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Zebra Mussels *Dreissena polymorpha* are an invasive bivalve species that are highly successful at dominating new environments. These invaders have been known to colonize substrate so thoroughly that all they have to attach to are members of their own species. When large mats of interconnected mussels completely cover a riverbed, it can have a drastic effect on the surrounding environment. This study serves to create a better understanding of the hydrologic parameters needed for Zebra Mussels to create these ecosystem-transforming colonies known as druses. Data was collected on a small stretch of the Upper Mississippi River in Northern Minnesota, previously known to be a Zebra Mussel hotspot. The goal was to search for correlations between substrate size, water depth, and water velocity that create optimal zones for Zebra Mussels to form these huge colonies.

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Introduction

Zebra Mussels Dreissena polymorpha started their invasion of Lake Bemidji in 2018 (Kao et al. 2021). Since then, larval Zebra Mussels have used the currents of the Mississippi River to escape the lake and migrate downstream. This form of invasion takes advantage of the stream-pool-stream dynamic that makes up the headwaters of the Mississippi and its connected lakes (Horvath et al. 1996). Many articles have been written focusing on the formation of Zebra Mussel colonies in lentic systems but there is a surprising lack of information on how they take over the benthic layer of streams and rivers. The purpose of this study is to search for correlations between water velocity, depth, and substrate size that allow Zebra Mussels to form these substratesuffocating clusters.

Species Taxonomy and Identification

Reaching a maximum shell length of only 1-2 cm, Zebra Mussels are one of the smallest freshwater bivalves in the Mollusca family. They feed and respirate by filtering massive amounts of phytoplankton and bio-matter from the water column (Depew et al. 2021). One individual can filter as much as 1 to 2 L of water per day (Aldridge et al. 2006). These hardy mollusks have multiple attributes that give them a competitive edge over native species. One of these advantages comes in the form of sticky structures called byssal threads used to latch onto substrate (Farsad and Sone 2012). Byssal threads make Zebra Mussels the only

freshwater mollusk capable of holding on to solid objects (James et al. 2021). Being able to cling to substrate is an advantage that Zebra Mussels have used to take over thousands of water bodies in very little time.

Behavior and Ecological Impacts

When Zebra Mussels reproduce, one female can release up to one million eggs per reproductive season. The eggs hatch and release planktonic larvae called veligers that settle to the bottom and cling to whatever hard substrate is available (Ram et al. 2011). Veligers are so lightweight that they have multiple modes of transport at their disposal such as clinging to fish, birds, amphibians, and other aquatic animals, as well as being able to float down river systems, or hitching a ride in boats and other vehicles that transport water from one body to the next (Benson 2013).

When veligers eventually settle to the bottom, they often end up landing on the shells of their predecessors. This eventually leads to the formation of large clumps or mats called druses, that can completely cover the beds of lotic environments (Farsad and Sone 2012). When druses form there is a multitude of ecosystem-wide impacts that begin to occur. For example, druses have been known to completely cover native mussels, effectively keeping them from being able to move, feed, or reproduce (Ricciardi et al. 1998). Other species in the environment, like predators within the water column, are also affected negatively when their bottom-dwelling prey becomes increasingly harder to find under the solid shell of protection that the druses form.

Related Studies and Findings

A study of Zebra Mussel habitat preferences within a river ecosystem in Spain showed that Zebra Mussels have a strong preference for water velocities between 0.3 and 0.8 m/s. The same study showed habitats with velocities higher than 1.2 m/s were almost completely void of Zebra Mussel habitation (Sanz-Ronda et al. 2014).

Zebra Mussel druses have been shown to alter aquatic food webs by giving some species of aquatic invertebrates more surface area to thrive while simultaneously making it more difficult for predators to forage for them (Beekey et al. 2004). To minimize the potential catastrophic implications for native aquatic ecosystems, it is crucial to collect as much data as possible in order to identify measures that can be taken to combat the invasion.

Methods

The study area was an approximately 180 m long, 25 m wide stretch of the Mississippi River east of Bemidji, Minnesota. This site included the stretch of river between a hydroelectric dam and its nearest downstream bridge. The dam belongs to Otter Tail Power Company and is the first dam on the river. This site was chosen due to a combination of having known benthic mats of Zebra Mussel druses, a large variation of water velocities, and areas of substratum from silt (<2 mm) to cobble (65 - 256 mm) to boulders (257 – 4096 mm) (Wolman 1954). For the headwater region of the Mississippi, the mean flow rate is 1.2 m/s. As stated in the Sanz-Ronda et al. (2014) study, 1.2 m/s is the known topmost limit for Zebra Mussel presence.

Data collection spots were chosen based on velocity reading. The data collector attempted to get multiple readings of as many different velocities as possible within the study area. At each data collection point four metrics were recorded: 1) velocity (m/s), 2) depth (m), 3) average substrate size (mm), and 4) Zebra Mussel status. Zebra Mussel status was based on a categorical scale of 1-3. 1 being no Zebra Mussels present, 2 being Zebra Mussels present but only in a single layer (not in a druse), and 3 being Zebra Mussels present in druses.

Water velocity was recorded using a Hach FH950 portable velocity meter. The meter's sensor was placed at the lowest possible point on the sensor rod to be as close to the streambed as possible. The front of the meter was always pointed in the up-river direction. In events where water was not traveling down river (eddies and pools) the velocity reading would be negative. Zebra Mussel status was determined by scraping the top layer of sediment into a canvas reinforced D-frame dip net using a flat headed shovel. The net was emptied onto a board and its contents were studied to visually determine which of the three categories the mussels (or lack thereof) fell into.

Average substrate size was determined by randomly selecting a representative sample of 10 pieces of sediment from the nets contents and measuring each piece by its median axis in mm. If substrate size was deemed too large to fit into the net, average substrate size was determined by reaching into the water and grabbing the first rock that was touched by the collector's pointer finger. This process was then repeated for the ten measurements needed to get an average. *Data Analysis*

After the collection event, the data was entered into a working excel spreadsheet with columns for velocity, sediment size, depth, and mussel status. The data was checked for any errors, missing values, or outliers. Shapiro-Wilk tests done on each of the three variables showed significant p-values for sediment size and depth, but not for velocity. Upon further investigation through Q-Q plots and histograms it was discovered that none of the three variables were normally distributed. This was to be expected as the method for selecting measurement locations was not random. Non-normal data called for the use of descriptive statistics and nonparametric tests to for significant differences in habitat variable among the three mussel statuses.

Results

Velocities among all three categories ranged from -0.70 to 1.46 m/s and had a mean of 0.51 m/s (SD = 0.43; Figure 1). Average substrate sizes for all three categories ranged from 1 to 156.1 mm and had a mean of 46 mm (SD = 35.87; Figure 2). Depths ranged from 0.06 to 1.10 m and had a mean of 0.43 m (SD = 0.28; Figure 3).

Areas where Zebra Mussels were completely absent were few and far between but always had either very small substrate (mean = 7.67 mm; SD = 10.17) or velocities that were negative or near zero (mean = 0.13 m/s; SD = 0.29).

Areas where Zebra Mussels were in a single layer tended to be where high velocity (mean = 0.73 m/s; SD = 0.45) met large sediment size (mean = 78.82 mm; SD = 29.66).

Areas that were completely covered by druses made up most of the study area and had medium velocities (M = 0.46 m/s, SD = 0.29) and medium to large sediment sizes (M = 30.62 mm, SD = 10.17).

When comparing mussel configuration to velocity, Bartlett's Test for Homogeneity of Variance showed that there was not equal variance between samples. Welch's one-way ANOVA revealed that there was a statistically significant difference in velocity between at least two groups of mussel configuration. A Turkey-Kramer Pairwise multiple comparisons test showed that all pairwise comparisons were different at the $\alpha = 0.05$ level. This suggests that the differences in velocity among the different mussel configurations are statistically significant.



Figure 1. Box plot showing the distribution of velocity measurements for the three mussel configurations, 'No Mussels', 'Single Layer', and 'Druse'.



Figure 2. Box plot showing the distribution of sediment size measurements for the three mussel configurations, 'No Mussels', 'Single Layer', and 'Druse'.

When comparing mussel configuration to depth, Bartlett's test for homogeneity showed equal variance among samples. A Kruskal-Wallis test was performed to compare the effect of depth on mussel configuration. The test did not reveal a statistically significant difference in depth between the three groups ($\chi 2 = 2.064$, df = 2, p = 0.356). Therefore, we can conclude that there is no evidence to suggest that depth is a significant factor in the formation of druses based on the data from this study.

When comparing mussel configuration to sediment size, Bartlett's Test for Homogeneity of Variance did not suggest equal variance between samples. Welch's one-way ANOVA revealed there was a significant difference in sediment size between at least two groups of mussel configuration. Finally, a Turkey-Kramer Pairwise multiple comparisons test showed that all pairwise comparisons were different at the $\alpha = 0.05$ level. This suggests that the differences in sediment size among the different mussel configurations are statistically significant.



Figure 3. Box plot showing the distribution of depth measurements for the three mussel configurations, 'No Mussels', 'Single Layer', and 'Druse'.

Overall, the results suggest that, on their own, velocity and substrate size, are important predictors of the presence and configuration of Zebra Mussels in the study area, while depth does not appear to have a significant effect.

Discussion

Water velocity plays a crucial role in the formation of Zebra Mussel druses. Velocity and druse formation were positively correlated, with the highest incidence of druses occurring between 0.2 and 0.7 m/s. These parameters take the findings of Sanz-Ronda et al. (2014) one step further by looking at the formation of druses rather than only looking at the presence or absence of mussels. This velocity range is likely the sweet spot where strong enough current for adequate filter feeding meets weak enough current to allow individuals to adhere to one another.

Zebra Mussels' ability to colonize smaller siltlike substrates in this stretch of river was obviously limited as shown by almost zero mussels being found in these substrates. Even when mussels were grouped in druses, they tended to avoid the sand beds. This is different from the benthic mats talked about in the Lake Eerie druse study by Berkman et al. (1998). The results indicate that while druse formation is an effective invasion mechanism, it may only work in lentic environments like Lake Eerie that have very little current compared to river and stream environments.

The Zebra Mussel druses in this study completely matted expansive sections of riverbed. There were areas where druses could be the reason that the smaller pebble-sized sediment underneath wasn't being washed downstream like it might normally have been under normal exposure to the current. This disruption to the natural hydrologic processes of sediment loading and movement within river systems can have significant impacts on native ecosystems. Consequently, additional research is necessary to quantify the extent to which large colonies of mussels are capable of impeding sediment transport downstream.

In conclusion, this study provides important insights into the factors that contribute to the formation of Zebra Mussel druses in the Mississippi River. The findings suggest that water velocity, and substrate size, are important determinants of Zebra Mussel druse formation. These findings have important management implications for the control of invasive Zebra Mussel populations and may help to guide efforts to prevent further invasion and spread of this ecologically destructive species.

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