JOURNAL OF
EMERGING INVESTIGATORS

Article

Assessing Materials' Short-term Effectiveness on Controlling Zebra Mussel (*Dreissena polymorpha*) Attachment

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SUMMARY

Zebra mussels, an aquatic invasive species, cost millions of dollars each year to control due to their extensive attachment on essential industrial structures and detrimental effects on the native ecosystem. Reducing mussel attachment would allow researchers to control what surfaces these mussels attach to, and this reduction would prevent dangerous situations in industrial piping that occur when mussels prevent water flow. The goal of this research was to identify nontoxic materials that were effective in preventing or reducing the strength of zebra mussel attachment. We tested two materials, Sharklet and Netminder, that were designed to prevent biofouling of aquatic organisms, as well as two control materials, PVC pipes and a lake rock. The first experiment determined that Sharklet cannot prevent adult mussel attachment, but there was a statistically significant difference in attachment strength between Sharklet, the uncovered PVC pipe, and the rock. The second experiment determined that Netminder likewise cannot prevent adult mussel attachment and does not have a statistically significant difference in attachment strength with an uncoated PVC pipe. Since the Sharklet demonstrated some ability to deter mussel attachment, the material, along with other micro-engineered surfaces, warrant further study. Netminder likewise requires future study to determine its effect on zebra mussel veligers in a natural environment.

INTRODUCTION

Invasive species are a diverse group of organisms that have been transported to a location that is not in their native range. These organisms are usually extremely well adapted to these new environments; consequently, they spread rapidly with little hope of eradication (1). One of the most prominent aquatic invasive species in the United States is the zebra mussel *Dreissena polymorpha*. Zebra mussels are freshwater mussels that inhabit lakes, streams, rivers, and reservoirs where they attach to stable substrates which often include manmade materials, other animals such as crayfish, and even other zebra mussels (2). Zebra mussels live 3–9 years and have a shell length of 36–46 millimeters (2, 3). These mussels are native to the Black, Caspian, and Azov Seas in eastern Europe but became invasive in 1986 when they were introduced to the Great Lakes after being transported through the ballast water of ships (3, 4). Zebra mussels are now widespread in the United States and are currently found in locations ranging from the Great Lakes Region and the Mississippi River to Laurel Lake in western Massachusetts where they were discovered in July 2009 (5, 6).

Once zebra mussels become established in ecosystems in which they are not native, their superior adaptations in filtration and breeding allow them to thrive at the expense of native shellfish and other organisms. Zebra mussels are prolific breeders; a female produces up to a million eggs each year, and once fertilized, microscopic larvae known as veligers develop and are planktonic for three to four weeks before settling on a suitable substrate (2). This planktonic stage allows for widespread colonization of zebra mussel larvae and rapid spread within and between water bodies. Adult mussels are able to detach and drift short distances to find a more suitable substrate and can spread over land by adhering to boats (2, 3). Zebra mussels commonly reach densities of over 200,000 mussels per square meter and can filter around one liter of water per day (2, 3). As a result of dense zebra mussel colonization, native microorganisms, shellfish, and fish, both adults and larvae, are severely disrupted (2).

In addition to having a profound impact on natural ecosystems, zebra mussels significantly impact industrial operations. Zebra mussels readily adhere to materials such as concrete, carbon steel, and stainless steel, which are common construction materials (3). The strength of attachment varies depending on the substrate, although it generally increases with surface roughness due to increased penetration of the adhesive layer (3). As a result, the mussels often colonize water supply pipes of hydroelectrical and nuclear power plants, public water supply plants, and other industrial facilities in a process known as biofouling (2). This biofouling causes numerous problems that include restricted water flow and intake, decaying organisms in the water, corroding metal and concrete, and reduced flow of cooling water to electric power plants due to pipe blockage (2, 7). Such blockages, corrosion, equipment failures, and possible lake closures are expensive for these facilities and cost millions of dollars per year (7).

Zebra mussels attach to substrates by byssal thread formation, which generally increases with the size of the mussel (3). The byssal threads contain plaques, threads, stems, and roots (8). The adhesive plaques attach to the substrate in a continuous layer that is electron dense and around 10–20 nanometers thick (8). Threads attach to the individual plaques and the stem holds these threads in a bundle (8). The root is attached to the bundle of threads (8; **Figure 1**). Like many marine mussels, zebra mussels are thought to utilize 3,4-dihydroxyphenylaline (DOPA), an uncommon amino acid, to adhere to substrates (8). The distinct composition of the adhesive layer and the spatial distribution of proteins within it are unknown, which limits the understanding of the adhesion process (8). It is understood, however, that mussels use DOPA-mediated bidentate hydrogen bonding to attach to a wide variety of surfaces, which is double the strength of a normal hydrogen bond, metal/metal oxide coordination, or oxidative cross linking (9). Mussels can form bidentate hydrogen bonding when the material has hydrogen bonding sites. Coatings that contain epoxy, urethane, and urea linkages are susceptible to this type of bonding (9).

Figure 1: Byssal threads. An image of zebra mussel threads when a mussel was being removed from a lake rock at Laurel Lake.

Researchers have been attempting to create a surface or coating that prevents biofouling; however, there is currently no product that is fully capable of preventing biofouling while remaining both safe for the environment and durable. Some methods, such as copper-based heavy metal coatings, are effective and durable but hazardous to the environment (3, 5). Antifouling paints also commonly contain biocides such as copper and only last 1–2 years in flowing water (5). Other foul release coatings, such as silicone, are nontoxic but are also susceptible to abrasion and eventual depletion (3).

The goal of this research was to identify coatings or materials that are durable, environmentally friendly, and could be used to control zebra mussel adherence on industrial surfaces such as pipes. We tested Sharklet, a micro-engineered surface, and Netminder, a self-polishing water-based release coating. These materials were chosen primarily due to their availability, but also due to their nontoxic properties, which separate them from heavy metal coatings and antifouling paints.

Sharklet is a micro-engineered material with an embedded primary surface pattern that is diamond shaped with rows 3 microns tall and 2 microns wide. The pattern and dimensions can be modified depending on the application and the pattern can either protrude outward from the surface or be recessed into the surface (11). This pattern is based on shark skin, which naturally prevents biofouling in a marine environment (11). Sharklet is part of a group of materials known as micro-engineered surfaces. These materials can be based on surfaces in nature and improve performances of foul release coatings, but the optimal size of the surface pattern is different among organisms (3). Thus, the width and spacing of topographical patterns may need to be tailored to specific organisms (10).

Netminder is a water-based and self-polishing silicone release coating that is marketed as a nontoxic alternative to copper, zinc, or organotin based products (12). This coating is photoactive and requires an illuminated environment to work properly (12). When in oxygenated water, Netminder generates low levels of peroxides at its surface that are said to control the settlement of larvae and adults of marine biofouling organisms (12). The peroxides then dissociate into water and oxygen, making the coating nontoxic (12). Netminder most closely resembles a foul release coating, specifically a silicone foul release coating. This type of antifouling coating works either by slowly eroding and removing biofouling or minimizing attachment strength due to the unique chemical properties of the coating (3). These coatings are generally considered safe for the environment because they do not use biocides; however, some materials in the paint itself such as zinc are considered toxic (3). Silicone based foul release coatings are generally considered the best performing foul release coatings due to their low elastic modulus that releases organisms by peeling instead of shearing (9). Some silicone foul release coatings can prevent mussel attachment, and these coatings are generally considered nontoxic compared to antifouling paints but are susceptible to abrasion. In addition, these coatings sometimes get fouled with algae and aquatic vegetation, which then leads to zebra mussel attachment (3). Increased water flow can further decrease the lifespan of biocide based foul release and antifouling coatings (3).

We hypothesized that due to their unique properties as micro-engineered surfaces or water-based release coatings, Sharklet and Netminder would negatively affect the strength of zebra mussel attachment when compared to a common industrial piping surface (PVC).

RESULTS

This study tested the short-term effectiveness of two materials, Sharklet and Netminder, on preventing adult zebra mussel attachment in a laboratory setting. The research was divided into two experiments: Experiment 1 compared Sharklet to control surfaces, and Experiment 2 compared Netminder to a control surface, with each experiment being divided into two trials. It is important to note that each experiment has its own control because Experiment 1 began in May and Experiment 2 began in August and each used a different set of mussels. In all trials, some mussels did not attach to the target material; therefore, the data from Trials 1A and 1B were combined in the reported results of Experiment 1 and the data from Trials 2A and 2B were combined in the reported results of Experiment 2.

In Experiment 1, we tested the mussel attachment on the Sharklet covered PVC compared to a rock and an uncovered PVC pipe in two trials, Trial 1A and Trial 1B. Over two trials, 12 mussels attached to the rock out of 20 total mussels (60.0%). Out of 40 total mussels, 17 mussels attached to the uncovered PVC pipe (42.5%). For the Sharklet covered PVC pipe, 12 mussels attached out of 40 total mussels (30.0%; **Figure 2**). Mussels that were not found on the target material were either attached to the glass, other mussels, rocks holding the PVC pipe down, or had become inactive or dead. This occurrence happened in both trials of the experiment. Experiment 1 also compared the mussel attachment strengths to the rock, PVC pipe, and Sharklet (**Figure 3**). On the rock, the mean attachment strength was 0.82 pounds, the median attachment strength was 0.74 pounds, and the standard deviation was 0.39 pounds. On the uncovered PVC, the mean attachment strength was 0.63 pounds, the median attachment strength was 0.59 pounds, and the standard deviation was 0.39 pounds. On the Sharklet covered PVC, the mean attachment strength was 0.37 pounds, the median attachment strength was 0.32 pounds, and the standard deviation was 0.24 pounds. The attachment strength data in Experiment 1 tended to be skewed right possibly due to attachment strength having a natural limit of zero and the presence of a high outlier in the Sharklet group. This asymmetry happened with most data sets except for the rock (**Figure 3**), indicating that in a natural environment zebra mussel population attachment strength could be normally distributed. A one-way ANOVA test was used to test for a statistical significance in the measured attachment strengths of the three different surfaces with the significance level set at 0.05. The *p*-value of the test was 0.012, indicating that there is a statistically significant difference in the mussel attachment strengths on the three surfaces.

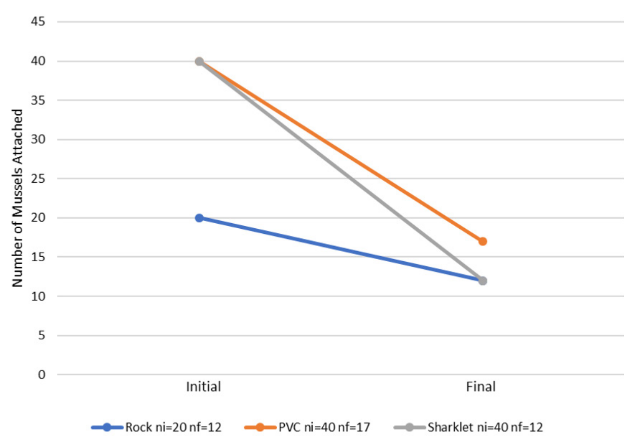

Figure 2: Mussel attachment rates in Experiment 1. The rock (blue), PVC (orange), and Sharklet (gray) mussel attachment rates are shown. The initial point represents the total original number of mussels placed on the material (ni) and the final point represents the total number of mussels that were attached at the conclusion of the trials (nf).

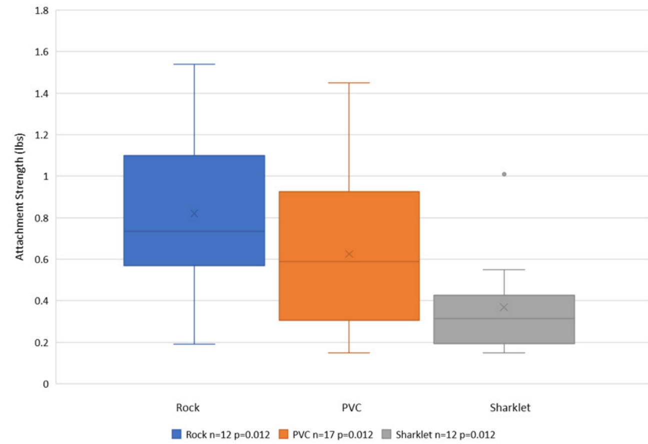

Figure 3: Comparing the distributions of attachment strength in Experiment 1. The distributions of mussel attachment strength values are displayed with the number of mussels (n) and the p value of the ANOVA test (p) displayed below each labeled material. The line in the middle of the box represents the median attachment strength, the x in the box denotes the mean attachment strength, and any dots outside the box and whiskers represent outliers.

Experiment 2 tested PVC pipe coated with Netminder against uncoated PVC pipe. Out of 60 total mussels, 27 total mussels attached to the uncoated sections of the PVC pipes (45.0%) and 28 total mussels attached to the Netminder coated sections of the PVC pipes (46.7%; **Figure 4**). On the uncovered PVC, the mean attachment strength was 0.24 pounds, the median attachment strength was 0.19 pounds, and the standard deviation was 0.13 pounds. On the Netminder covered PVC, the mean attachment strength was 0.32 pounds, the median attachment strength was 0.24 pounds, and the standard deviation was 0.23 pounds (**Figure 5**). Similar to Experiment 1, the attachment strength data in Experiment 2 tended to be skewed right possibly due to attachment strength having a natural limit of zero and the presence of two high outliers on both the Netminder and PVC. Using the assumption that zebra mussel attachment strength in the wild could be normally distributed due to the earlier results from the rock, a two-sample *t* test was conducted. The two-sample *t* test tested for a statistical significance in

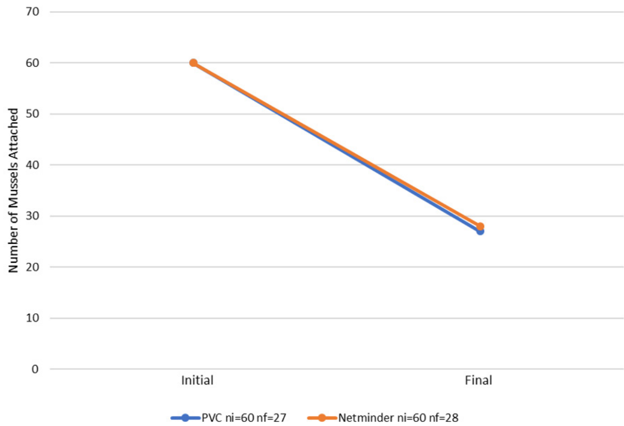

Figure 4: Mussel attachment rates in Experiment 2. The PVC (blue) and Netminder (orange) mussel attachment rates are shown. The initial point represents the total original number of mussels placed on the material (ni) and the final point represents the total number of mussels that were attached at the conclusion of the trials (nf).

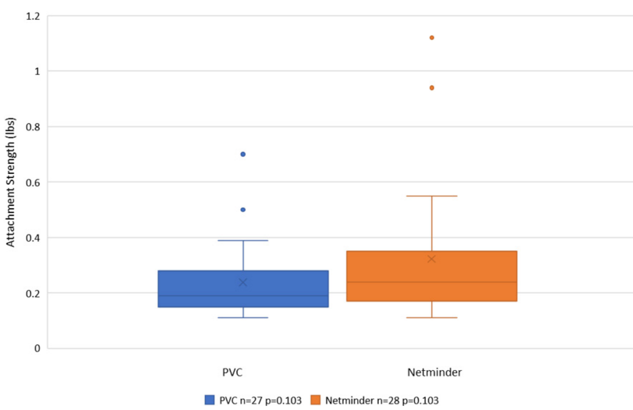

Figure 5: Comparing the distributions of attachment strength in Experiment 2. The distributions of mussel attachment strength values are displayed with the number of mussels (n) and the p value of the two-sample t test (p) displayed below each labeled material. The line in the middle of the box represents the median attachment strength, the x in the box denotes the mean attachment strength, and any dots outside the box and whiskers represent outliers.

the mussel attachment strengths to the Netminder and the uncoated PVC pipe, with the significance level set at 0.05. The *p*-value of this test was 0.103, indicating that there is no statistically significant difference in the attachment strengths of mussels on Netminder coated PVC and uncoated PVC.

DISCUSSION

Zebra mussels were able to attach with varying strength to all materials used during this study. We did not expect that any one material would be able to completely prevent mussel attachment; however, we hypothesized that Sharklet's unique properties as a micro-engineered surface would negatively affect zebra mussel attachment. The results of Experiment 1 suggest that Sharklet does lower the attachment strength of zebra mussels compared to their natural environment and a common industrial material, as it had lower average attachment strengths and there was a statistically significant difference in attachment strengths among those three surfaces. While Sharklet did have the lowest mean and median attachment strengths, it cannot be determined that it was the surface that caused the statistical significance. In Experiment 1, the mussels occasionally moved off the target material to other materials in the tank including the glass, various rocks, and often other mussels. Although the mussel attachment rate on Sharklet was lower, it was not significantly different from the mussel attachment rate on PVC; therefore, it could not be determined which of the two materials the mussels preferred. It is unknown whether Sharklet has been previously tested on zebra mussel attachment strength, as zebra mussel attachment values could not be found in any literature researched, and it is mainly marketed as a method to control the settlement of microorganisms and not freshwater mollusks.

While not able to prevent mussel attachment, Sharklet could still be a promising material for future research. Sharklet demonstrates that its micro-engineered pattern causes lower attachment strength when compared to manmade and natural materials. Although this particular pattern does not prevent attachment in adult mussels, it might be useful for reducing or preventing the settlement of veligers, the microscopic larvae of zebra mussels that are one of their main avenues of colonizing new surfaces. Sharklet's pattern is primarily designed to prevent the colonization of bacteria and other microscopic organisms and has been shown to significantly decrease the settlement of *Ulva*, which are 5 micrometers, and *S. aureus*, which are 0.6 micrometers (10). Since zebra mussel veligers have shell lengths of 70–350 micrometers (3), they could interact with the Sharklet topographical pattern in a similar way to other microorganisms during their initial attachment attempt. The adhesive plaques on adult mussels' byssal threads are one millimeter wide (8) and may be too large to interact with the Sharklet pattern. This hypothesis could be feasibly tested by verifying the presence of veligers in a body of water as well as determining if juvenile mussels attach on the Sharklet material over time. Other micro-engineered patterns might also work better with adult mussels. For example, researchers have developed a mushroom-shaped micro-engineered surface that controls marine barnacle attachment (13), indicating that an ideal pattern may be different between organisms and there is possibly an ideal pattern for zebra mussels.

Similarly, we hypothesized that Netminder's properties as a water-based foul release coating would negatively impact the strength of mussel attachment. Due to the fact that zebra mussels attached to Netminder with similar strength to uncoated PVC, this hypothesis was not supported in the experiment. Experiment 2 suggests that byssal thread formation and attachment among adult zebra mussels is unaffected by the release of peroxides by Netminder in the illuminated environment on a short-term basis. Similar to Experiment 1, mussels did not attach to the desired surfaces for multiple reasons, including being unresponsive or attached to other surfaces that included the glass, rocks, or other mussels. Since zebra mussels attached to both the Netminder and the uncoated PVC at almost equal rates (46.7% and 45.0% respectively), they did not prefer to attach to either material.

It is also unknown whether Netminder has been tested on zebra mussels before, as no attachment strength values could be found in the literature. In one study conducted by the U.S. Bureau of Reclamation, silicone-based release coatings tested had a maximum attachment strength of 0.40 pounds (5), although these values are hard to compare as the measurement procedure was conducted using a different instrument and the mussels were allowed to attach for a longer period of time in a natural environment. Future research therefore also needs to be conducted on Netminder. The use of peroxides, which dissociate into oxygen and water, is safer for the environment than traditional biocides such as copper. Hydrogen peroxide is also known to control zebra mussel adults and veligers in high doses (7). Although Netminder readily allowed adult zebra mussel attachment, veliger interactions with Netminder's surface would be more prominent in a natural environment; therefore, controlling veliger interactions may be more important in controlling overall mussel attachment. A similar long-term experiment in the natural environment of the mussels as proposed for Sharklet could further determine Netminder's ability to control zebra mussel veliger attachment.

Throughout both experiments, byssal threads were frequently seen on all materials after the attachment strengths were measured and the mussels removed, with the adhesive plaques present on the material and threads still attached to them. Particularly, remaining byssal threads were present on both sides of the Sharklet sheets, indicating that neither side was more effective than the other. The presence of byssal threads demonstrates that for some of the mussels, the attachment strength was only a measure of how strong the threads were; and the actual attachment strength (strength of adhesive plaque to surface attachment) would be a higher value. Another interesting observation to note is the difference in attachment strength values between Experiment 1 and Experiment 2, particularly with respect to unaltered PVC, which was the only common material used in both experiments. In Experiment 1, the PVC had a mean attachment strength of 0.63 pounds while in Experiment 2 its mean attachment strength was 0.24 pounds. It is important to note that this difference could potentially be due to the fact that the mussels were collected at different time of the year. The difference in mussel attachment strengths depending on the time of year suggests that Laurel Lake's conditions change throughout the summer and ultimately impact the zebra mussel population's ability to attach effectively. This difference is not expected to impact results because within the individual experiments the sets of mussels used were collected from the lake at the same time. Overall, while both Sharklet and Netminder were hypothesized to decrease mussel attachment strength, there was only evidence that there was a significant difference in attachment strength between Sharklet and the two control materials tested against it. Both materials warrant future research, however, due to their unknown effect in a more natural environment and on zebra mussel veligers and juveniles.

MATERIALS AND METHODS

This study researched the effect of two materials, Sharklet and Netminder, on preventing or reducing the strength of adult zebra mussel attachment in a laboratory setting. Zebra mussels were obtained on two occasions from Laurel Lake at the boat launch shoreline in Lee, Massachusetts. Zebra mussels were obtained with the permission of Jim Straub of the Massachusetts Department of Conservation and Recreation who also advised the disposal of water and mussels after the experiment was completed. Fifty mussels were collected for Experiment 1 in May 2018 and 61 Mussels were collected for Experiment 2 in August 2018. All mussels were collected by removal from shoreline rocks by hand in shallow water under supervision. Waders were worn to minimize contact with the lake water that contains zebra mussel veligers. Zebra mussels were transported from Laurel Lake to the laboratory in a bait bucket, a small circular container with a removable top. The mussels remained in this bait bucket with aeration for three to four hours until the experiments were set up, at which time they were moved into the aquarium. For both experiments, approximately 20 gallons of lake water were taken and used to house the mussels. A flat rock on the shoreline was also taken during the first collection to provide a control surface during Experiment 1 and to hold a PVC pipe underwater during Experiment 2.

Zebra mussels were contained in an Aqueon brand 20-gallon fish tank (30.25" x 12.50" x 12.75") for the duration of both experiments with no filtration. The aquarium received aeration from an air pump that fed air through plastic tubing to two air stones, which released air into the water that could be controlled by valves. In Experiment 1, the tank was covered by a plastic bin cover; however, a light was needed in Experiment 2, so a cover was not used. In Trial 1A, powdered *Chlorella* algae was fed to the mussels; however, this method polluted the tank water so live *Chlorella* algae was bought and cultured for the remaining trials. Once the culture was sustainable, live *Chlorella* algae was fed to the mussels daily. This method was used in Trials 1B, 2A, and 2B of experimentation.

Experiment 1

Experiment 1 tested the effectiveness of Sharklet in preventing short-term adult zebra mussel attachment and incorporated the combined data from Trials 1A and 1B. The Sharklet was cut into multiple small sheets. The Sharklet pattern was confirmed by shining a laser through the sheets, which made a distinct light arrangement due to the surface pattern (**Figure 6**). Three-inch diameter PVC pipes were used in both experiments as control substrates since they have been extensively tested and zebra mussels are known to attach to them with intermediate strength (14, 15). The PVC pipes were bought from a local vendor and cut into 20-inch segments. The pipes were cut in half so that the top of the pipe would be open, and the zebra mussels could be accessed to measure attachment strength. In both experiments, rocks were put on top of the PVC pipes to hold them down at the bottom of the aquarium since they float. This setup still allowed the mussels to have full access to the entire surface of the pipes (**Figure 7**).

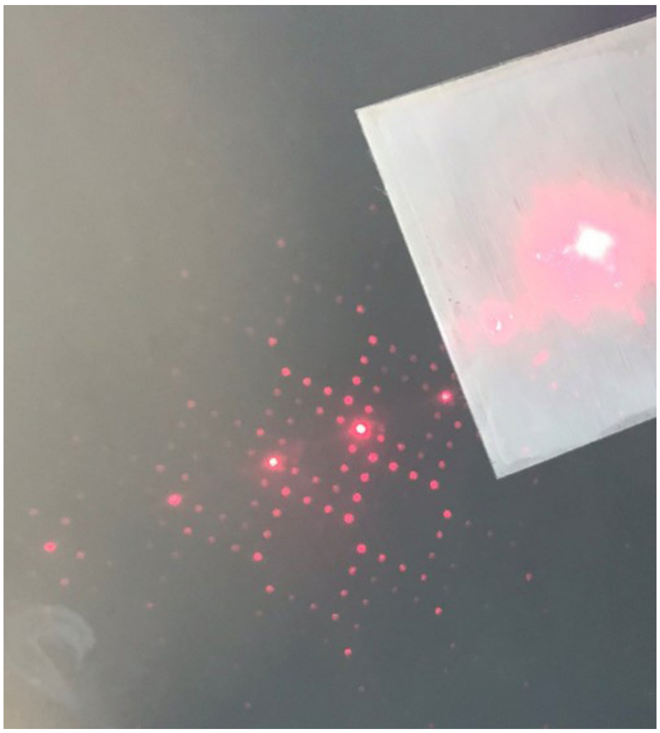

Figure 6: Sharklet Laser Pattern. This original image displays the distinct light pattern created by Sharklet's micro-engineered surface when a laser is shined through it.

Trial 1A was set up with two PVC pipes, one covered with Sharklet and one without it, and the flat rock from Laurel Lake. The PVC pipe with Sharklet was covered by eight sheets of Sharklet, four of which were approximately 4.5" x 2.25" and the remaining four were approximately 3.0" x 2.0." Both PVC pipes and the rock were separated by a plastic mesh tank divider that was anchored to the walls of the aquarium using suction cups. 20 mussels were placed on the Sharklet covered PVC pipe, 20 mussels were placed on the uncovered PVC pipe, and 10 mussels were placed on the lake rock. A smaller number of mussels was placed on the rock due to its small surface area. The mussels were placed by hand and evenly spaced along the length of the covered and uncovered PVC pipes and the rock. The lake rock was used as a control to make sure mussels would behave in the same way as in Laurel Lake. Ten days after the day of placement, the attachment strength of the zebra mussels was measured. The measurements were taken in pounds using a Quarrow Fish Lip Grip Digital Fishing Scale (50-pound capacity) and the data was recorded. The scale usually measures a pulling force, but it was not practical to attach the stainless-steel jaws to a mussel and pull it off a surface. Instead, the pushing force was measured since the scale can read pushing forces as negative numbers. The negative sign was ignored for the experiment. A Magic Sliders brand felt pad was cut into a half circle and glued onto the end of the steel jaws to prevent shell breakage and provide a greater surface area for measurement. When measuring attachment strength, the scale was first zeroed, and then light pressure was applied against the mussel's shell by pushing the felt pad against the shell. Increasing force was applied to the mussel's shell until the mussel became dislodged from the surface it was attached to. The highest reading before the mussel became dislodged was taken as the attachment strength. If an individual mussel was attached to a surface and had other mussels attached to it, this was known as a cluster. If the mussels attached could be removed, the individual's attachment strength was measured; however, this was inconsistent as sometimes the arrangement of the cluster did not allow for removal or all mussels became dislodged with the removal of one mussel. Since the mussels could move freely throughout the PVC area and the glass bottom and sides of the aquarium, the number of mussels on each surface were recorded as well. The same procedures for measuring attachment strength for individuals, clusters of mussels, and counting the number of mussels on each surface were followed in both experiments. The PVC pipes and Sharklet sheets were cleaned of any waste, algae, or other debris before the next trial began.

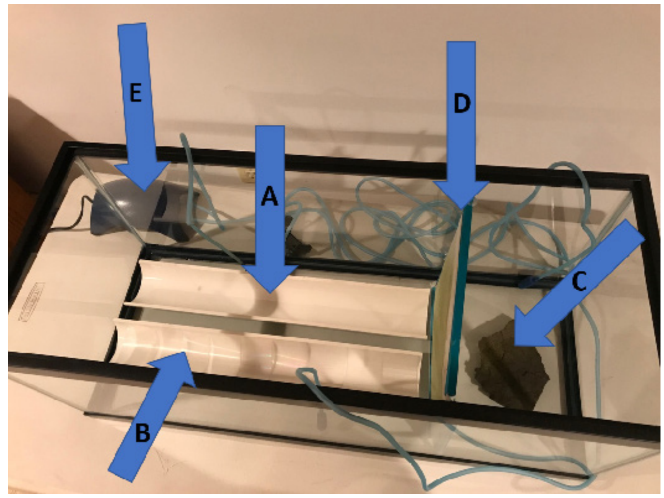

Figure 7: Experiment 1 Design. This original image displays all components of the design of Experiment 1: (A) the uncovered PVC, (B) Sharklet covered PVC, (C) lake rock, (D) tank divider, and (E) aeration system.

Trial 1B was set up in the same way as Trial 1A, with 20 mussels on the uncovered PVC pipe, 20 mussels on the

PVC pipe with Sharklet and 10 mussels on the lake rock and all components in the same positions. The mussels on each section were not necessarily the same mussels from Trial 1A as they were picked randomly during placement. The only difference in the setup was in the placement of the Sharklet. The four small sheets of Sharklet became dislodged from the PVC pipe in Trial 1A so they were not used in Trial 1B. Since no more large sheets were available, the four large sheets were placed together on one side of the pipe and all mussels were placed on these sheets. To achieve similar spacing on the uncovered pipe, the mussels on that pipe were also hand placed in the same corresponding area of the pipe. Ten total days after placement, the attachment strength was measured and recorded using the same procedure as Trial 1A. In both trials, some zebra mussels moved off the target material, were unresponsive, or attached to themselves to form clusters. These numbers were included in the analysis of the attachment rates of mussels but decreased the number of data points to analyze for attachment strength. Therefore, the data from Trials 1A and 1B were combined in the reported results of Experiment 1.

Experiment 2

Experiment 2 tested the effectiveness of Netminder at preventing short-term adult zebra mussel attachment. Experiment 2 was also divided into two trials, Trial 2A and Trial 2B, with each trial again lasting 10 days. Thirty mussels were placed on each material. Sixty total mussels were placed on both the PVC pipe and the Netminder during this experiment. A one-quart sample of Netminder was generously provided by Alex Walsh, the Director of Research and Product Development of ePaint. Since Netminder is photoactive, a Coralife 6700K 96-watt light was used in both Trial 2A and Trial 2B. The right end of the light rested on one five-gallon bucket while the left end of the light rested on the right edge of the aquarium and was suspended nearly halfway across the right side of the aquarium. In between the right side of the aquarium and the five-gallon bucket was the *Chlorella* algae culture that also needed the light to continue growing. The light provided lighting to the whole aquarium, but the right side received stronger light than the left. The light ran on a 12-hour timer to ensure that the same amount of light was received during each trial. Netminder was applied by brush to the right half of three new PVC pipes that were cut in the same way as those from Experiment 1. One coat was applied by brush under supervision following the package instructions. 3M Nitrile gloves, goggles, and a mask were worn to protect from the paint and fumes.

Trial 2A was set up with the three PVC pipes secured by rocks, including one lake rock from Experiment 1. The pipes were arranged so that they aligned horizontally. The Netminder-coated half of the pipes were oriented into the stronger light (**Figure 8**). On each PVC pipe, 10 mussels were hand placed on the coated half and 10 mussels were placed on the uncoated half. In total, 30 mussels were on the Netminder coated sections of the PVC pipes and 30 mussels were on the uncoated sections. The remaining mussel was placed on the glass away from the PVC pipes. Mussels could move freely between the two halves of the PVC pipes. After 10 days of placement in the tank, the attachment strength of the zebra mussels was measured using the same procedure, the data was recorded, and the pipes were cleaned.

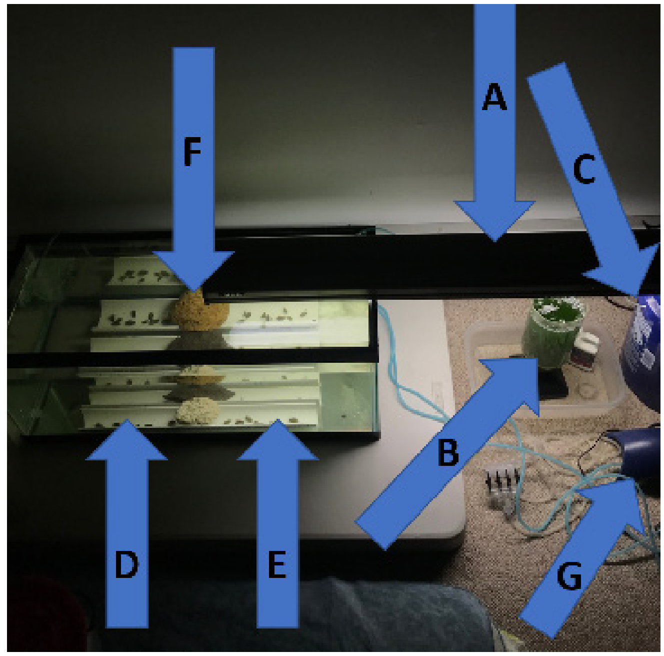

Figure 8: Experiment 2 Design. This original image displays all components of the design of Experiment 2: (A) The light, (B) *Chlorella* algae culture, (C) five-gallon bucket, (D) PVC, (E) Netminder coated PVC, (F) rocks, and (G) aeration system.

Trial 2B was set up in the same way as Trial 2A. A total of 30 mussels were hand placed on the Netminder coated section of the PVC pipes and 30 total mussels were placed on the uncoated sections. On each pipe, 10 mussels were hand placed on each section. One mussel from Trial 2A died (its shell was open and would not close); therefore, that mussel was replaced with the extra that was placed on the glass in Trial 2A. The mussels were picked randomly when placed so the same individuals were not necessarily in the same places as Trial 2A. The light was raised approximately 1.5 feet due to concerns that intense light was causing increased mussel mortality; however, 7 total mussels died on the uncoated PVC and 9 total mussels died on the Netminder coated PVC by the conclusion of Experiment 2. The mussel attachment strength was measured by the same procedure as in previous trials. Similar to Experiment 1, the movement of mussels off the target materials and the increased mortality of mussels decreased the number of mussels that could be used to measure attachment strength. As a result, the data from Trial 2A and Trial 2B were combined in the reported data of Experiment 2.

Safety Procedures

Throughout both experiments, certain procedures were followed to eliminate the potential spread of zebra mussels or veligers into the water supply or nearby bodies of water. The aquarium was kept covered during Experiment 1, but this was not possible in Experiment 2. Waterproof gloves were worn when filling the tank with water, handling the mussels or experiment equipment, and measuring mussel attachment strength (except when removing mussels from another mussel individually attached to a surface) to minimize contact with lake water. Gloves were not worn when the mussels were collected from Laurel Lake or placed on the PVC pipes in the aquarium because these tasks required increased accuracy that could not be replicated when the gloves were worn. Occasionally water did make contact with skin or the gloves did not adequately prevent water contact. In these circumstances, hands were not immediately washed as this could spread veligers into the water supply. Instead, hand sanitizer containing 70% Ethyl Alcohol was used to kill potential veligers.

The following disposal procedures were required by Jim Straub to ensure no spread of invasive species. The experiment was conducted away from drains, sinks or other areas that connect to the water supply. After Experiment 1 concluded, all lake water was disposed of in the middle of a grassy yard. The PVC pipes, Sharklet, waders, gloves, scale, rocks, containers, buckets, aquarium, and other materials used were dried inside between the two experiments. The zebra mussels were counted and confirmed as 50, the original number. After confirmation, the mussels were placed in two sealed plastic bags and disposed of in the trash along with other disposable materials that were used during Experiment 1. After Experiment 2 concluded, the number of mussels was confirmed as 61, the same as the original amount; and they were disposed of in the same way as Experiment 1. The aquarium water and extra lake water were again disposed of outside in a yard rather than down a drain. Some materials, including the gloves, air pump tubing, air stones, and the tank divider were disposed of in the trash. Other materials, including the aquarium, towels, five-gallon buckets, and covers were placed inside during rainy days and outside during dry days to dry. These materials were dried outside for a minimum of seven days, not necessarily consecutively, as advised.

ACKNOWLEDGEMENTS

Thank you to Mr. Jim Straub of the Massachusetts Department of Conservation and Recreation for giving permission to collect zebra mussels and advice about disposal; otherwise this project would not be possible. A special thanks goes out to Mr. David W.H. Wong of the Massachusetts Department of Environmental Protection who gave advice about where to collect the mussels and how to obtain permission to do so. Another special thank you goes out to Mr. Alex Walsh, who generously provided a sample of Netminder to test. Thank you to Mr. John Willson for giving crucial advice for the project. A final thank you goes out to Mrs. Doris Brennan who provided the device to grow the algae in and gave advice for the paper and Mr. Chris Rando who cut the PVC pipes.

Received: October 29, 2018
Accepted: January 30, 2019
Published: June 3, 2019

REFERENCES

1. Worden, David. “Aquatic Invasive Species Assessment and Management Plan.” Massachusetts Department of Conservation and Recreation, October 2010, https://www.mass.gov/files/documents/2017/10/10/aquatic.pdf.
2. “State of Michigan’s Status and Strategy for Zebra and Quagga Mussel Management.” State of Michigan, 2014, https://www.michigan.gov/documents/deq/wrd-ais-dreissenids_499881_7.pdf.
3. Sytsma, Mark, and Steven Wells. “A review of the Use of Coatings to Mitigate Biofouling in Freshwater.” Portland State University: Centers for Lakes and Reservoirs, 2009, http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.458.7807&rep=rep1&type=pdf.
4. Baldwin, Wen, Shawn Gerstenberger, Bryan Moore, and Wai Hing Wong. “Settlement and Growth of Quagga Mussels (*Dreissena rostriformis bugensis* Andrusov, 1897) in Lake Mead, Nevada-Arizona, USA.” *Aquatic Invasions*, vol. 7, no. 1, 2011, pp. 7-19. http://www.aquaticinvasions.net/2012/AI_2012_1_Wong_etal.pdf.
5. “Coatings for Mussel Control-Results from Six Years of Field Testing.” U.S. Bureau of Reclamation, July 2014, https://www.usbr.gov/mussels/docs/MERL2014-64Coatings.pdf.
6. “Zebra Mussel Phase I Assessment: Physical, Chemical, and Biological Evaluation of 20 Lakes and the Housatonic River in Berkshire County, Massachusetts.” Biodrawversity LLC, November 2009, https://www.mass.gov/files/documents/2017/10/10/phase%20i.pdf.
7. Glomski, L.M. “Zebra Mussel Chemical Control Guide Version 2.0.” US Army Engineer Research and Development Center, July 2015, https://erdclibrary.erdc.dren.mil/xmlui/bitstream/handle/11681/6966/ERDC-EL-TR-15-9.pdf?sequence=1&isAllowed=y.
8. Farsad, Nikrooz, and Eli D. Sone. “Zebra Mussel Adhesion: Structure of the Byssal Adhesive Apparatus in the Freshwater Mussel, *Dreissena polymorpha*.” *Journal of Structural Biology*, vol. 177, no. 3, pp. 613-62. doi: 10.1016/j.jsb.2012.01.011.
9. Merten, Bobbi Jo, Allen D. Skaja, and David Tordonato. “Coatings for Invasive Mussel Control: Colorado River Field Study.” *Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States*. 2015, pp. 451-466. doi: 10.1201/b18447-37.
10. Brennan, Anthony B., Scott P. Cooper, and Chelsea M.

Magin. “Non-toxic Antifouling Strategies.” *MaterialsToday*, vol. 13, no. 4, 2010, pp. 36-44. *ScienceDirect*. doi: 10.1016/S1369-7021(10)70058-4.
11. Sharklet Technologies, www.sharklet.com.
12. Netminder Aquaculture Coatings, www.netminder.us.
13. Allen, Michael. “Barnacles are no Match for Mushroom-shaped Microstructures.” *PhysicsWorld*, 27 August 2018, physicsworld.com/a/barnacles-are-no-match-for-mushroom-shaped-microstructures/.
14. Gerstenberger, Shawn L., Sara A. Mueting, and Wai Hing Wong. “An Evaluation of Artificial Substrates for Monitoring the Quagga Mussel (Dreissena bugensis) in Lake Mead, Nevada-Arizona.” *Lake and Reservoir Management*, vol. 26, no. 4, 2011, pp. 283-292. doi: 10.180/07438141.2010.540700.
15. Coyle, Benjamin P., Paul H. Lord, and Wai Hing Wong. “Monitoring Zebra Mussel Colonization: PVC and Cast Iron Recruitment.” http://www.oneonta.edu/academics/biofld/PUBS/ANNUAL/2014/14%20Coyle%20Adherence%20Monitoring.pdf.