# ***Dreissena polymorpha*: An Observational Chemical Attachment Study**

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Abstract

Zebra Mussels (*Dreissena polymorpha)* are an invasive species that have taken over many of the waterways within the United States, specifically the waterways of the Great Lakes region. Due to its ability to attach to almost any substrate both in and on the water's surface with its byssal threads, *Z. polymorpha* are responsible for destabilizing docks, sinking marker buoys, weighing down watercraft, and clogging water facility intake pipes, costing consumers millions of dollars per year. In this study, Zinc and Magnesium multi-vitamin tablets were crushed and physically applied to different PVC pipe substrates. Three separate three day trials of this experiment were conducted to make a comparison with how effective each chemical was in preventing byssal thread attachment to the substrate. It was found that zinc was more effective than magnesium in preventing attachment. The importance of this experiment is that some chemicals are more effective in preventing zebra mussel attachment than others. This information can help researchers and engineers in designing anthropogenic substrates infused with chemicals that can minimize zebra mussel attachment.

**Introduction**

Invasive species are one of the major causes why extinction of native organisms occur, costing an estimated $120 billion of economic loss as a result of the introduction of non-native species (DeFazio 2013). An invasive species, defined by the US Fish and Wildlife Service, is an organism in which is not native to an ecosystem, and causes damage to the environment, humans, and the economy (USFWS 2012). Invasive species can be a variety of different organisms including animals, plants, insects, as well as pathogens, and can either be terrestrial or aquatic invasive species.

An example of an aquatic invasive species that has been introduced unintentionally is the Eurasian watermilfoil, a macrophyte commonly known as milfoil (*Myriophyllum spicatum)*. Introduced to North America in the 1940's originating from Europe and Asia , *M. spicatum*, is a macrophyte, resembling a feather like structure that has been sold as an aquarium plant, and was introduced to  the U.S. waterways as a result of aquarium disposal (WAECY 2013). In high densities, milfoil forms thick mats at the water's surface, becoming entangled in watercraft rudders and propellers, and is transported as a result of boaters not checking and washing their watercraft before entering a new body of water (MNDNR 2013).  It has no predators residing in North America at the moment, resulting in declines of native freshwater macrophytes, due to competition (MNDNR 2013). A study conducted in Indiana has a projected number of $1.2 million per year losses in that state alone as a result of trying to manage and control the exotic plant (INDNR 2009).

Some aquatic animals are an equal menance. An example of an aquatic animal invasive species which results in similar environmental impact and costs is that of the zebra mussel (*Dreissena polymorpha). D. polymorpha* is a mollusk of the family Dreissenidae that is invasive to the continent of North America. It is a filter feeder mollusk and can inhabit almost any freshwater source. Zebra mussels are native to Russia (Ludyanski et al.1993) and reports have shown that they are have been established in the Great Lakes as an invasive species in the last twenty-five years (Peyer et al. 2011) specifically beginning their invasion in the 1980s when they entered the St. Lawrence seaway via ballast water on ships (Appledorn et al. 2006). Similar to most invasive species, zebra mussels have no natural bird and fish predators living in North America (Schloesser et al. 1994), and are able to attach to any surface, known as a substrate (KDWPT 2013). A substrate can be a natural surface within the aquatic body of water including a rock, an aquatic plant or animal, (including other mussels), or a man made surface including piers, water craft, marker buoys, and pipes from industrial facilities located along the shores of a water body (Cibrowski 2007).

Zebra mussels are a major issue in United States waterways for a variety of reasons. Zebra mussels have the ability to latch onto any man-made surface that resides in or floats on top of the water surface. Due to *D. polymorpha* having the ability to attach to any substrate, they are a concern in almost every aspect of the water environment (KDWPT 2013), both for the ecosystem and humans alike economically *D. Polymorpha* attach to safety and navigational marker buoys floating in the water, sinking them below the surface resulting in recreational hazards (Williams 2007). Zebra mussels also latch onto boat hulls, adding unnecessary weight to the water craft, resulting up to a fifty-percent increase in both cost and fuel consumption (Stanzcak 2013). In addition to being a nuisance on public beaches and shores, zebra mussels also infest drinking water and clog factory pipes and waterways by forming colonies, or large groups of mussels (Sea Grant 1991) causing water to not be able to flow as easily through the pipes, which may lead to water not being able to flow at all (KDWPT 2013). As a result of *D. polymorpha* clogging intake pipes, they cause obstruction and harm interior plant structures in which are needed to produce clean drinking water, and produce energy for electricity (Rhode Island Sea Grant 1991). It has been estimated that about $1 billion annually will be spent on both removal and control methods to try to minimize the spread of zebra mussels as much as possible (Peyer et al. 2010).

A wide variety of control methods and management techniques have been used over the years to try to resolve the impacts of this invasive species caused by their fast dispersion and establishment. Manual removal, surface chemical-coatings, radio wave exposure, and thermal techniques all have been implemented to try to decrease the numbers of *D. polymorpha (*Ludyanski 1993) and their ability to attach to substrates. Even though manual removal by scraping or power washing the mussel off the substrate is a quick and safe solution that doesn't impact or harm native species, manual removal is very time consuming, laborious, and is only a temporary solution that will constantly need to be replicated. A number of studies have been conducted using a wide array of chemicals to inhibit the attachment of zebra mussel colonies and infestations to a substrate. A study conducted by Cope et al. 1997, suggested that chemicals with antioxidant properties inhibit attachment of zebra mussels to substrates due to interfering with the catalytic enzymes involved with byssal thread development ranging in gradients from 0.4 to 5.4mg/L. Some of the chemicals that Cope used that may limit byssal thread development include tannic acid, butylated hydrxyanisole (BHA), and Ethoxyquin, to name a few (Cope et al. 1997). It is unclear how effective different chemicals are when manually applied to a substrate.

The goal of this study is to gather information that will test the effectiveness of chemicals to inhibit byssal thread attachment. This study will focus on using chemicals to prevent attachment of the mussels to substrates. By using the information obtained, this information can help inform management agencies to be able to see what chemicals are the most effective for the longest period of time when applied directly to the pipe substrates.

## Literature Review

Similar to most invasive species, the *D. Polymorpha* invasion has lead to multiple political, economic, and environmental issues. As a result of the spread of zebra mussels out of the Great Lakes waterways, other regions of the United States have had to implement laws and regulations as a way to try to further prevent the dispersal of these “aquatic cockroaches” (Ludyanski 1993). Implemented and passed in 2009, known as the “Director’s Orders”, the state of Arizona issued the “Don’t move a mussel-NOW it’s the LAW” regulation in hopes to try to limit and bring awareness to the state about the aquatic invader (AZGDF 2013). The states within the Great Lakes region have implemented laws as well to try to attempt to decrease the impact and prevent any further zebra mussels invasions. In the state of Minnesota it is illegal to travel on public road with zebra mussels attached to the watercraft, and boaters must clean their boat of any aquatic vegetation, and discharge ballast water before leaving the zebra mussel infested water (Minnesota Waters 2013). In both Wisconsin and Minnesota is it illegal to collect, transport and keep *D. polymorpha*, unless an invasive species permit is obtained from the local DNR (WIDNR 2013). By implementing laws and regulations like this, the society will become an active player in the role of assisting with the invasive bivalve. If laws became an active role in the invasive species problem earlier, less money would have to be spent on the damages in which occur from them. It has been estimated that between the years of 1993 and 1999, that over $3.1 billion was spent by the power industry as a result of zebra mussels interrupting and disturbing the ecosystem by displacing native species, and cost industries, businesses and communities over $5 billion (AZGDF 2013). The only logical way in which to avoid the cost is to simply prevent the spread, and therefore the attachment of the zebra mussels.

## Life Cycle/Biology

The biology of *Dreissena polymorpha* is a contributing factor as to why they are an issue in United States’ waterways. The first stage in the zebra mussel life cycle is known as the planktonic veliger state. This phase can last between one and two weeks (New York Sea Grant 1991) depending on environmental conditions, including water temperatures ranging between 10 and 24 $^{o}$ C (Ludyanskiy et al. 1993). The veligers, or free swimming larvae, travel by drifting with the currents of the water by living in the water column (Ludyanskiy et al. 1991). During this larval phase, the veligers begin to grow their bivalve shell, marked with alternating white and black lines, giving the zebra mussels their famous trait and name. The bivalve shell begins to grow and become more heavy in weight causing the zebra mussels to no longer be able to stay buoyant, causing them to sink (DNR 2013). During the time of the maturing of the bivalve shell, another new body part begins to form on the veliger. An appendage, or a tiny foot develops (EPA 2004) marking the end of the veliger state. The appendage, sometimes referred to as a functional foot (Ludyanskiy et al. 1993) allows the sinking mussels to able to attach to a substrate in order to survive its next stages of life. According to the Department of Natural Resources, this is the stage when the free swimming larvae develop into anchored mussels (DNR 2013). With the development of the appendage, the mussels enter the pedivelilger phase. During this state, the juvenile mussels are capable of moving and crawling along the substrate with their foot reaching speeds over 3.8cm per hour (New York Sea Grant 1991). The postveliger state occurs next which consists of a few different stages. Postveligers form a shell with a triangular shape and can range between 250 and 700 microns (Ludyanskiy et al. 1993). Once the postveliger settles and finds a spot on the substrate, they form thread-like structures in which are secreted from byssal glands. These byssal glands are located in the appendage and contain schleroproteins which harden and solidify in the water, allowing the zebra mussels to firmly attach to the substrate in which they are positioned on (Ludyanskiy et al. 1993).

*Dreissena polymorpha* are sexually mature between one and two years of life (North Carolina Grant 2013) but can vary due to both geographical and environmental conditions (Luyanskiy et al. 1993). Studies have shown that zebra mussels residing in Europe sexually mature during their second year of life after reaching ten millimeters in size, but have been known to be able to sexual reproduce at sizes as small as three millimeters on the continent of North America, specifically Lake Erie and Lake St. Clair, reaching maturity earlier than their relatives in Europe (Ludyanskiy et al. 1993). Sexual maturity age most likely varies from location to location by favorable weather conditions, as well as amount of predators, or lack thereof.

For successful spawning and hatching to occur, environmental conditions must be favorable. Zebra mussels prefer a temperature range between 55-77 F (13-25 $^{o}$C), a pH of 7.5 or greater for growth and reproduction,  salinities of 0.21 to 1.47 ppt ( parts per thousand) and a current speed between 0.5-1.6 ft/s or 1.5-0.5 m/s for best growth (North Carolina Sea Grant 2013). Other optimal and favorable water conditions for reproduction and growth include a Calcium content and alkalinity greater than 3mg/L, and a dissolved oxygen content with 90 percent saturation (North Carolina Sea Grant 2013). If all of these parameters are met, the conditions for zebra mussels are favorable for both their growth and their reproduction cycle. Weather and environmental conditions change hourly, making zebra mussels being able to conform, or generalize to the specific area in which they are inhibiting.

Male and female zebra mussels are separately sexed from each other (Sea Grant New York 1991). Once female zebra mussels reach maturity, they can produce between 30,000 and one million eggs per year (DNR 2013). This process is known as spawning. Spawning can occur anywhere between two and five times a year, with an individual female zebra mussel releasing more than half of her eggs during her first spawn of the season directly into the water (Sprung 1990 as cited by Ludyanskiy et al. 1993). The eggs then drift through the water being carried by the water currents.  Even though there is a high mortality rate of 97% of veligers (Ludyanskiy et al. 1993), the high number of veligers still alive in the water, accounts for one of the main reasons as to why zebra mussels are so abundant.

## Ecological Impacts

A major impact and problem of *Dreissena polymorpha* is how it consumes its food and energy, affecting many other species in the freshwater ecosystems. Zebra mussels are filter-feeders who use a biological formation known as a siphon to vacuum in water (Smith 1997). As the water enters the mussels' siphon, plankton, and algae are retained within the mussel, with the water being discharged into the lake (DNR WI 2013). The majority of the food web depends on these primary producers for food, energy and nutrient uptake, and thus are located at the bottom of the food chain (DNR WI 2013). When plankton and algae numbers decrease, this causes shifts in species native to the lake that are dependent upon plankton and results in an unbalanced ecosystem. In a study conducted by Appledorn et al. in 2006, three native mollusk species were displaced by the invasive species of *Dreissena* polymorpha shifting the community structure by outcompeting the native and present mussels (Appledorn et al. 2006).

*Dreissena polymorpha* are causing another problem within the aquatic ecosystem in regards to their filter-feeding abilities. As a result of using their siphon to filter water within their shell, zebra mussels are greatly reducing the turbidity of water. Turbidity, usually measured by a secchi desk, is how turbid, cloudy, or how hazy the water is as a result of the amount of algae and suspended particles that are in the water (USGS GA 2013). Zebra mussels filter the algae out of the water, resulting in a cleaner, more clarified water, but results in local decreases of algae densities (Mellina et al. 1995, as cited by Ciborowski 2007). When the turbidity of water decreases, additional sunlight is able to penetrate the surface of the water, resulting in warmer limnetic zones, while increasing the amount of photosynthesis that macrophytes can undergo (NPS 2013). Other studies have shown that *Dreissena polymorpha* also negatively impact chlorophyll a content. In a study conducted in Lake Erie, researchers have found that water clarity has increased from a two to three fold and that chlorophyll a content within the water has dramatically declined due to phytoplankton (New York Sea Grant 1991). In 2010, another study conducted by Higgins and Vander Zanden, showed that zebra mussels caused an impact on phytoplankton. They found that zebra mussels decreased the amount of water column phytoplankton biomass by 35%-78% in bodies of water were zebra mussels were present (Kirsh et al. 2012). In another experiment conducted at the Kellogg Biological Station in Michigan in 1999, the scientists performed a mesocosm study in ponds, to determine if the presence of zebra mussels had an effect on phytoplankton volume. After one week into the study, it was found that when *D. polymorpha* are present, phytoplankton biovolume decreased by 53% (Wilson 1999). Algae are food to other organisms within the aquatic ecosystems, but as the zebra mussels continue to filter feed out these sources of food in which organisms throughout the ecosystem need in order to survive dramatic changes are occurring. Higgins and Vander (2010) analyzed data on the impact of *D. polymorpha*, and discovered that these invasives are causing a 40-80%

decline in the entirety of the food chain located in the pelagic zone, but had a 2-10x increase of the food chain located in the benthic zone (University of Wisconsin 2008).

One of the major negative impacts that *Dreissena polymorpha* cause is known as feces and pseudofeces which change and alter both the physical and biological composition of the benthic zone within the aquatic system (Stewart et al. 1995, as cited by Perry et al. 2003). Similar to all organisms, zebra mussels excrete waste products in the form of feces. Feces particles are larger in size than particles of food which were eaten, and dynamically change the structure of benthic zone by causing feces buildup in this area of the lake (Cibrowski 2007). By the zebra mussels excreting the feces on the bottom of the aquatic area, this transfers the energy in the ecosystem from that of the open water pelagic zone, which was once in the form of phytoplankton, to the benthic/bottom area of the lake, now in the form of feces (Cibrowksi 2007). Due to zebra mussels being filter feeders, they filter in particles in which are contaminated, specifically algae contaminated by polychlorinated biphenyls (PCBs), which in turn are released during the excretion of feces, and then consumed by benthic invertebrates, who are eaten by larger and bigger organisms in the food chain, transferring the PCBs to these other organisms (Cibrowski 2007). Known as a process called bioamplifcation, the PCBs concentrations increase as you move up the food chain resulting in these larger organisms containing higher amounts of the PCBs (Van der Hoop 2013).

Of the two types of feces, pseudofeces negatively impacts the aquatic ecosystem the most by being an undigested waste product (Roditi et al. 1995). Pseudofeces are materials which collect on the gills of the zebra mussels and aren't filtered through to the mussel's gut as a result of rejection (Cibrowkski 2007). Pseudofeces can also consist of particles covered in mucus and then discharged (New York Sea Grant 1991). A very interesting fact about zebra mussels in regard to pseudofeces is that they are able to reject or inhibit inedible plankton from their system. A study conducted at the Great Lakes Environmental Research Laboratory (GLERL) located in Ann Harbor, Michigan found out that many species of blue green algae aren't favorable for zebra mussels and other aquatic organisms (Cibrowski 2007). *Microcystis aeruginosa*, a type of blue-green algae is harmful due to its ability to form colonies and mats on the surface of the water resulting in high amounts of the toxin microcystin into the water (OEHHACA 2009). The researchers discovered that the zebra mussels have selective feeding habits, and will filter any other particle out of the water except, *Microcystis*, which is filtered back into water, but its predators are filtered by the zebra mussels. *Microcystis* is toxic to other aquatic life when high levels of it are reached (Cibrowski 2007).

Even though zebra mussels are causing very negative impacts on the majority of the aquatic ecosystem, there has been some research showing that these invasives can be a positive impact on some macroinvertebrates. Macroinverterbrates are organisms that can live in complex structures, or areas within in a river or a stream with many tight crevices in which to hide and live. Examples include various species of snails and caddisflies. In a study conducted by Horvath et al. (1998), the scientists were interested in seeing if the abundance of zebra mussels on a substrate would increase the abundance of macroinvertebrates as a result of zebra mussels providing more surface area (shells) for the macroinvertebrates to live in and upon (Horvath et al. 1998). The researchers had this expectation because *Dreissena polymorpha* increases the surface area and living space for macroinvertebrates. The experiment lasted a total of twenty-eight days, and their results supported their hypothesis that an abundance of zebra mussels on rocks significantly increased the abundance of macroinvertebrate living in the stream (Horvath et al. 1998). The number of macroinvertebrates increased with the rise of *D. polymorpha* because the organisms had more surface area to procreate, hide from predators, and had more benthic organic matter (BOM) from the feces of the mussels to thrive in (Horvath et al. 1998).

Although *Dreissena polymorpha* increases the surface area and living space for macroinvertebrates, other aquatic organisms in the ecosystem are impacted in a negative way. The Eurpoean bitterling, *Rhodeus amarus*, a fish species native to British fresh waters, is one such species that has been impacted. *Rhodeus amarus*, spawns and lays its eggs in unionid mussels, or common clams, that have bilateral symmetry (GPNC 2013) which are being outcompeted by zebra mussels both for habitat and food (Ermassen et al. 2010). The researchers completed an experiment in which quantified the impact of zebra mussel attachment to the unionids which directly correlated to the amount of eggs that were layed by *Rhodeus amarus* in the native mussels (Ermassen et al. 2010). One of the main reasons why *Dreissena polymorpha* is such a large issue is of it's ability to be able to attach to any surface or substrate in it's path, known as biofouling.

By definition biofouling is the attachment of an organism to a surface or a substrate that is in contact with the water over an extended period of time (Stanczack 2013). Due to the zebra mussels strong attachment ability to any substrate with byssal threads causes it to be an extremely harmful and expensive biofouler. By an estimation conducted by the US Fish and Wildlife service, biofouling as a result of zebra mussels disturbing industrial, utility, municipal and water use facilities could reach about $5 billion in the Great Lakes region (Ludyanski et al. 1993). As a result of biofouling on marine transportation, fuel costs and consumption increases up to 50% (Stanczack 2013). Not only is biofouling resulting in losses in the economy, zebra mussel biofouling is also harming native aquatic species. In a study conducted by Appledorn et al. (1993) in Douglas Lake in Michigan, the researchers found that one native species of snail, *Elimia liveseens*, traveled 1.5-1.6 times farther in areas where zebra mussels were not present (Appledorn et al. 1993). With this particular species of snail, when *D. polymorpha* are present, they inhibit the traveling capabilities of this snail to be able to move from place to place easily to find food, and even potential mates. When zebra mussels are present, native species of snails and mollusks are negatively impacted because the *D. polymorpha* may interfere with reproduction habits, predator avoidance and evasion, as well as trying to avoid unfavorable environmental conditions (Appledorn et al. 1993). They also tested the mobility of two native species of unionoid bivalves (*Ligumia nasuta* and *Anodonta grandis)* and found that areas without zebra mussels present result in more mobility, concluding that zebra mussel biofouling on the native mollusks may be a direct result in these species declines due to the invasive mussels attaching and adding excess weight to the native ones (Appledorn et al. 1993). The zebra mussels may cause localized extinctions of the native species of unionids because the excess weight causes mobility constraints on the natives not allowing them to disperse (Appledorn et al. 1993).

Biofouling of *Dreissena polymorpha* is a major issue due to the amount of the invasives within our waterways. Biofouling is very costly, both to the environment, as well as to the water facility industry, and boating industry. As a result of zebra mussels colonizing in fresh-water intake pipeways, water flow has been restricted and may result in polluted waterways. Control methods can be used, but are generally very time consuming and costly.

**Management Techniques to Control Zebra Mussels**

Several actions and techniques have been created, researched, and implemented in order to slow down and stop the dispersal of *Dreissena polymorpha.* One of the most important and simplest ways to attempt to avoid continued dispersion of zebra mussels is that of education. Education and awareness is key to any problem dealing with invasive species. Due to *Dreissena polymorpha* attaching to hulls of ships, boats, and being transferred by ballast water, it is vital to inform boaters of the high risks involved with invasive species (Cibrowski 2007). A project titled the Lewis and Clark Project, created by the Pacific States Marine Fisheries Commission is a six step program that brings awareness to boaters by having a program that includes regional publicity, containment strategies, as well as quarantine plans, has shown great promise in leading the prevention of zebra mussels westward (ANS Task Force 2004 as cited by Cibrowski 2007). Projects similar to the Lewis and Clark Project inform boaters that it is vital to have important boat hygiene (USGS 2013). One of the simplest ways to practice this is for boaters to wash and clean their boat with warm soapy water before transferring the boat to another body of water (USGS 2013). In addition to cleaning and disinfecting their boat, boaters should also inspect watercraft, including seines, buckets, motors, trailers, and pumps if boating in zebra mussel infested water (North Carolina Sea Grant 2013). By bringing awareness to the boaters about zebra mussel infestations can decrease the dispersal rate of these invasive organisms.

Although it is too late for many of the freshwater waters in our area to prevent the infestation of *Dreissena polymorpha*, there are methods in order to try to reduce this nuisance organism by interfering with its attachment ability by its byssal threads to substrates. Manual scraping of the zebra mussels off of their substrates is one of the main ways to stop attachment. Manual scraping involves physically removing the mussel from its substrate by scraping its byssal threads off of the substrate. This is the safest way to remove the invasive because it doesn't interfere directly or harm other forms of aquatic life. Manual scraping, or sometimes referred to as mechanical removal, is a very laborious, time consuming technique that is only effective for a certain amount of time (USGS 2006). It will only be effective until a new spawning of zebra mussels comes to that area or substrate, and attaches their byssal threads to it.

Thermal application is another technique that has been used to try to eliminate *Dreissena polymorpha* colonies. Studies have shown that zebra mussels cannot survive in bodies of water where temperatures exceed roughly between $32^{o}C$-$39^{o}C$ (Elderkin 2005) (Cornell University 2008). This technique is implemented by flushing the colonized area and substrate with hot water reaching these temperatures which is the main control method used by industrial facilities to rid of the mussels from clogging their water intake pipes (Cornell University 2008). Industrial facilities that use this method expose the invasives for a minimum of four-hundred and nineteen minutes in order to obtain 100% mortality rate of the mussels (Cornell University 2008). Although this technique is efficient in eliminating the aquatic nuisance, non-target species such as fish and native mussels may be at risk if they are in the same thermal application area.

Another control method that has been studied to try to decrease the current colonies of *Dreissena polymorpha* is called extremely low frequency (ELF) radio waves. Scientists believe that these ELF waves can kill and eliminate zebra mussels without causing any harm or disturbance to the environment or other species (Long 2001). It is considered a technique in which is nonchemical, and economically sound in reducing the amount of zebra mussel infestations by dying within forty days of being exposed to the ELF waves (IL-IN Sea Grant 1999). Researchers even believe that mussels that are exposed to the ELF waves will not be able to undergo normal growth patterns, specifically mussels in the larvae and veliger state, if exposed to these waves will be unable to develop and grow normal bivalve shells ultimately resulting in not being able to mature, develop, and reproduce (IL-IN Sea Grant 1999). The ELF waves interfere with the zebra mussels biology destroying their ability keep and absorb calcium needed in order grow (Long 2001). Although researchers believe that the ELF waves will not harm any other species in the aquatic environment, further studies will need to be conducted before this technique can be successfully implemented to ensure other calcium building organisms are not affected.

Scientists have also been trying to intervene with zebra mussel success and infestation rates by manipulating their ways of spawning and reproduction. Researchers have found that the chemical, Serotonin, may lead to zebra mussels artificially spawning when exposed to this chemical (Michigan Sea Grant 1991). If this technique is successful, scientists may be able to manipulate zebra mussels into spawning when levels of phytoplankton are low, thus having little food availability when the larvae hatch from their eggs, leading to die off before the veligers have a chance to settle on a substrate (Michigan Sea Grant 1991). It is unclear how the exposure of serotonin can affect non-target species and humans if they are exposed to this chemical.

Even though scientists are still in the development stages of creating and inventing ways to manipulate zebra mussels by using ELF waves and interrupting their spawning cycle, researchers have developed other management techniques that can be a temporary resolution to zebra mussel biofouling. Scientists have used surface coatings to try to eliminate zebra mussel colonies on anthropogenic substrates. Sometimes referred to as antifouling coatings, surface coatings have been primarily used in water systems (Boelman et al. 1997). Although surface coatings are known to leach aquatic toxins, specifically cuprous oxide, they are effective for a two to five year time span (Boelman et al. 1997). A different type of coating known as foul-release coatings placed on the pipes or any other type of water facility equipment are considered to be a slippery surface that reduces the adhesion of the byssal threads of the zebra mussels and its ability to attach to the substrate (Boelman et al. 1997). Reports have shown that chemicals have been used to minimize or stop the reattachment of zebra mussels to substrates (Cope et al. 1997).

Over the years, scientists have employed numerous techniques using chemicals to try to deter mussel attachment abilities as well as their development abilities. In a study conducted by Cope et al. 1996, their objective was to be able to identify candidate chemicals that could either inhibit the development of byssal threads or their attachment abilities. The scientists exposed a range of chemicals to zebra mussels with gradients ranging from 0.4 to 5.4mg/L (Cope et al. 1996). The researchers found that eleven of the forty-eight chemicals used inhibited the zebra mussels reattachment abilities, but further studies would need to be conducted to see if these chemicals impacted nontarget species, including fish (Cope et al. 1996).

In recent years, scientists have been able to identify specific metals in which inhibit mussel attachment. Most recently, the Bureau of Reclamation have made positive progress toward in finding that silicone foul release coatings had the ability to reduce zebra mussel settlement, as well as being able to easily remove exposed attached zebra mussels (Soeth 2012). However, the problem with the silicone foul release coatings is that they aren't stable and durable due to them being soft and easy to damage as a result of mechanical abrasion and floating debris (Soeth 2012). More research needs to be conducted to see if silicone has any negative environmental impacts. In previous studies, the common mussel *Mytilus edulis*, has been exposed to zinc and reattachment was deferred (Hietane et al. 1988). In a study conducted by the US Army Corps of Engineers, it was found that zinc may be of a toxic substance to that of zebra mussels because when exposed to zinc with a 5-ppm concentration for twenty-four hours, a 5% mortality rate of adult zebra mussels occurred (Race et al. 1992). However, evidence suggests that zebra mussels are unable to attach to zinc based/zinc painted surface coatings which is one of the main reasons zinc is used to surface coat concrete, steel, as well as because it can be one of the least expensive and universal applicable anti-fouling surface coatings (Race et al. 1992).

Magnesium, an alkaline earth metal is a very abundant metal within the earth and its ecosystems. It is the eight most abundant chemical element that is in the earth's crust, and is very essential for both plant and animal life (ELC 2008). Similar to zinc, magnesium is a chemical that is needed within the cells and tissues for biological functions (Dietz et al. 1994) Byssal thread composition and pathways for development are biologically and chemically made within the mussel due to glands and cells contributing to the formation of the byssal threads (Cope et al. 1997). The chemical zinc inhibits the enzymatic catalyzed reaction needed to carry out the oxidation reaction in order to produce the proteins needed to create the byssal threads (Cope et al. 1997). It is unclear how effective magnesium will be in reducing zebra mussel attachment abilities. Due to zinc being a toxic, but an effective chemical in controlling zebra mussel populations by their attachment abilities, an experiment will be conducted to test the mussels' reattachment ability using both Magnesium and Zinc in organic multi-vitamin form. It is hypothesized that magnesium will not be as effective as zinc in preventing *Dreissena polymorpha* attachment to a substrate.

**Methods**

Before the collection of *Dreissena polymorpha*, an invasive species permit had to be obtained from the Wisconsin Department of Natural Resources (DNR). A letter was written to Ms. Maureen Ferry, the Water Resources Management Specialist, in order to be issued a permit that allows one to collect, transport, and posses *Dreissena polymorpha.* The letter specified details including the approximate quantity of specimen needed, that the applicant was a student conducting a senior thesis research experiment under advisor's supervision, where the specimen would be collected from, how they would be disseminated and prevented from spreading into the wild, and how the experiment being conducted was an observational reattachment study. Within a week, the invasive species permit was issued electronically.

Collection of *Dreissena polymorpha* was conducted on Saturday, October 26th, 2013 from Silver Lake located in Silver Lake Wisconsin. Silver Lake was chosen due to its history of having high infestation rates of zebra mussels all throughout the lake, including near the boat launches, as well as in more shallow woody waters. This location was also chosen as a result of high success rate for finding zebra mussels for experiments, as well as the high amount of boating and recreational activity from both locals and tourists alike.

Zebra mussels were collected from any substrate which they were attached. Some of the mussels were attached to rocks located on the shallow bottoms of the lake located near the shoreline. Other mussels were collected from fallen tree limbs and branches either floating on the surface of the water, or laying on the bottom of the shallow water's edge. A number of mussels were collected from both substrates by manual scraping removal methods by using a razor blade or a knife. Three hundred mussels were collected in total. Once the mussels were obtained from the substrates, they were placed into a five gallon bucket with water from Silver Lake. An additional five gallons of Silver Lake water was also collected in a separate container to be able to feed the mollusks during the duration of the experiment with the suspended particles algae and plankton in the water.

Nine separate sixty-four ounce jars were used for the experiment each containing about one liter of water from Silver Lake. A filter was then placed in each bowl to circulate the water and to make sure that water was circulating and moving in order for the zebra mussels to have dissolved oxygen in order to survive as seen in *Figure 1*.

**Figure 1- Set up of nine separate bowls containing PVC pipes and zebra mussels.**

Each jar had ten mussels placed upright onto the substrates as seen in *Figure 2*. Before the mussels were placed into the rest of the bowls, the substrates had to have chemicals applied to them. Nature Made brand Zinc and Magnesium 50mg powder vitamin tablets were used. The tablets were crushed, and then weighed. About 5.4mg of Zinc was physically applied to one substrate by spreading it across a four inch long by a ¼ inch wide pvc pipe. 5.4mg of Magnesium was then physically applied to a separate pvc pipe of the same size. The third pvc pipe contained no multi-vitamin supplement. Before the pipes were placed into the tanks with water, the substrate was set aside to dry for a twenty-four hour period. Each of the pvc pipes were then placed in separate jars on the bottom, replicated three times.

**Figure 2-Zebra mussels placed on PVC substrate pipe.**

Ten zebra mussels were placed into each bowl sitting upright on the pvc pipe. The mussels were checked every day for a three day period to see if reattachment occurred in this initial period. Three day trials were used because mussels have a tendency to reattach within a twenty-four to forty-eight hour period. After the completion of this trial, two more separate three day trials took place, for replication, with different sets of mussels to see if a trend occurred with any of the chemical treatments. Different mussels were used for each trial to ensure that the attachment ability of the mussels were not inhibited by being disposed to chemicals in prior trials.  In each trial, three different measurements were taken making a total of nine measurement periods for the experiment. During each measurement, a count was made to determine if the chemicals prohibited reattachment of the mussels to the substrate. To see if the mussels attached or not, the substrate was lifted, turned over, and shaken gently five times. If mussels fell off, they were considered unattached, and if they stayed on, they were considered attached.

When the experiment was completed, the mussels were killed with bleach and were then placed in a biohazardous waste receptacle to ensure that the zebra mussels used in the experiment were not let back into the environment.

An ANOVA, or an Analysis of Variance, was run to determine if there was any correlationship between the success and failure rate of each chemical at Silver Lake, in order to identify statistical significance. The treatment variables measured were the control pvc pipe with no chemical as well as the chemicals, zinc and magnesium. The response variables measured were how many mussels each treatment prevented attachment of the mussels to the substrate. The ANOVA generated p-values based on both group variation and within group variation. By being able to compare these numbers, it helped determine if there was a statistical difference if the between group variation was greater than the within group variation, as well as differences greater than random chance, due to the treatments of zinc and magnesium. If the p-value was greater than 0.05, the null hypothesis was rejected, but if it was less than 0.05, the null hypothesis will be accepted and means there is a statistical significance, or a 95% certainty that there is a relationship between the variables being tested. If the null hypothesis is rejected, that means there is no statistically significant difference between the success and failure rate of each chemical that was used.

**Results**

At the conclusion of the experiment, 180 mussels were exposed to the chemical of zinc and magnesium. Zinc was more effective in the reattachment ability of the zebra mussels than magnesium, as well as with no chemical at all. Out of the three separate trials, four mussels attached to substrates applied with zinc, while 86 did not attach as depicted in table 1. The mussels exposed to the magnesium had equal amounts of attached mussels and unattached mussels each totaling 45. Only 4% of the mussels exposed to zinc attached to the pvc pipe, whereas 50% of the mussels attached to the pipe with magnesium. The control pvc pipes with no chemical application had a 98% success rate of the reattachment of the zebra mussels.

Figure 1 compares and contrasts the mean amount of mussels that attached to each kind of treatment substrate. For the entire experiment, zinc had the lowest amount of mussels that attached to the substrates. The averages of attached mussels for the control, zinc, and magnesium applied substrates were 28.67, 1.33, and 15, respectively. The zebra mussels set upon the zinc applied substrate had a mean standard deviation of ±0.57. The mussels in which were attached to the magnesium applied substrate had a standard deviation of ±9.165 and the mussels that attached to the control PVC pipe had a standard deviation of ± 1.555.

The data retrieved from running an ANOVA in SPSS included a p-value with all of the data joined together and had a p-value of.141. Due to this value being $p>.05$, there is no statistical difference between the variables. Overall, zinc had higher rates than magnesium and the control in preventing zebra mussel attachment to the substrates with their byssal threads.

**Table 1-Percentage of zebra mussels that did reattach to the substrate.**

| | **Zinc** | **Magnesium** | **Control** |
|---|---|---|---|
| **Amount that Reattached (**Out of 90) | 4 | 45 | 88 |
| **Amount that did not reattach** (Out of 90) | 86 | 45 | 2 |
| **Percentage** | 4% | 50% | 98% |

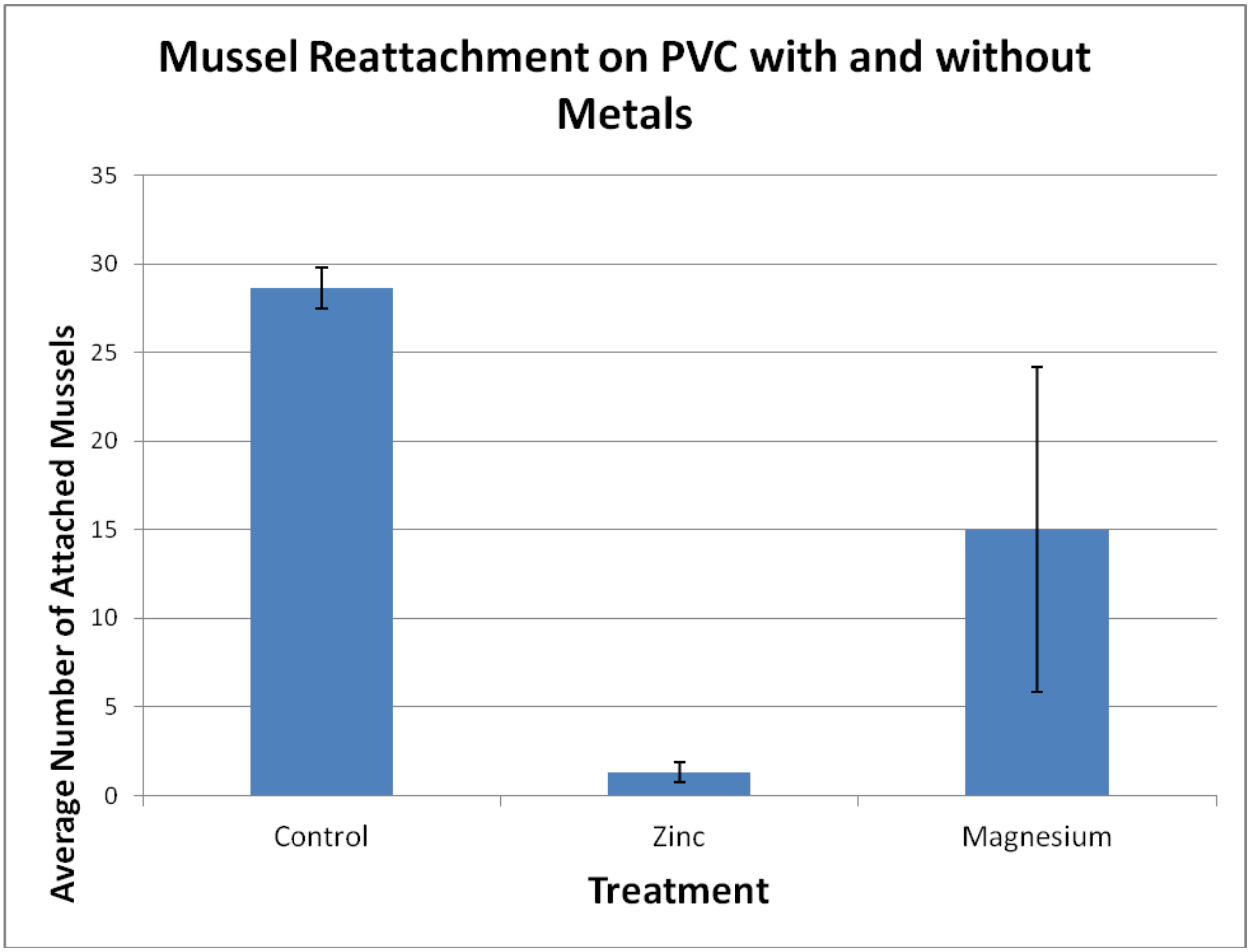


**Figure 1.** This graph demonstrates the average amount of attatched mussels per applied treatment as well as the standard deviation.

**Discussion**

The results obtained supported the hypothesis that magnesium will not be as effective in preventing *Dreissena polymorpha* attachment to a substrate by the mean values of the mussels that attached to the substrates. In the experiment, 50% of the mussels that were placed on the magnesium applied substrate successfully attached with their byssal threads, whereas only 4% of the mussels that were placed on the zinc applied substrate were able to attach.

The ANOVA test, as seen in Table 3. shows there isn't a statistically significant difference between the success rate of zinc and magnesium inhibiting the attachment of byssal threads to substrates. When all of the data from all three of the trials were joined together in a single ANOVA, the p-value was .141. Due to this value being greater than $p<.05$, there is no statistical significant difference between the treatment variables and the null hypothesis can be rejected. Although there isn't a statistical significant difference between the chemicals of zinc and magnesium, this doesn't mean that zinc is less effective than magnesium in preventing mussel attachment. The mussels that were set upon the zinc applied substrates, had a mean of 1.33 of successful attachment, whereas, the mussels that were set upon the magnesium applied substrate, had a higher mean of attached mussels being 15.

By citing these results, one can infer that zinc is a more powerful chemical than magnesium in preventing byssal thread attachment in zebra mussels. The chemical of zinc occurs as an effective deterrent to the mussels because they may have the ability to sense that it is a toxic chemical, and thus do not attach themselves to surfaces where zinc is present (CPS 2013). Magnesium may not be toxic or as toxic of a chemical as zinc is to zebra mussels which may be a reason why such a higher amount of mussels were able to attach to the substrate coated with magnesium. Higher amounts of zebra mussels may have attached to the magnesium substrates more than the zinc covered ones due to less enzyme interference in byssal thread formation and attachment than the chemical of zinc causes. Although magnesium was not as effective as zinc, it was more effective than having no chemical treatment at all applied to the substrate. The mussels that were applied to the control substrates had a total mean of 28.67 of attached mussels. This average is higher than both of the other means that were for the chemical treatments showing that substrates applied with chemicals have higher success rates in preventing mussel attachment than no chemicals at all.

The results collected in this study can be compared to other studies that used chemicals to try to prevent attachment of zebra mussels to substrates. One such study, as previously mentioned by Cope, et al. 1996, used a variety of different chemicals to try to inhibit mussel attachment. The mussels were collected from the field specifically in the waters of the Midwest and were brought back to the lab for experimentation. After exposing the mussels to a variety of different chemicals for a forty-eight hour period, they found that eleven of the forty-seven chemical tested inhibited mussel attachment. This study correlates with the current study that some chemicals prevent attachment of zebra mussels by their byssal threads better than others.

Another study that used chemicals to try to inhibit mussel attachment was by Costa, et al. 2008. In this study, the scientists used molluscicidal agents at various times during the year to see if the responses of the mussel to the substrate were different during the seasonal changes throughout the year. They found that Zebra mussels were more susceptible to these agents in the warmer months of June and July than they were during the rest of the year. This was important to their study because it may save consumers billions of dollars in chemical treatments if they are deployed only during these specific months of the year.

After analyzing and reviewing all of the data from this experiment on the success and failure rates of the chemicals zinc and magnesium in preventing zebra mussel attachment to substrates, a few implications can be created to see how well the chemicals work in natural environments. According to the results of this study, chemicals applied to substrates work better in preventing mussel attachment than no chemicals at all. Due to physical chemical application to substrates in the real aquatic world not being very ideal or safe for the environment, engineers and scientists can work together in creating products with infused organic zinc into these substrates. Zinc can be implemented into water facility intake pipes when they are created in the factory, to reduce the amount of mussels in which attach to the pipes resulting in clogging and slower water flow into the industrial facility. Scientists can also implement zinc on the bottom of boat hulls to reduce the amount of mussels that can attach to the hull, as well as reducing the amount of fuel consumption and costs that zebra mussels cause by attachment. Scientists could also work with engineers in creating marker buoys and docks with zinc implemented into their structure to reduce boating recreational hazards from sinking marker buoys and destabilizing docks into the water due to the zebra mussel infestation weight. If these implementations are taken into consideration and designed, zebra mussel infestations will be greatly reduced from the anthropogenic substrates, saving consumers millions of dollars in removal of these pests.

To improve this study, the experimental period should be greatly extended. All of the three trials took less than a total of two weeks to be completed. A number of different trials should also be added to improve the quality of the data. Chemical reapplication should also be done in future studies. In the real world waters, currents would wash away the applied chemicals from the substrate, so chemicals should be reapplied every few days to see how the numbers of attached and unattached mussels change. Due to limited space and short period of time to collect mussels, 300 mussels were collected all from the same body of water of Siler Lake. To validate the data better, one should have more mussels for this experiment in a number of trials from a variety of different locations. This would help validate the data that chemicals have the same or different affects on mussel attachment ability based on what region or body of water they are from. The more subjects there are, the more data there are, the better the data as a whole. Tanks more than a few ounces should also be used. Bigger tanks would be able to simulate lake communities more realistically. Other aquatic plant and animal species should also be placed in the tanks to determine if they are affected by the chemicals used in this study.

Besides extending the study period and having a larger number of mussels to test, other things should be done differently. Each jar had a different air pump connected to it for air circulation in the water. Six of the jars also weren't directly set up to the air pump itself, but were connected to an air tube by a t-valve connected to the air pump. This made the water current faster in some of the jars and slower in some of the others causing some of the mussels to not sit firmly on the substrates. The water current from the air pump at certain times appeared to move the zebra mussels from the substrates and onto the bottom of the jar. Some of the zebra mussels not only attached to the bottom of the jar, but also to the side of the jar, and at the top near the water's surface. Although water currents occur naturally in a natural lake or river setting, the air pumps may all have had different air flow settings resulting in varying air flow circulation in the tanks, causing some of the mussels to move throughout the tanks. This may have altered some of the results. Temperature, salinity, and the level of pH in the water was also not monitored in this experiment. The mussels were in water that were at room temperature which may have be warmer than their natural habitat and may have altered their response to the chemicals.

In the past few years, quagga mussels have also been invading the waters of the Great Lakes region where zebra mussels are present. Quaggas actually have been known to displace their invasive competitor the zebra mussel. Future studies should take into high consideration how quagga mussels are affected by the chemicals of zinc and magnesium. A recommendation would be to set up an experiment with both zebra and quagga mussels to be able to compare and contrast their responses are to these chemicals when physically applied to a substrate. Mussel samples should be from waters where both zebra and quagga mussels are present to be able to accurately compare and contrast results to see if one is more susceptible to a chemical more than the other.

**Conclusion**

Due to the zebra mussel *(Dreissena polymorpha)* invading the U.S. waterways at such a fast rate, environmental and commercial impacts have severely increased costing consumers millions of dollars per year. By gathering information on what the effects of zinc and magnesium on zebra mussel reattachment ability, this study has shown that different chemicals have different impacts on the mussel attachment ability to a substrate with zinc having higher success rates than magnesium, and both being more effective than no treatment at all. Using this information governmental agencies are one step closer in finding or creating a chemical that can completely stop zebra mussel attachment to substrates, impeding their invasive ability.

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