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As of November 2021, zebra mussels were confirmed in 270 Minnesotan lakes with another 232 lakes listed as infested due to their connection or proximity to where zebra mussels have been found. With lack of effective treatments to impede their spread, zebra mussels continue to colonize new areas causing ecological as well as economic harm. The goal of this study was to establish a length-weight relationship for the zebra mussel population in Lake Bemidji using shell length (SL) and wet weight (WW), as well as to assess substrate type and log transformed shell length as predictors of log transformed zebra mussel wet weight. The selected substrate types were driftwood, cobble-sized stones (64-256 mm in diameter), and native unionids to include a variety of bottom-types commonly found throughout the lake. The established length-weight relationship was as follows: $\log WW(g) =$ -9.2964 + 3.0998 log SL (mm). Regression analysis demonstrated that zebra mussel shell length alone was the best predictor of mussel wet weight. Substrate type showed relatively less support compared to other models with less predictive value overall compared to shell length.

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Introduction

The zebra mussel *Dreissena polymorpha* is a bivalve native to Eastern Europe that has become one of the most concerning invasive species in North America in recent decades, dramatically changing ecological systems and causing billions in damages. The species has quickly expanded its range across the United States since accidental introduction around 1986 when it was likely transported to the Great Lakes through infested commercial vessel ballast water (Vanderbush et al. 2021). Zebra mussels were first discovered in Lake St. Claire in 1988 (Hebert et al. 1989), and as of 2019 have spread to 31 states from coast to coast (Vanderbush et al. 2021).

Zebra mussels are highly effective suspension filter feeders that obtain nutrients from phytoplankton in the water column while disrupting natural movement and circulation of organic material. They filter nearly 100% of particles > 1 μ m, with much of the unused nutrients being excreted on the lake bottom as psuedofeces (Fanslow et al. 1995). This energy displacement from the pelagic to benthic zone can impact the entire food web of an aquatic system and be of detriment to native species. Zebra mussels have high reproductive rates, and strong byssal attachment to colonize a variety of man-made and natural surfaces (Marsden et al. 2000).

Although there are several existing studies on zebra mussel colonization of artificial substrates (Czarnołeski et al. 2004), little research has been conducted on natural substrates, and even less is known about length-weight relationships across natural substrates. Furthermore, previously reported length-weight relationships on zebra mussel populations have traditionally only used ash free dry weight (AFDW) as a biomass metric (Nalepa and Schloesser 1993; Nalepa et al. 1993). Therefore, the objective of this study is to establish a length-weight relationship using the biomass metric of mussel wet weight, and to investigate shell length and substrate type as predictors of zebra mussel wet weight in Lake Bemidji.

Methods

Study Area

Lake Bemidji is a 6,596-acre lake with a maximum depth of 76 feet, located next to the city of Bemidji in southern Beltrami County. It is connected to the Mississippi River with Lake Irving upstream and Stump Lake downstream. Zebra mussels were initially discovered on the north end of the lake in July 2018 and have since colonized much of the lake's hard substrate.

Lake Bemidji was chosen for this investigation due to the recency of zebra mussel infestation and lack of existing data on the status of the population. Additionally, as a part of the Upper Mississippi River watershed, it is particularly important to understand how the establishment of this invasive species may impact this waterbody as well as the rest of the Mississippi River Basin.

Sample Collection

Samples were taken from the southwest shoreline of Lake Bemidji adjacent to the Bemidji State University campus at approximately 47°28.888'N 94°52.3945'W. The wadable zone (~1 m) was the depth of focus and zebra mussels were collected from three different littoral substrate types.

Sampling occurred over three days, on November 5, 10, and 12, 2021. Using waders to locate substrates of interest along the shoreline, a paint scraper was then used to collect mussels from each substrate type: driftwood, cobble-sized stones, and native unionids. Samples were taken from each of the three substrate types in closest proximity to each other.

Data Analysis

Mussels that best represented the length-weight distributions for each of the substrate types were selected for analysis, with a total of 90 mussels (30 per substrate type) collected over the sampling period. Wet weight and shell length of the mussels were recorded. Before weighing on the scale, shells were cleaned of any debris using a cloth soaked in isopropyl alcohol. Wet weight was measured to the nearest hundredth of a gram. Shell lengths were measured to the nearest hundredth of a millimeter using an electronic caliper. The length-weight relationship for the sample was then calculated using this data.

Using Program R, regression models were tested to assess how substrate type and shell length play a role in predicting zebra mussel wet weight. Wet weights and shell lengths were log transformed for this analysis to allow existing relationships to be seen clearly. Akaike's Information Criterion (AIC; Akaike 1973) was used to compare the models and assess the best at predicting zebra mussel wet weights. The model with the lowest AIC score and highest Multiple R² value was selected as the best fitted to the dataset. An AIC score of at least two units lower than other models was considered significantly better fitted to the dataset.

Results

Three of the tested models had similar support in predicting wet weight. These included the log transformed length model, the interaction model of log transformed length and substrate type, as well as the log transformed length and substrate type model (Table 1). All three models had an AIC score within two units and R² values within one percent of one another. Due to this, the simplest of these is likely the best at describing the dataset. The simplest model included only the contribution of log transformed shell length on resulting log transformed wet weight. This model had an AIC score of -72.09 and R² value of 0.98.

The model that included only substrate type demonstrated relatively less predictive value than other models with an AIC score of 292.21 and R² value of 0.00 (Figure 1).



Figure 1. Non-linear shell length- wet weight relationship of Zebra Mussels sampled from Lake Bemidji color-coded by substrate type. (Red = cobble-sized stone, Green = native unionid, Black = woody debris)



Figure 2. Log transformed shell length-wet weight relationship of Zebra Mussels sampled from Lake Bemidji color-coded by substrate type. (Red = cobble-sized stone, Green = native unionid, Black = woody debris)

Table 1. Regression models tested as predictors of log transformed zebra mussel wet weight, including their AIC score and R^2 value. Zebra mussel shell length and substrate type were variables of focus. Both shell length and wet weight were log transformed to normalize their distributions for analysis.

Model	AIC	ΔΑΙΟ	Multiple R ²
Log (Length)	-72.09	0.00	0.982
Log (Length) * Substrate Type	-71.01	1.08	0.984
Log (Length) + Substrate Type	-70.36	1.73	0.983
Null Model	288.42	360.51	NA
Substrate Type	292.21	364.3	0.00

Based on the log transformed length-weight model, the length-weight relationship for the sampled population across substrate types was as follows: log WW = $-9.2964 + 3.0998 \log SL$ (Figure 2).

The null model with log transformed wet weight as a function of the intercept was also tested to ensure that length was in fact a good predictor of wet weight. Results of this analysis support that assumption.

Discussion

Shell length was found to have significantly higher value in predicting mussel wet weight than substrate type in this study. This finding is backed by considerable support for body size directional measurements such as shell length as predictors of biomass metrics in many mussel species including zebra mussels. Findings from a comprehensive study of 44 bivalve populations from Europe, the Americas, and Asia found shell length to be a good predictor of living weight, wet weight, dry shell weight, dry weight, and ash free dry weight with R² values ranging 0.82-0.96. Some variation was seen between the different populations, but shell length proved to be a highly reliable predictor for all biomass metrics with accurate conversion factors being established between the various biomass metrics (Coughlan et al. 2021).

In comparison, substrate type was found to have less predictive value than all other tested models in this study. Additionally, it was hypothesized that zebra mussels attached to native unionids would have greater lengths and weights compared to those sampled from other substrates due to potential nutrient siphoning from the native species, but this was not observed. Little is currently known about zebra mussel weights across substrate types, however, literature suggests that zebra mussels benefit from attachment to native mussels which may result in better condition and overall health (Pilotto et al. 2016). This was not apparent in this investigation and no significant differences in length-weight distributions were observed among substrate types. One possible explanation for this finding is that the current zebra mussel population in Lake Bemidji has not yet progressed to a stage where these patterns are present, and potentially length-weight relationships will become more variable as the infestation progresses. Without established literature on this area of study, further investigations are necessary to explore this possibility.

This study created a framework for understanding zebra mussel populations in Lake Bemidji by establishing a length-weight relationship and assessing the variables of substrate type and shell length in predicting zebra mussel wet weights. Future investigation into population trends and subsequent impacts on the surrounding watershed should be conducted to supplement these findings.

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