial organisms (Jarvie 2005).

Currently, there are no plans for remediation, so the tailings will continue to be at risk of moving down the shoreline. One major cause of this dispersal is the Keweenaw Current (Kerfoot et al. 1999). The Keweenaw current sweeps around the Keweenaw Peninsula and runs past the eastern side of the Peninsula, shifting the Gay tailings southwest into Keweenaw Bay. There is a concern of long distance displacement of tailings due to the current (Kerfoot et al. 1999).

Rasmussen et al. (2002) researched the shifting shoreline at Gay, Michigan, and produced a figure displaying how the shoreline has receded between 1938 and 1997 and the amount of stamp sand above the water level has decreased over time.

Previous Stamp Sand Research

Stamp sand varies from natural substrate in shape and chemical composition. Stamp sand is larger and coarser (approximately 4.76 mm in diameter) than natural beach sand but not large enough to provide the necessary interstitial spaces (Rasmussen et al. 2002) trout lake prefer when spawning. However, there has not been sufficient research focused on comparing stamp sand with natural Lake Superior beach sand. Additionally, stamp sand has elevated concentrations of copper, arsenic, lead, chromium, and cadmium (Jeong 1997). In addition to Torch Lake, there is quite a bit of information on copper-rich sediments in both Keweenaw Waterway and Portage Lake; however, little research has been done linking the Gay stamp sand dispersal with possible impacts on aquatic organisms.

Experimental Methods

Lake Sturgeon Growth Experiment

Additional lake sturgeon eggs were hatched in the lab and held for the growth study at the Ontonagon Streamside Rearing Facility (SRF). Prior to arrival at the SRF, the fish were kept in water from the Portage Canal, in Houghton, Michigan and fed brine shrimp twice daily. Six plastic bins were set up, three with Ontonagon beach sand and three with unwashed stamp sand from the Gay site. Twelve hundred grams of sand was placed into each bin. Water flowed into the bins from the Ontonagon River through PVC piping and out of the bins into a raceway that drained back into the river. Netting covered the outflow pipes so the fish would not be sucked out with the water.

Initial total lengths were collected and 10 fish were placed into each bin on June 28th, 2010. For the first four weeks, a collective weight was used, as the fish were too small to individually read on the scale (OHAUS CS-2000). Each bin received 2 grams of bloodworms to help alleviate handling stress. Outflow measurements were taken for each bin to monitor flow through the system. Fish were fed 0.25 teaspoon of brine shrimp and 2-3 grams of bloodworms twice daily, and any dead fish were removed and recorded. As of July 11, 2010, fish were fed only bloodworms twice daily. Outflow screens were removed and scrubbed daily to prevent the bins from backing up and overflowing.

Total lengths and collective weights were measured weekly, and new fish were added to the bins at this time to bring the total number of fish back to 10. After 3 weeks, no more new fish were added. An unidentified bacterial infection spread through the hatchery, and most of the fish grew ill and died. Final measurements were taken on August 11, 2010 and all remaining fish were returned to the laboratory at Michigan Tech.

Substrate Selection Experiments

Initial substrate preference studies were conducted using both rusty crayfish and earthworms. One 15 gallon glass aquarium tank was set up with three crayfish and another 15 gallon glass aquarium tank was set up with 5 earthworms. The bottom of each was divided evenly into two sections, with 2.5 cm of stamp sand on half and 2.5 cm of beach sand on the other half. The tanks were monitored for two weeks, and it was noted which half of the tank the organisms were found on at each observation point. The crayfish were given about 10 cm of water and an aerator continuously bubbled in the middle of the tank. Additionally, three plastic half-flower pot pieces were added to provide shelter. The substrate in the worm tank was moistened daily to prevent the worms from drying out.

Additional substrate preference studies were conducted using earthworms. Again, a 15 gallon glass aquarium tank was filled with 2.5 cm of stamp sand and 2.5 cm of natural beach sand; however, only 1 earthworm was placed in each. Four tanks were set up at a time and were monitored for 72 hours. Location at observation time was recorded and sand was moistened daily to prevent the worms from drying out. Worms were not used more than once during any of the substrate trials.

After the initial 72 hour period, we employed a different testing method to test substrate preference. A 15 gallon glass aquarium was partitioned into five equal compartments using 13 cm tall Plexiglas partitions. Each compartment contained 11 cm of one of the following substrate mixtures: 100% natural sand, 75% natural sand/25% stamp sand, 50% natural sand/50% stamp sand, 75% stamp sand/25% natural sand, and 100% stamp sand. Substrate was moistened prior to the trial and daily throughout the next 72 hours, and 3 worms were placed in each division, for a total of 15 study organisms. Prior to this trial, worms were allowed to feed on corn meal overnight.

Unfortunately, this method did not support earthworms through the complete 72 hour study, so we conducted a third trial using a new method. Using the same 15 gallon glass aquaria, potting soil and sand combinations were mixed and placed into each of three compartments. Three initial tanks were set up. All contained three compartments with one of the following substrate mixtures: 50% potting soil/50% natural sand, 50% potting soil/25% natural sand/25% stamp sand, and 50% potting soil/50% stamp sand (Figure A.1). The order of compartments was mixed in each of the three tanks to avoid influence by outside laboratory stimuli. Two earthworms were added to the center of each compartment (six earthworms per tank), and water was added until each compartment read to a moisture level of two on the soil moisture meter (General Model No. GLMM200). Finally, each tank was covered with Plexiglas and left for 72 hours. At the end of the trial period, the number of worms in each compartment was counted and recorded. Earthworms were removed and not used more than once in preference trials.

Finally, I attempted to monitor where each earthworm moved by marking individuals using both sutures and food coloring injections. Fewer worms survived following these attempts, and it was common for an injected earthworm to bind off the injected part of its body and re-grow its tail. The arrangement of substrate in each of the tanks was changed (Figure A.2) and the individual movements of worms was noted.

Results and Discussion

Lake Sturgeon Growth Experiment

Total length of lake sturgeon was measured weekly, starting on June 29, 2010 and ending August 11, 2010. The mean total length increase of sturgeon over natural sand was 28.4 mm (±6.5), and the mean total length increase of sturgeon over stamp sand was 22.7 mm (±8.7) (Figure A.3). While total length and combined weight was monitored weekly, the experimental design had a number of flaws. First of all, lake sturgeon were replaced weekly to bring the combined total to ten in each tank; however, the “extra” fish were kept in the raceway that each of the six bins drained in to. As a result, sturgeon used in the natural sand containers may have already been exposed to trace amounts of stamp sand by the time they were placed in a bin. The growth difference was not statistically different between substrate types and there was a bacterial infection that swept through the hatchery fish. Many fish grew ill and died, resulting in a rapid replacement. Few fish remained alive throughout the entire experiment, so the growth numbers are available but skewed, as each fish spent varying amount of time in the experimental containers.

Substrate Selection Experiments

Earthworms were marked using both suture arrangements and food coloring injections. Worms moved an average of 1.5 compartments (Figure A.4). Mortality was much higher during these marked trials than in the unmarked trials and a number of earthworms dropped the part of their body that was manipulated. After the initial marking trial, I removed the sutured worms and used only food coloring to mark. Four more trials were run in this manner. I was not able to find literature on how to successfully mark earthworms, and my experiments showed that neither sutures nor food coloring is an efficient way to monitor movement and keep the organisms alive.

Lake Trout Experiments

Total number of hatched lake trout and mean total length was measured for each compartment (A, B, C) within each Frigid Unit. The number of hatched embryos did not vary among compartments (Figure A.5 (a)), and the mean total length did not vary among compartments (Figure A.5 (b)). As a result, I can report with confidence that proximity to the circulator did not influence hatch success or early growth in lake trout.

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Appendix A Figures

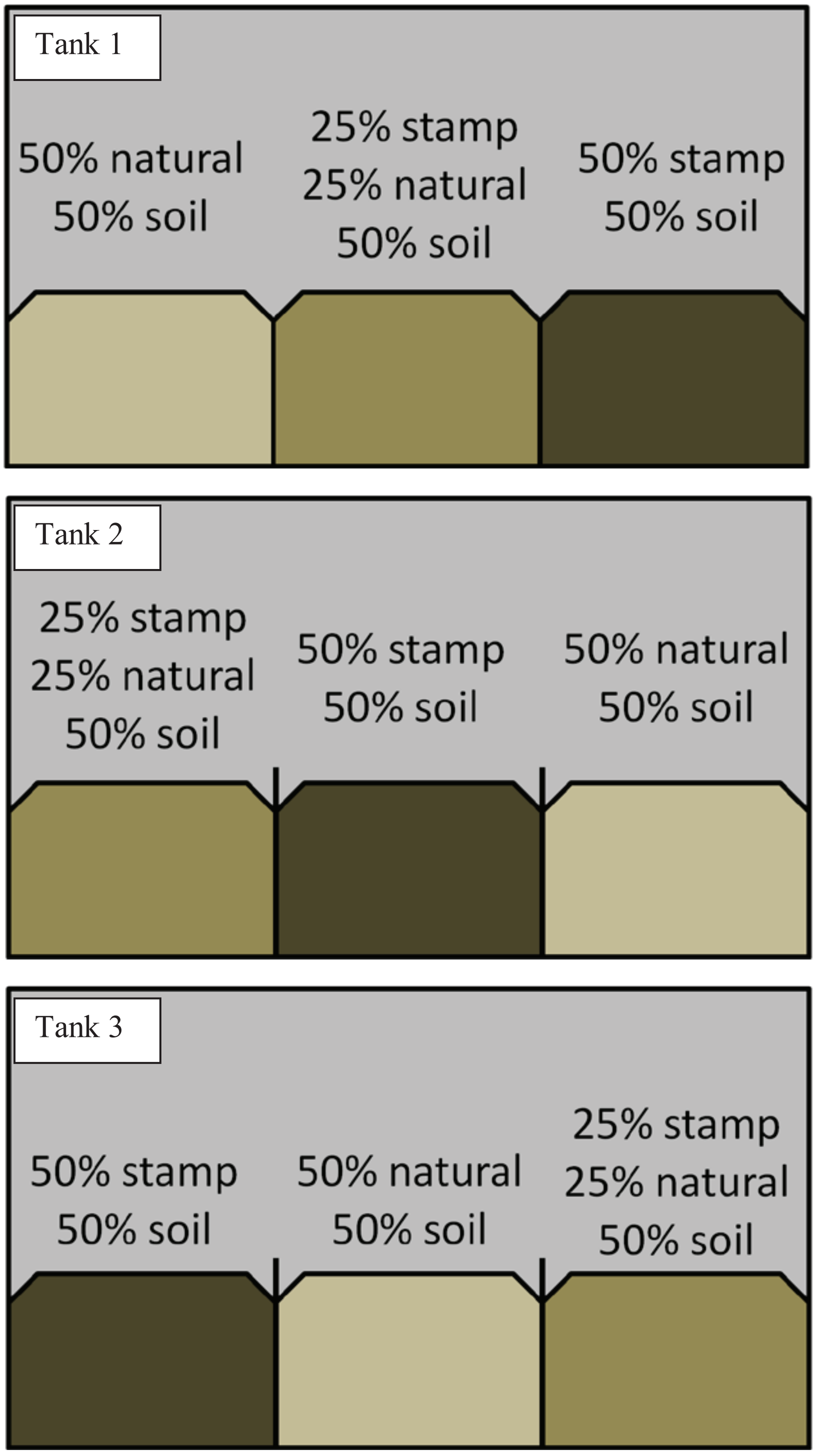


Figure A.1. Arrangements of substrate in each aquaria for first five trials of earthworm selection experiments. Earthworms were not marked in these trials.

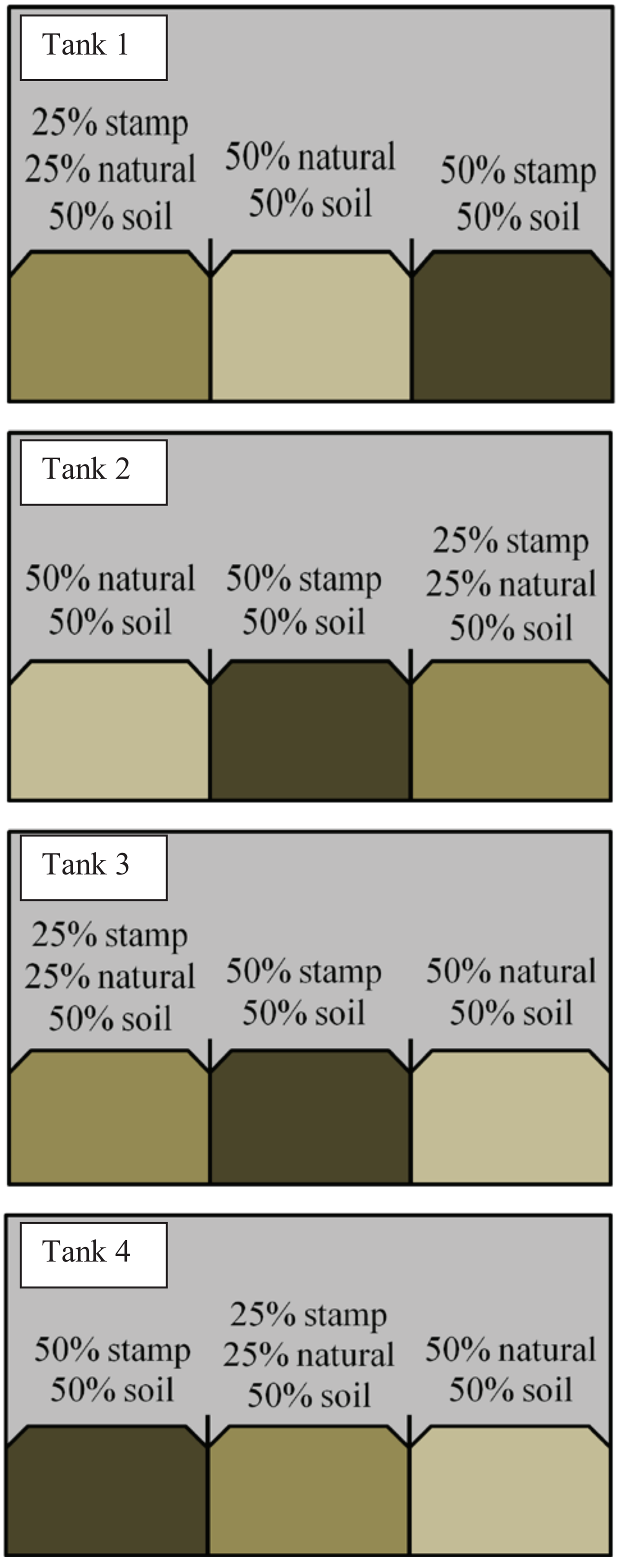


Figure A.2. Substrate arrangement in each tank for five earthworm substrate selection trials. Earthworms were marked in each of these trials.

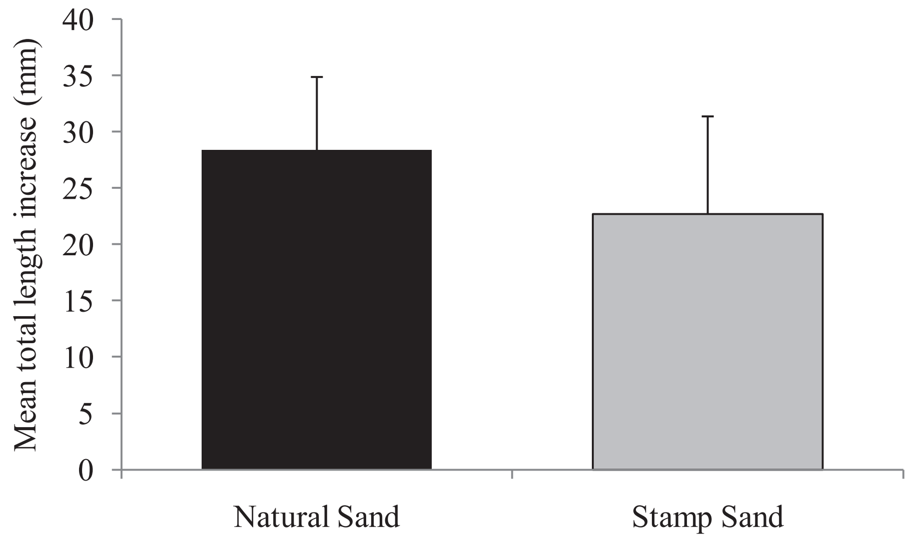


Figure A.3. Mean total length increase (mm) of lake sturgeon during growth experiment at Ontonagon streamside rearing facility. Weekly measurements started on June 29, 2010 and ended August 11, 2010.

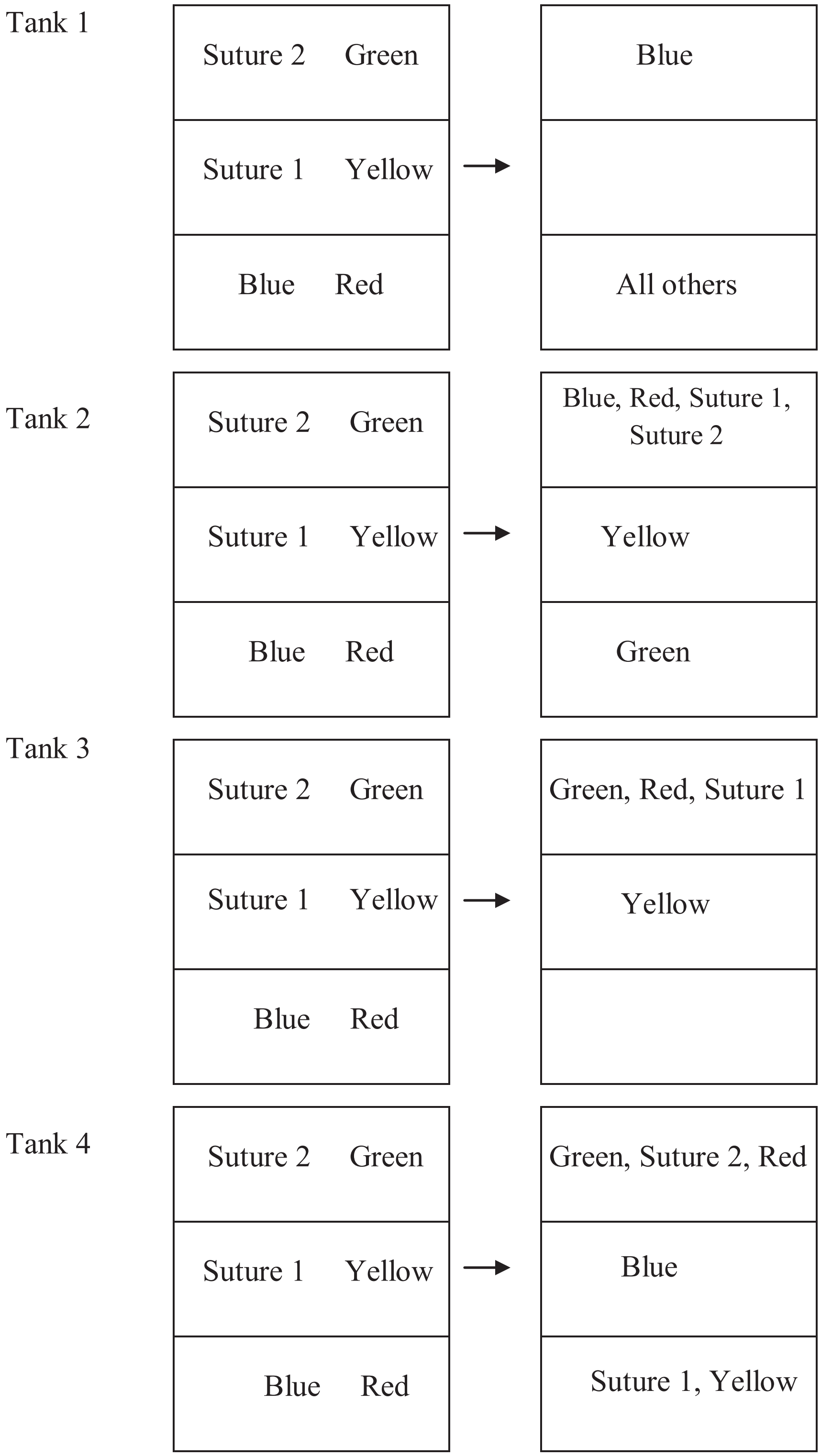
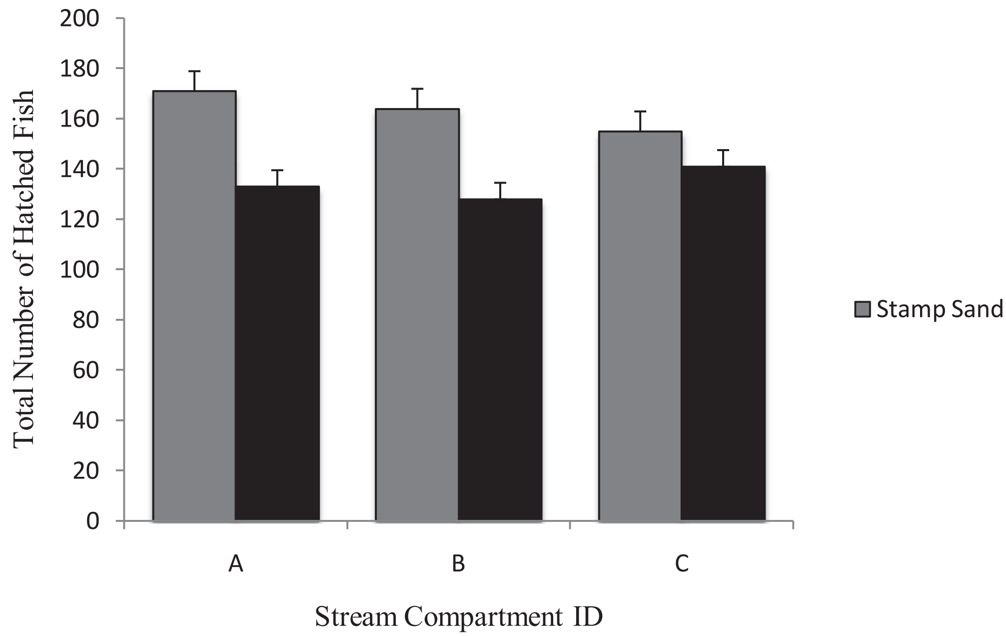


Figure A.4. Original placement of each earthworm is indicated in left column. After 72 hours, worms were located, as shown in the right column. Two worms died during the above trial (one of five total).

a.



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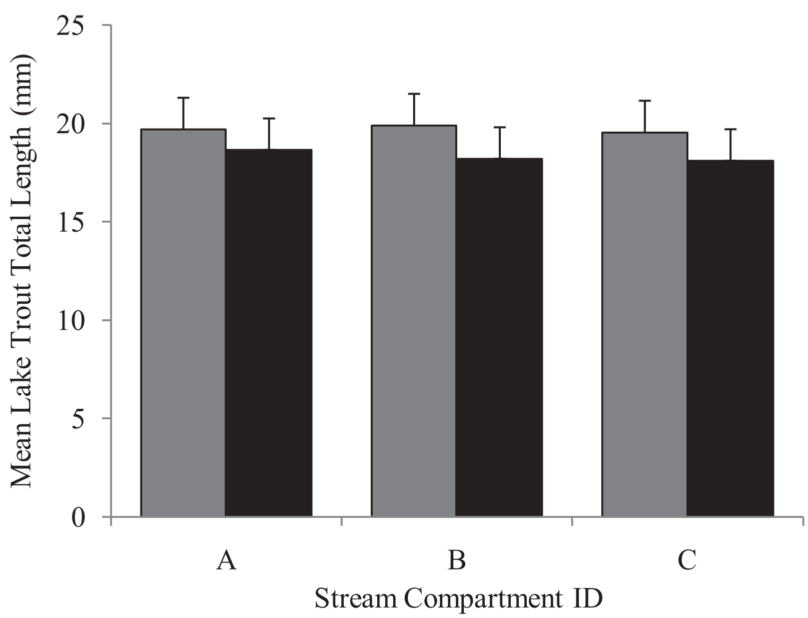


Figure A.5. Total number of lake trout embryos (±1 SD) hatched per stream compartment (a) and mean initial total length (mm) (±1 SD) of lake trout in each stream compartment (b) (A, B, or C). Compartment A is closest to the circulator and compartment C is farthest.

Appendix B

AP Biology Experiment

In the fall of 2009, preliminary stamp sand research was conducted as part of an AP Biology class experiment. In this experiment, amphipods, bloodworms, and planaria were placed in containers with either 200 grams of stamp sand or natural sand and two liters of lake water and survival was monitored for six weeks.

In six containers, 20 planaria were suspended over the two substrate types, three containers for each stamp sand and natural sand. All of the planaria survived the entire six week experiment.

Six more containers housed 10 amphipods and 10 bloodworms. The amphipods were fed *Elodea* every other day and allowed to roam free throughout the containers. The bloodworms were housed in mesh drawstring bags with 10 grams of leaves and washed in the containers.

Both the natural sand and stamp sand bloodworms experienced population declines of 90% during the first two weeks. All of the bloodworms had died over natural sand by week five of the experiment, while the remaining 10% of the stamp sand bloodworms survived until the end of the experiment (Figure B.1a).

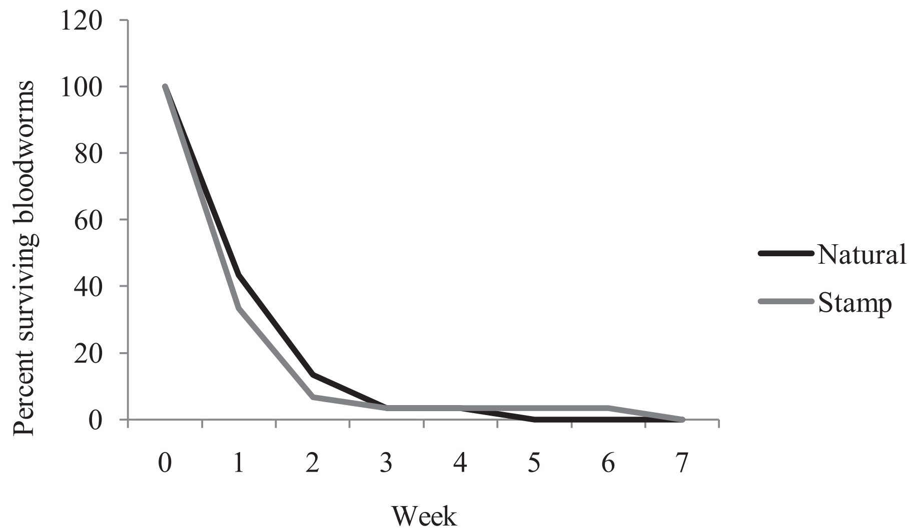
The amphipods had higher survival rates than the bloodworms, and the amphipods housed on natural sand had higher success rates than the stamp sand organisms. Amphipods in both groups survived to the end of the experiment (Figure B.1b).

This preliminary study allowed us to test our experimental design and examine the effects of stamp sand on secondary producers. Additionally, we performed initial substrate selection experiments using both earthworms and rusty crayfish. Five earthworms were placed into one five-gallon aquarium that had two inches of stamp sand covering half of the bottom and two inches of natural sand covering the other half of the bottom. Worm location was noted multiple times daily over the course of two weeks, and it was found that the earthworms strongly preferred the natural sand over the stamp sand. Distilled water was added daily to keep the sand moist. This initial experiment was the foundation of our later successful earthworm substrate selection experiments.

Three rusty crayfish were placed into one five-gallon aquarium filled with five liters of water and similar substrate as the earthworm tank. Four plastic flowerpots were cut in half and placed upside down to provide shelter for the crayfish. The crayfish did not display a clear preference during this study, and the results were inconclusive.

Appendix B Figures

a.



b.

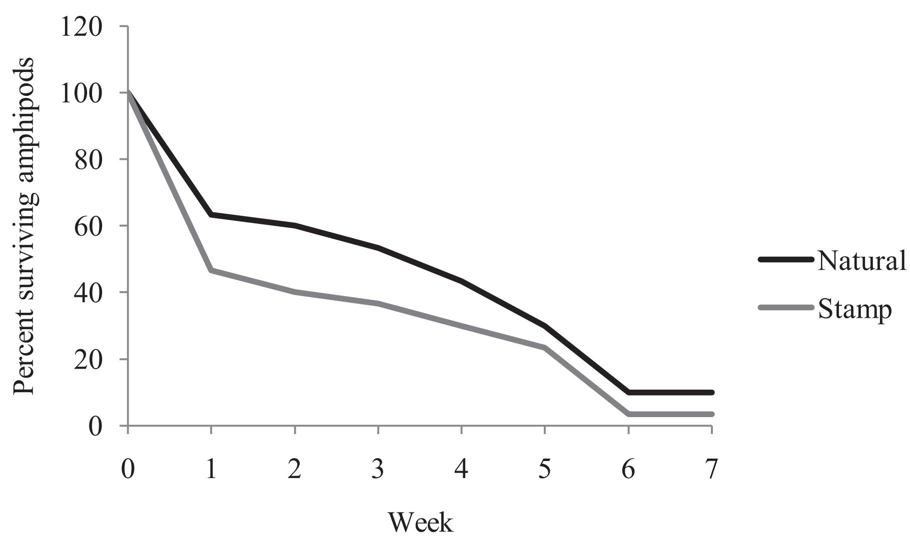


Figure B.1. Percent surviving bloodworms (a) and amphipods (b) during duration of initial stamp vs. natural sand study in Fall 2009.