TWO INVADERS, ONE ECOSYSTEM: EXPLORING INTERACTIONS BETWEEN *DREISSENA POLYMORPHA* AND *HYDRILLA VERTICILLATA*

by

Emily Lorkovic, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
May 2023

Committee Members:

Astrid N. Schwalb, Chair

Monica McGarrity

Jason Martina

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Astrid Schwalb, for giving me the opportunity to conduct science from the ground up, for encouraging me along the way to execute this project and bring it to completion. I would also like to extend my appreciation to my committee members, Monica McGarrity and Dr. Jason Martina, for their invaluable contributions in formulating and refining this study.

I would like to express my heartfelt appreciation to the small village of people who supported me throughout this challenging journey. First, I want to thank my graduate lab-mates, Kiara Cushway and Meghan Martinski, who slogged through this journey with me, we will forever be connected in this way. A big thank you for to Dr. Mariana Perez-Rocha, without whom I might still be adjusting ggplots. Finally, I want to thank all the lab assistants: Kenya Johnson, Marlee McDaniel, Daniel Hayes, Kelsie Phelps, Mitchell Sabourin, Seth Ross, Caleb Clarkson. Also, a big thanks to the Freeman elite, who have helped me in countless ways, Ericah Beason, Ashley Cottrell, Dr. Tonya Ramey, Dr. Weston Nowlin, Kenneth Sparks, Caroline Mierzejewski.

I want to thank my family for the immeasurable support and reminding me that I am a real person in the heat of stress. I want to give special thanks to Kasey, my partner, for his unwavering support throughout my graduate studies. His encouragement and belief in me, even during the most challenging times, is the reason I'm here today.

TABLE OF CONTENTS

Page

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Tables **Page**

ABSTRACT

Invasive species in aquatic ecosystems can alter ecosystem processes, detrimentally affect native species, and facilitate the invasion of other species. One infamous aquatic invader, the zebra mussel (*Dreissena polymorpha*) is known to cause declines in phytoplankton through their filtering activity and to facilitate the subsequent spread and growth of macrophytes by increasing water clarity. In turn, submerged macrophytes may provide substrate for settlement of zebra mussels, which require a firm substrate to settle on (i.e., not mud or sand). The invasion of Canyon Lake, TX by zebra mussels has occurred relatively recently, and since then, the spread of an invasive submerged macrophyte, *Hydrilla verticillata*, has also been observed. The goal of this study was to examine variation in the distribution of both zebra mussels and Hydrilla in relation to sediment composition, to each other, and over time (summer vs. fall), and potential facilitation between the species in laboratory and field experiments. To investigate the distribution of each species, dive surveys were conducted in June 2022 and September 2022 using sampling quadrats along a transect at eight sites at Canyon Lake, TX. Field and lab experiments used a fully crossed experimental design to examine the impact of Hydrilla on zebra mussels and vice versa with controls (Hydrilla only and zebra mussels only), and treatment (Hydrilla + zebra mussels). For the surveys, Hydrilla densities tended to be higher in muddy compared to rocky sediment. In contrast, zebra mussel densities tended to be higher in rocky (on Hydrilla and rocks) compared to muddy

habitat (only on Hydrilla). Within the rocky habitat, zebra mussels attached to Hydrilla had significantly higher densities and a smaller size than those attached to rocks. However, only a small fraction of zebra mussels remained on Hydrilla in September, almost exclusively representing a new settlement cohort based on their size distribution. Hydrilla biomass did not change significantly between summer and fall. The experiments did not detect any significant impact of zebra mussels on growth and condition of Hydrilla and positive effects of Hydrilla on zebra mussels was limited to Hydrilla presence in low densities. Nevertheless, Hydrilla may directly facilitate zebra mussel dispersal, especially in spring, as mussels attached to plant fragments can be transported by currents downstream or by human activities, such as entanglement in boat propellers or trailer bunks.

1. INTRODUCTION

Biological invasions pose a major threat to ecosystems globally (Vitousek et al., 1997; Ricciardi, 2007). Although dispersal of non-indigenous species is a natural occurrence, modern human travel has facilitated long-distance geographic distribution at faster rates (Ricciardi, 2007; Hulme et al, 2008). Freshwater ecosystems are especially vulnerable to invaders because they are sites of high biodiversity and support nutrient cycling (Kalff, 2001). Natural dispersal into and among freshwater aquatic systems is somewhat limited to regional watersheds as many taxa lack the ability to spread across long distances overland (Havel et al., 2015). However, some species have characteristics that can facilitate long-distance jumps into novel habitats. For instance, desiccation resistance of nesting eggs can facilitate zooplankton dispersal far from native environments (Hairston & Cáceres, 1996). Similarly, invasive mussels and aquatic vegetation can survive short-term desiccation and be transported overland on watercraft (Johnson et al., 2001; De Ventura et al., 2016; Drake et al., 2017). The United States is now home to more than 1,300 non-indigenous aquatic taxa, including 268 in Texas (http://nas.er.usgs.gov/). High numbers of species introductions increase the likelihood of aquatic ecosystems housing multiple nonindigenous and potentially invasive species (Hulme et al., 2008).

The Invasional Meltdown Hypothesis (IMH) suggests that when invader species disrupt ecosystems, these disruptions can lower community resistance and facilitate further invasions by other species (Simberloff & Von Holle, 1999). An already present invader can benefit a novel one in different ways. For example, an invasive reef-building tubeworm (*Ficopomatus enigmaticus*) was able to alter estuarian community assemblages

by increasing substrate complexity which favored non-native fauna (Heiman & Micheli, 2010). Another example is when invaders reduce competition. For instance, an invasive snail (*Pomacea maculata*) may facilitate establishment of an exotic wetland plant (*Alternanthera philoxeroides*) by preferentially consuming native plants, reducing competition resulting in local increase of biomass of *A. philoxeroides* (Meza-Lopez & Siemann, 2015).

Zebra mussels (*Dreissena polymorpha)* are often considered one of the most problematic invasive species in North America. Originally from Eurasia, their initial introduction in North America is believed to have occurred through the release of ballast water and fouled anchors from oceangoing vessels (Ussery & McMahon, 1994; McMahon 1996), which was also the most likely mode of introduction of another invasive congener, the quagga mussel (*Dreissena rostriformis bugensis*). Zebra mussels were first found in the United States in 1986 in Lake Erie of the Laurentian Great Lakes Basin (Carlton, 2008; Benson, 2013). Zebra mussels have since spread throughout the Mississippi River Basin and adjacent regions of North America primarily by boats carrying attached adult individuals (Strayer, 2009; Cole et al., 2019), although downstream dispersal of free-swimming larvae, called veligers, from river impoundments has also contributed significantly to their spread in some areas, including Texas (Olson et al., 2018).

One key feature of zebra mussel populations is the formation of aggregates, called druses, which accumulate on hard surfaces. Mussel bio-fouling clogs pipes and coats infrastructure leading to large economic costs in the drinking water and recreational industries (Strayer, 2009). Zebra mussels quickly establish populations in new habitats

because they broadcast their gametes into the water column in high abundances. In ideal conditions, a single female mussel can release approximately one million eggs per spawning event (Borcherding, 1991). These larval mussels, of the free-floating veliger type common to bivalve mollusks, eventually settle on submerged biotic and abiotic surfaces, and attach to surfaces by producing proteinaceous byssal threads. Although zebra mussel densities are generally higher on abiotic surfaces, settlement on biotic substrate such as plant surfaces can also support substantial portions of populations, especially juveniles (Londo et al., 2022).

Zebra mussels are "ecosystem engineers," which exert significant effects on ecosystems via transfer of nutrients from the water column to the benthos by filtration and excretion, causing a benthification of ecosystem energy dynamics (Sousa, 2009; Higgins & Vander Zanden, 2010). The excretion of nutrients at the bottom can benefit benthic aquatic plants and algae in the littoral zone, which can increase primary production as well as respiration causing fluxes in environmental oxygen and carbon dioxide concentrations (Figure 1, Zhu et al., 2006; Armenio et al 2016; Jeppesen et al, 2016). Several studies have documented the facilitation of macrophytes by dreissenid mussels. Increased water clarity caused by high filtration activity of zebra mussels and a subsequent decline in phytoplankton were associated with lake-wide increases in macrophyte production and diversity in lake Oneida, NY (Zhu et al., 2006). Reduced turbidity from plant and mussel activity can shift ecosystem equilibria to a clear water state in shallow lakes (Scheffer et al., 1993). Also, quagga mussels appeared to facilitate rapid range expansions of an invasive macrophyte, *Elodea nuttali*, into deeper waters in a reservoir in Germany (Wegner et al., 2019) and in laboratory conditions zebra mussel

presence promoted the growth of *Elodea nuttali* when grown in monocultures and mitigated the competition between this species and another invasive plant, *Elodea canadensis* (Crane et al., 2020).

Macrophytes can provide biotic surface for attachment of zebra mussels which may be especially important in areas with soft substrate, where zebra mussels otherwise would not be able to attach (Figure 1, Londo et al, 2022). Attachment of a morphologically similar invasive bivalve, the golden mussel (*Limnopterna fortuni*) has been observed on Hydrilla (*Hydrilla verticillata*) possibly facilitating the spread of the golden mussel in Brazilian reservoirs (Michelan et al., 2014). However, plant surfaces may not be sites of permanent settlement for zebra mussels. In temperate regions, plant senescence can trigger mussel abandonment of tissues (Martel, 1993; Londo et al. 2022). Zebra mussels may just release and fall to sediments or engage in a process called "post-metamorphic drifting," which is a form of passive locomotion where mussels can detach from plant surfaces and drift on a mucosal filament in the water column (Martel, 1993; Ricciardi and Hill, 2023).

To the best of my knowledge, interactions between zebra mussels and Hydrilla have not been studied yet. Hydrilla is a submerged aquatic plant native to Southeast Asia that has spread to multiple continents, including North America where established populations persist in Eastern and Southern River Basins (USGS 2022). On a local scale, co-occurrence of Hydrilla and zebra mussels has been observed in Canyon Lake, TX, (E. Lorkovic personal observations) where stands of plants were first described in 2020, three years after the initial detection of zebra mussels, and increased in density by 2022 (TPWD Vegetation Report, personal communication Patrick Ireland). This reservoir is a

highly trafficked location for recreational boaters, and therefore is likely to experience high propagule pressure from other locations in the basin that resulted in invasions by both zebra mussels and Hydrilla. Zebra mussel distribution in Canyon Lake has been monitored since 2017 (Schwalb et al. 2022), but not in relation to Hydrilla.

Hydrilla spreads easily by multiple propagation methods including fragmentation, tubers, and turions that help disperse propagules by birds and boats (Langeland, 1996; Zhang et al 2013; Patrick & Florentine, 2021). Hydrilla is often found rooted in benthic substrate as large as gravel (Wentworth, 1922), which allows the species to spread and persist in locations with rocky substrates (Nichols, 1992). In addition, the tuber organs are resistant to desiccation and freezing which make the plant difficult to control via physical removal and water level manipulations once established (Langeland, 1996). Individual plants can grow approximately 2.5 cm/day under ideal conditions forming canopies up to 4m tall, that outcompete native plants and phytoplankton for sunlight (Langeland, 1996; Bianchini, 2010, Figure 1). Hydrilla has additional characteristics that promote establishment which include high stem density at surface, high water content of tissues, high salinity tolerance, broad pH tolerance, ability to persist at depths with 1% Photosynthetically Active Radiation (PAR), and ability to switch carbon uptake species (i.e., free CO2 or bicarbonate) (Langeland, 1996). Furthermore, Hydrilla sequesters nutrients from sediments and the water column, decreasing root to shoot ratios (Tang et al., 2019). These macrophytes can engineer ecosystems primarily by increasing vertical habitat complexity and increasing water clarity (Langeland, 1996). Hydrilla may also increase carbon sedimentation by natural decomposition (source of organic carbon) of plant tissues and reducing local flow (inorganic carbon) as occurs with a morphologically

similar aquatic invasive species, Brazilian waterweed (*Egeria densa* Planch.) (Drexler, 2021). On a local scale, environmental oxygen concentrations may oscillate diurnally in densely packed stands of Hydrilla, where daytime concentrations are high from photosynthesis and nighttime concentrations dip near anoxia due to respiration (Spencer et al., 1994; Sousa, 2011). However, studies are limited on Hydrilla's effects on local ecology in respect to sedimentation and oxygen fluctuations, and further research is warranted.

The goal of this study was to examine variation in the distribution of both zebra mussels and Hydrilla in relation to substrate, each other, and over time (summer vs. fall) and to examine potential interactive effects in field and lab experiments. Specifically, my first objective was to examine how (1) substrate composition affects the distribution of zebra mussels and Hydrilla.

In areas with muddy substrates, we predicted that Hydrilla biomass would be highest, with moderate mussel densities on Hydrilla tissues (Table 1, 1a). Conversely, in rock-dominated areas overall mussel densities would be higher because both benthic rocks and Hydrilla tissues would support mussel densities. (Table 1, 1b). Secondly, I examined how the density and size distribution of mussels would differ between those attached on rocks versus Hydrilla and between summer and fall (Table 1, 2a-b). I expected higher zebra mussel densities on Hydrilla than on rocks but mostly consisting of newly settled smaller juveniles. In respect to differences between summer and fall (Table 1, 3a-b) I expected an increase in Hydrilla biomass and a decrease in zebra mussel densities over that period.

Finally, we explored possible effects of mussel presence on growth and tissue condition of Hydrilla, as well as Hydrilla effects on zebra mussels (Table 1, 4a-b). We anticipated

that mussels would increase the biomass and growth of Hydrilla, but we did not expect Hydrilla presence to significantly affect zebra mussels.

2. MATERIALS AND METHODS

Study Locations

This study was conducted in Canyon Lake, a eutrophic reservoir with over 24 kilometers of shoreline located in the Guadalupe River basin in south-central Texas. A total of eight sites were surveyed between the 16th and 23rd of June 2022; with four sites consisting mostly of muddy sediment (a mixture of corbicula shells, organic particles, and silt), whereas the other four sites were dominated by rocky sediment (larger pebbles and cobbles) (Table 2). Two sites (BR 1 G and BR1 Cove H) were located near the dam, where water depth can reach up to 42 meters (Fig. 1, USACE Bathymetric Map, 2000). Another four sites (Potters C, Potters A, Marina E and BR 23) were in the transitional zone of the reservoir, displaying much shallower water than sites near the dam (i.e., maximum depth 16 to 24 meters, Fig. 1, USACE Bathymetric Map, 2000). The last two sites (Crane A & Crane B) were located near a major channel bend (maximum depth up to 16 meters) and were the most upstream sites in our study. Between the 15th to 18th of September 2022, 6 sites were sampled again (except Marina E and BR 23 due to time constraints) in transects adjacent to quadrats removed in June. Field experiments were carried out between the 25th of August and the 1st of October 2022 in a submerged experimental plot 0.5km east of Boat Ramp 23 (Fig. 1). This location is shallow on the eastern shore but deepens on a gradient toward the east. The benthic substrate was dominated by coarse sand and Hydrilla monocultures were densely rooted across the site. The location was not used by recreational swimmers but frequented by anglers. The year of 2022 was an “exceptional drought” year in Texas (NOAA, 2022), which resulted in less flow and subsequent reservoir drawdown, dropping the water levels 1.2 meters between June and September during the survey period, and water levels dropped to 0.4

meters between August and October 2022 at the field experiment site (Texas Water Development Board, 2022).

Field Survey & Laboratory Techniques

Hydrilla and zebra mussel surveys were carried out by scuba diving using five sampling quadrats (25 x 25 cm) at every two meters along 10 meter transects located parallel to the shore at a water depth between 2.7 to 4.0 meters in June and 1.7 to 2.7 meters in September. Inside each sampling quadrat, all Hydrilla tissues present in the water column (from surface to lake bottom) were removed by hand using garden shears. Immediately after collection, Hydrilla tissues samples were placed in 0.5-millimeter mesh bags. Rocks colonized by mussels were sampled by collecting 1/4 of the sampling quadrat (25 x 25 cm) for rocky sites in June only. Plant and mussel samples were transported in aerated lake water (to reduce stress) to the laboratory and immediately frozen at

-12°C.

Hydrilla samples were thawed at room temperature for processing, after which zebra mussels were carefully removed from plant tissue and washed with deionized water. Subsampling was performed in the following way: zebra mussels were removed from 1/3 of each plant sample (derived from one sampling quadrat). If fewer than 50 mussels were found, then an additional 1/3 of the plant sample was inspected for more individuals. If 50 mussels were still not reached in the subsamples, then the entire plant sample was inspected for individuals. All zebra mussels (removed from Hydrilla and collected from rocks) and Hydrilla samples were oven dried at 60°C for 24 and 48 hours,

respectively, until a constant weight was achieved. Zebra mussel individuals were counted and measured with calipers to the nearest 0.1 mm, and Hydrilla samples were cooled to room temperature and further weighed to determine their dry biomass.

At each field survey, temperature (°C), dissolved oxygen (mg/L^{-1} & %) and specific conductance ($\mu S\ cm^{-1}$) were measured with a multisonde (YSI model ProDDS). The water depth of each sampling quadrat was determined during diving to the nearest 30 cm (1 feet) with a SCUBA depth gage (Cressi miniconsole 2 imperial gage). Water samples were collected from the water surface during surveys in June and September and at 3- and 2-meters depth in June and September, respectively. Turbidity (NTU) and chlorophyll-a (Chl-a) concentrations (µg/L) were determined in laboratory using a fluorometer (Trilogy, model 7200-000) and calculated using a linear regression as described in Robertson and Schwalb (2016). Total suspended solids (TSS) were measured by filtering a volume of lake water through an ashed 1 µm filter (Pall A-E Glass-Fiber Filters) and dried in the oven at 101°C for 1 hour. After dehydration, suspended solids were weighed on a microbalance (Sartorius Lab Instruments ENTRIS124-1S).

Field Experiment

The field experiment was carried out for 5 weeks between August 25th and October 1st, 2022. A fully crossed experimental design was used to examine the impact of Hydrilla on zebra mussels and vice versa with two controls (Hydrilla only and zebra mussels only), and one treatment (Hydrilla + zebra mussels) with 6 replicates, summing a total of 18 treatments and controls. To carry out this field experiment, a 3 x 6 meter experimental plot was selected near site BR23 in Canyon Lake (Fig. 1) and all Hydrilla

was removed by pulling up stalks and root structures by hand. A wooden frame was submerged in the plot and weighted to the bottom with large boulders sourced from the lake shore (mussel free). The plot was sub-divided by wooden crossbeams into 18 experimental units (1x1m quadrats) for different treatments (controls and Hydrilla+zebra mussels).

Each replicate control for Hydrilla consisted of a ceramic pot (dimensions 13 x 15 cm, volume 2,558cm^3) placed in the middle of the quadrat and filled with 5 centimeters of topsoil scraped from the surface of a nearby shore location (mixed well by hand). Three pebbles (4 to 6 centimeters intermediate axis) were placed on top of the sediments inside the ceramic pots. Treatments including hydrilla contained 3 strands of the plant which ranged from 10-15 centimeters length. Before planting in the center of each plot, root structures were rinsed with lake water to remove residual sediments, and all zebra mussels were removed from Hydrilla surfaces for both treatments. For each replicate, pebbles were placed on top of sediments in the pots to support plant stems and elevate mussel sampling bags, for treatments with zebra mussels approximately 10% of the rocks' surface was covered with attached mussels (to increase mussel densities) and for hydrilla control treatments same sized rocks mussels were absent. In addition, cages containing 40 mussels divided equally into two class sizes, small (5-14.9mm) and large (15-25mm), were placed in 0.5mm mesh bags and zip tied to the upper surface of one of the pebbles.

The field experiment was run for 37 days. Starting at day 2, the submerged plot was monitored for damage to Hydrilla once a week as well as zebra mussel mortality. All regrowth of vegetation within and 1 meter around the wooden frame was removed. To

check for mussel mortality, cages were removed from treatments and dead mussel shells were discarded at each weekly monitoring event. At day 16, 40 Hydrilla stems were propped up with a 46 cm coated wire with eyelet to keep apical stems pointing toward the water surface and prevent breakage. The following environmental characteristics were measured initially, then at day 2 and every week thereafter: temperature, dissolved oxygen (percentage and concentration) and conductivity. Also, water samples were taken to be analyzed for TSS, Chl-a, and turbidity. Water samples were processed in same manner as and mentioned in field surveys.

At the end of the experiment, all biotic material and structures were removed from the experiment site. Hydrilla above and below sediment structures were carefully removed from pots and placed in 0.5mm mesh bags and returned to the laboratory at Texas State University for further analyses. Hydrilla samples were frozen within 4 hours of removal. For sample processing, Hydrilla samples were thawed at room temperature and lengths were measured using a tape measure (nearest 1mm) and tissues were dried and weighed as for Field Surveys. Carbon and nitrogen analysis were performed on Hydrilla, where all stems per sample were homogenized using a grinder (IKA Works A 11 Basic Analytical Mills) and approximately 3-5mg was subsampled and weighed using a microbalance (Mettler Toledo MX5). The amount weighed was then packaged in 30mm tin disks to measure carbon and nitrogen concentrations using a FlashEA 112 Series – NC Soil Analyzer. Zebra mussel samples were not processed because mortality was too high over the experiment duration.

Laboratory Experiment

A laboratory experiment was conducted to test the effects of zebra mussels on hydrilla growth and vice versa under controlled conditions. The experiment was carried out using a fully crossed design with 5 replicates of the following treatments: Hydrilla high density + zebra mussels, Hydrilla low density + zebra mussels, and controls for each of the treatments (zebra mussels only, Hydrilla only in low and high density), plus three with water only to control for water parameters. Each independent experimental unit consisted of a 1.5 L tank filled with filtered lake water (50um mesh) from Canyon Lake. The experiment was carried out for 16 days (November 7th to November 23rd, 2022). Zebra mussels and Hydrilla were collected from Canyon Lake, Texas at the start of the experiment near boat Ramp 23 (Fig. 1). Apical stems of Hydrilla were clipped at 5cm total length and bottom 0.5 cm wrapped in cotton gauze where they were attached individually to rocks using cotton thread. High density Hydrilla tanks received a total of 9 plant strands and low density Hydrilla tanks received 3 strands per tank. All tanks received a total of 3 rocks (2-3 cm intermediate axis) for standardization. Initially, a total of 10 mussels (3 each sizes 7 & 12 mm and 2 each size 8 & 13mm) were placed in the bottom of the tank in all mussel inclusive treatments. At day 5 the number of zebra mussels was increased 3 times initial densities (30 mussels per tank) to buffer mortality and increase treatment effects, wet biomass was extrapolated to match low-density treatments in Crane et al. (2020).

Temperature was continuously measured using temperature loggers (HOBO pendant Temperature/Light 64K data logger) in four randomly selected tanks across the treatments. PAR was calculated from lux measured in the tanks by regression analysis

(y=0.0138x, R^2=0.677) using a PAR meter deployed on the roof of the Freeman Aquatic Biology building at Texas State University (LI-190R Quantum Sensor, LI-COR, Lincoln, NE, USA). Three Hygger Programmable Aquarium LED Lights (HG-957, 36 watts) were placed between each set of two rows and set to a 16:8 day/night schedule. All treatments were aerated using 4mm silicone tubing and 1 inch air stones. Temperature, dissolved oxygen, and specific conductivity was measured once daily (at noon) in each tank using a multisonde (YSI proDDS), and pH was measured with a smaller probe (Hannah HI 98195/10). Mussels were inspected daily for mortality and replaced if found dead. Daily mussel filtration rates were calculated to be approximately 3L per day based on biomass (Bunt et. al. 1993). Two water changes were conducted on day 2 and 4 to feed mussels. However, at day 5 and every other day for the remainder of experiment, mussels were switched to a mussel food consisting of 2.4 mL of diluted 2:1 shellfish diet and *Nannochloropsis* liquid food (Reed Mariculture) to ensure mussels were getting adequate food.

On the last day of the experiment, all Hydrilla was measured for length (0.1mm) and both zebra mussels and Hydrilla were rinsed with de-ionized water before being frozen. The processing of Hydrilla plants was similar to methods described above for survey samples. Mussel samples were dried using the same methods as laid out in the field survey section. Additionally, dried mussels were measured for length, weighed on the microbalance, and burned in the Muffle Furnace (Barnstead Thermolyne 600 Furnace) at 500°C for 4 hours, then weighed again to determine Ash Free Dry Mass (AFDM).

Data Analysis

All statistical analysis was performed in R version 4.1.0 (2021). First, normality of data was assessed using Shapiro-Wilk's test for normality, and homogeneity of variances was evaluated using Levene's test. When normality assumptions were violated, logarithmic or cube-root transformations were applied to normalize the distribution. To examine the effect of benthic composition type on Hydrilla biomass and the densities of zebra mussels attached to Hydrilla, a Nested Analysis of Variance (ANOVA) was conducted, with site serving as a covariate. One-way ANOVA was employed to evaluate differences in Hydrilla C:N ratios (lab and field experiment), and zebra mussel AFDM (lab experiment). To test for significant differences among groups, Tukey's honestly significant difference post-hoc tests were performed on all ANOVAs. Pearson correlation coefficient was computed to examine whether there was a significant correlation between total zebra mussel densities and Hydrilla biomass at rocky and muddy sites. Pairwise t-tests were used to compare summer and fall surveyed Hydrilla biomass, assess differences between Hydrilla-bound and rock-bound mussels at rocky sites, and evaluate treatment effects of zebra mussels on Hydrilla change in biomass and growth in the field and laboratory experiment. Non-parametric Mann-Whitney U tests were employed to test for zebra mussel density changes between summer and fall, size differences between mussels found on Hydrilla tissues and rocks, and pairwise comparisons between controls and associated treatments plus low-density and high-density Hydrilla biomass in the laboratory experiment.

3. RESULTS

Distribution of Hydrilla and zebra mussels in relation to sediment composition

The ANOVA detected significant effects of substrate and site on both zebra mussel density and Hydrilla biomass (Fig. 2). In accordance with prediction 1a and 1b, zebra mussel densities tended to be higher at rocky sites, whereas Hydrilla biomass tended to be higher at muddy sites (Fig. 2, 3). A wide range of hydrilla biomass was observed at both muddy and rocky sites; only muddy sites had very high hydrilla biomass (> 400 g m^{-2}) and mussel densities below 10,000 individuals m^{-2}, except for one quadrat at Marina E, a rocky site (Fig. 3).). Total zebra mussel densities was significantly correlated with Hydrilla biomass at muddy sites ($r=0.73$, $p<0.001$) but not at rocky sites ($r=0.20$, $p=0.41$, Fig. 3).The highest Hydrilla biomass was observed at a muddy site in the riverine zone of the reservoir (Crane A), where biomass was 3.5 times higher than Potters D, the lowest biomass muddy site near the main channel (Fig. 2, 3). The lowest Hydrilla biomass was observed at sites located near the main channel of the reservoir, where Hydrilla was restricted to a narrow strip in shallow water near the shore (Crane B, rocky site) or was found in isolated patches (Potters D, muddy site, Fig. 2, 3). Like Hydrilla, zebra mussel densities were also lowest at the muddy site Potters D ranging between 64 to 1024 individuals m^{-2} attached to Hydrilla, (Fig.2, 3). Highest densities of mussels attached to Hydrilla were found at rocky site BR 1H, where values ranged from 11,156 to 46,729 individuals m^{-2} (Fig.2).

Zebra mussel densities and size differed between those attached to rocks compared to Hydrilla. At rocky sites in June, zebra mussel densities were significantly higher attached to Hydrilla compared to those attached to rocks at all sites (Fig. 4) which

supports prediction 2a (Table 1). The biggest difference was found at rocky site BR1 H, where zebra mussel densities on Hydrilla were 14 times higher compared to rocks ($T=11.19$, $p<0.001$, Fig. 4). Following prediction 2b, (Table 1), zebra mussels were significantly larger on rocks (6.1 ± 3.1 mm, mean ± SD) compared to mussels on Hydrilla (3.4 ± 0.8mm,); the size difference was significant for all 4 rocky sites (Fig. 5, compare boxplots for rocks in June vs. Hydrilla in June).

Variation between June and September

Between June and September zebra mussel densities declined by an average of 94.7% between all sites, whereas Hydrilla biomass did not change significantly (Fig. 6a). The decline in zebra mussel densities was statistically significant at all sites except Crane B (Fig. 6b, Table 1). Mussel size on plant tissues did not significantly change at resampled sites between June (3.4 ± 1.4 mm) and September (3.0 ± 1.5mm, Fig. 5, compare boxplots for rocks in June vs. rocks in September). Site Crane A was an exception, where zebra mussels were approximately 1.2 mm larger in September (4.2 ± 1.5) than June (3.5 ± 1.6).

Field & Laboratory Experiment

In contrast to prediction 4a zebra mussel presence did not have a significant effect on Hydrilla biomass in the field experiment (Student's T-test, $T=1.37$, p-value=0.20, Fig. 7a) or laboratory experiments (Mann-Whitney U test, Low Density, $W=20$, $p=0.14$; High Density, $W=9$, $p=0.53$, Fig. 7b, c; Table 1). In the laboratory, Hydrilla biomass increased at higher densities compared to lower densities regardless of zebra mussel presence (Mann-Whitney U Test, $W=100$, $p<0.001$, Fig 7b,c) but the opposite was observed for growth (Student's T-test, $T= -2.96$, $p<0.01$, Fig. 8b,c). Hydrilla growth was also not

significantly higher in zebra mussel presence compared to the control in the field, (Student's T-test, T= 0.74, p =0.47, Fig. 8a) nor in the lab (Low density T= -0.25, p=0.90, high density T=-0.69, p=0.51, Fig. 8b,c). Additionally, carbon to nitrogen (C/N) ratios in the lab experiments did not differ significantly between treatments or to the initial measurement (ANOVA $F_{4,18}$= 0.70, p-value = 0.60, Fig. 9b). In the field experiment, in contrast to prediction 4a, C/N ratio of Hydrilla where nitrogen content increased 7.9% during the experiment for Hydrilla controls but did not change for treatments when compared to initial condition (ANOVA $F_{2,12}$ = 11.41, p-value < 0.01, Fig. 9a). It should be noted that the field experiment started with lower C:N ratios in August (mean 1854.20 ± 114.75 SD mgC/mgN) than lab experiments in November (mean 2715.46 ± 473.5 SD mgC/mgN).

In contrast to prediction 4b, mussel biomass was higher when Hydrilla was present under laboratory conditions (ANOVA $F_{2,11}$ = 4.338, p= 0.043, Fig. 10). The difference was statistically significant for the low-Hydrilla density treatment (Tukey's p=0.04), but only marginally significant at the high-density treatment (p=0.09), where variation was higher. Zebra mussel growth and condition were not measured for the field experiment because mortality was high for both large and small size classes (Fig. 11). Mortality during the 16 day lab experiment ranged between 0 and 3 mussels, except for one mussel-only control tank, which experienced a mortality of 19 mussels and was subsequently excluded from analysis. The tank was located nearest to the air outtake for the electric air pumps but experienced only slightly higher temperatures (+0.8°C) and conductivity (+14.68 μS/cm) than the other four tanks.

Environmental Conditions

At the survey sites, temperature averaged 29.3±0.7 °C (mean ± SD) in June and 28.7 ± 0.85 in September (Table 2). Dissolved oxygen (DO) was high at all sites not dipping below 7.10, however samples were measured during daytime. Measurements of DO in dense Hydrilla over two nights dropped to near 3mg/L at temperatures of approximately 30°C.

Chlorophyll-a was very low at most sites for both months (0-1.51 µg/L) except at site Crane A in September (43.48 µg/L, Table 2). Turbidity was very low at all sites in June but slightly elevated in September at three sites: Crane B, Potters C and BR1 Cove G (15.47-22.74 NTU, Table 2).

Average water temperature during lab experiments (measured continuously) was 20.6±1.4 °C over both light and dark periods (1hr increments), whereas average water temperatures during field experiments (measure weekly) were 7.8°C higher (28.4±1.3°C). Light intensity (PAR) in the center of the light fixture averaged 61.4±3.86 µmol/m²/s during daytime, which is well above sufficient light (30 µmol/m²/s) for photosynthesis in similar submerged macrophytes (Mielecki & Pieczyńska, 2005). In the field, PAR was not measured but turbidity remained low (<8.7 NTU) except on week three where it spiked to 51.2 NTU.

4. DISCUSSION

Studies on invasive species often focus on a particular species, although interactions between invasive species may be important (Simberloff & VonHolle, 1999). This is the first study to examine variation in the distribution of both zebra mussels and Hydrilla in relation to substrate, to each other, and over time (summer vs. fall). It was also the first to quantify zebra mussel densities attached to Hydrilla. Hydrilla occurred in both muddy and rocky substrates, but their biomass tended to be higher in muddy sediment. Zebra mussels require substrate they can attach to and cannot settle on muddy substrate, but they occurred on Hydrilla in areas with muddy sediment. In contrast to Hydrilla, zebra mussel densities tended to be higher in rocky compared to muddy habitat, but within the rocky habitat zebra mussels attached to Hydrilla had significantly higher densities and a smaller size than those attached to rocks. However, only a small fraction of zebra mussels remained on Hydrilla in September, almost exclusively representing a new settlement cohort based on their size distribution. Hydrilla biomass did not change significantly between summer and fall. The study did not detect any significant direct impact of zebra mussels on growth and condition of Hydrilla and only limited effects of Hydrilla on the condition of mussels in both field and lab experiments.

Our results support findings by a few studies that macrophytes support transient populations of small zebra mussels (Londo et al., 2022; Bodamer & Ostrofsky, 2010). The mussels found on Hydrilla in September were most likely the result of lower settlement occurring during summer months (Schwalb et al. 2022), because there was no significant difference in size compared to June although growth should have occurred. The decline of juvenile mussels on hydrilla could have been caused by voluntary drift,

mortality, or inability to remain attached due to their size. Whether zebra mussels are physically able to remain attached to Hydrilla as they grow larger remains to be tested. It is known that juvenile mussels ≤ 2mm may drift in water especially at sites with high wave action (from wind or boaters) and a juvenile mussel of 5.9 mm was recently observed to be attached on a lake chub (Ricciardi and Hill, 2023). However, such transport mechanisms are unlikely to explain the substantial decrease observed on Hydrilla in Canyon Lake by an average of -95% (thousands of zebra mussels per m^2). Substantial declines of -56% were observed in the same year (2022) on cumulative settlement monitors (bricks) located near marinas on the lake. In the past, large declines up to -85% (at one site in 2021) and by -54 to -58% in September 2019 and August 2018 were observed in Canyon Lake which was attributed to high summer temperatures (Schwalb et al. 2022) although predation by fish such as catfish may have also played a role.

Summer mortality of zebra mussels can also be caused by low oxygen, which can be low in dense Hydrilla stands at night due to respiration of macrophytes and bacteria (Sand-Jensen, 1989; Spencer et al., 1994; Sousa et al., 2011). In Canyon Lake, dissolved oxygen was observed to decline to near 3mg L^{-1} in dense strands of Hydrilla when water temperatures were approximately 30°C. This is higher than reported by another study in Mississippi (1.5 mg L^{-1}; Miranda & Hodges, 2000), but could have contributed to zebra mussel mortality in combination which higher temperatures as demonstrated by lab experiments (Schwalb et al. 2022). However, dips in oxygen concentration in macrophyte beds are short-term and can oscillate to very high oxygen concentrations during the day, which may be tolerable by zebra mussels (Ventura et al., 2016).

Zebra mussel densities attached to Hydrilla in this study were moderate compared to other studies. For example, we found up to 46,729 ind. m^{-2} hydrilla dry mass, whereas densities up to 750,000 ind. m^{-2} were found on *Potamogeton perifoliatus* (clasping-leaf pondweed) and *Myriophyllum spicatum* (Eurasian watermilfoil) and other native plants in Lake Balaton, Hungary (Muskó & Bakó, 2005). Some studies suggest that veligers selectively settle on plants with sturdy stems (Londo et al., 2022), but future studies should test whether zebra mussel densities are lower on native macrophytes compared to Hydrilla, ideally in the same waterbody.

Higher densities of Hydrilla in muddy compared to rocky substrate support our hypothesis in this study. However, the presence of hydrilla observed in rocky areas further corroborates past evidence that hydrilla is often found in rocky areas although sediment nutrients may be limited (Jain et al., 2018). In our study, the upstream site had visibly thick sediments (Lorkovic field observations), possibly owing to greater sediment loading in the riverine zone of the reservoir, displayed the highest Hydrilla biomass. This site is located where the Guadalupe River dramatically curves and flow velocity declines facilitating settlement of finer transport. Several studies have reported that the presence of aquatic vegetation increases sediment deposition while reducing resuspension (Zhu et al., 2015; Li et al., 2016). This could be detrimental to zebra mussels, which require hard substrate for settlement and their attachment on hydrilla appeared transient in nature.

In previous studies Hydrilla above-ground biomass peaked in early August (Madsen & Owens, 1998), but in Canyon Lake, Hydrilla did not appear to grow between June and September in summer 2022. The early warming in Spring of 2022 may have

triggered earlier spring growth of Hydrilla. Optimum growing temperatures for Hydrilla start at 20°C, which were reached in early March 2022, compared to mid-April in 2020 and 2021 (True-Meadows et al. 2016; Schwalb et al. 2022), however, water temperatures may have been too hot over the mid-summer growth period.

Zebra mussels have been shown to increase the physiological condition of Eurasian Watermilfoil and Eelgrass (Zhu et al. 2007), but such positive interaction was not found in our lab and field experiments. However, our results may differ because C/N ratios were measured in this study and not protein content. In our field experiment, we observed higher C/N ratios of Hydrilla in treatments with zebra mussels compared to only Hydrilla, which means that leaf nitrogen was lower where mussels were present. These results contrast our expectations and suggests zebra mussels may have competed with Hydrilla for nutrients. However, no change in C/N ratios were experienced during the lab experiment which may have been affected by removal of roots and stolons from Hydrilla stems.

Mussels may benefit physiologically as shown in our laboratory experiment where mussels improved body condition when Hydrilla were present; this was observed in plant densities were both high and low, however this was only significant at low Hydrilla densities. One possible explanation is that macrophytes can provide attachment surface for biofilms and other bacteria with large particle size which can be utilized as a food source for zebra mussels (Silverman et al., 1996). Anecdotally, all the laboratory tanks with zebra mussels (with and without Hydrilla) had extensive microbial growth

after one week, but Hydrilla surfaces also supported colonies of microbes which could have benefited mussels in our study.

Our experiments did not detect a significant impact of zebra mussels on Hydrilla growth, suggesting this may not be the mechanism of invasional meltdown in Canyon Lake. Still, zebra mussels (the primary invader) may have played a role in facilitating the invasion of Hydrilla in Canyon Lake, by reducing phytoplankton and increasing water clarity (Robertson & Schwalb, 2019), expanding the open niche for the invasive macrophyte, particularly in the absence of major competition from long-term established native macrophytes. (e.g., Zhu et al. 2006, Wegner et al. 2019).

Reservoirs, as man-made ecosystems, provide a habitat for diverse plant and animal species, making it crucial to effectively manage invasive species to preserve their biodiversity. Hydrilla may directly facilitate zebra mussel dispersal through transport of plant fragments downstream by currents or human vectors as plants with attached zebra mussels are left on boat propellers or trailer bunks (Horvath & Lamberti, 1997). In turn, hydrilla can support more zebra mussels during certain periods of the year, which means that risks for zebra mussel spread between water bodies increases during these periods.

Continued research efforts are needed to further understand the potential impacts of invasive species on native species and ecosystems. Zebra mussels evolved in temperate climates, life-history strategies, population dynamics and ecosystem effects are different in Texas at the edge of their southern distribution than in northern regions like the great lakes. In the future, accumulation of mussel shells from mortality events may allow zebra mussels to colonize muddy sediments including those underneath Hydrilla,

but this remains to be studied. We conducted experimental investigations during fall, which may have influenced the uptake ability of our plants. We suggest that field and lab experiments be repeated during spring to capture interactions when Hydrilla growth rates are high and condition during the natural annual cycle. Future investigations should assess the effects of macrophyte-induced depletion of dissolved oxygen on zebra mussel juveniles and adults, and sedimentation rates of Hydrilla on the availability of benthic hard surfaces for zebra mussel habitat in reservoirs.

Table 1. Overview of rational and predictions of study outcomes including results from field surveys plus laboratory and field experiments.

RATIONALE	PREDICTIONS	SUPPORTED?	FINDINGS
1) Benthic composition of reservoirs is likely to influence the distribution of Hydrilla and zebra mussels. (a) Hydrilla can directly uptake nutrients from the sediment (e.g., mud) to increase biomass, however they can also persist where sediment composition is rocky. (b) Zebra mussels cannot settle on soft sediments, but they will utilize rocks to establish populations. Alternatively, zebra mussels may also settle on plant surfaces for settlement.	a) Hydrilla biomass will be higher where benthic composition is dominated by mud.	Yes	Hydrilla biomass tended to be higher at muddy sites.
	b) Zebra mussel densities on hydrilla will be higher where benthic composition is dominated by rocks.	Yes	Zebra mussel densities tended to be higher at rocky sites.
2) When benthic sediment is dominated by rocks, zebra mussel densities found on Hydrilla are expected to be larger than rocks because Hydrilla drastically increases habitat complexity and surfaces area for veliger settlement. However, zebra mussel sizes are expected to be smaller on Hydrilla compared to rocks because Hydrilla tissues do not maintain maximum above-ground biomass year-round.	a) Local zebra mussel densities will be higher when attached to Hydrilla rather than rocks.	Yes	Greater densities of zebra mussels were measured on Hydrilla tissues than rocks.
	b) Zebra mussels will be smaller (juveniles) on Hydrilla than rocks (adults).	Yes	Zebra mussels attached to Hydrilla were mostly juveniles and majority of adults were found attached to rocks.
3) Zebra mussels which originate from temperate zones (mild temperatures) will experience high mortality during summer (e.g., high temperatures and low dissolved oxygen) in subtropical climates. However, Hydrilla which originates from tropical zones is more adapted to tolerate higher temperatures and continue to grow well into summer conditions.	a) Zebra mussel densities will decrease between summer and fall.	Yes	Mussel densities decreased between June and September.
	b) Hydrilla biomass will increase between summer and fall.	No	Hydrilla biomass did not increase between June and September.
4) Zebra mussel excretion can increase nutrient content to benthos which may directly facilitate growth and condition of Hydrilla. On the other hand, Hydrilla is not likely to affect zebra mussel growth and body condition, because indirect negative effects (Hydrilla reducing light availability for algae) may cancel indirect positive effects (reducing direct sun exposure).	a) Hydrilla growth and tissue condition will increase in the presence of zebra mussels.	No	Hydrilla growth or tissue condition was not affected by the presence of zebra mussels in both laboratory and field experiments.
	b) Hydrilla will have no effects on zebra mussels body condition.	Yes, but only at high densities	At high densities of Hydrilla zebra mussel body condition did not increase. However, at low Hydrilla densities zebra mussels body condition increased.

Table 2. Physical characteristics of the eight survey locations arranged in order (Top to bottom) from far to near distance from the dam, stars denote sites with sediments dominated by rock. Sites highlighted in grey were sampled in June only. Distance from dam (DD) and physiochemical parameters include temperature (Temp), dissolved oxygen (DO), specific conductance (SPC), Chlorphyll-*a* (Chl-a), Turbidity (Turb) and total suspended solids (TSS).

Site	DD (m)	Month	Average Depth (m)	Temp (°C)	DO (mg/L)	SPC (µS/cm)	Chl-*a* (µg/L)	Turb (NTU)	TSS (g/L)
Crane A	13070	June	3.2	28.9	7.22	334.9	1.51	1.51	0.0
		September	1.8	28.5	8.51	338.0	43.48	0.00	3.0
BR 23 F	11965	June	3.0	31.0	NA	320.7	0.07	6.90	0.3
Crane B *	11936	June	2.7	28.7	7.75	339.6	0.93	8.03	0.7
		September	2.3	29.5	8.57	345..5	1.43	15.4	0.9
Marina E *	10110	June	3.5	29.6	7.54	364.3	0.12	0.475	0.9
Potters C *	9833	June	2.9	29.3	8.73	361.9	0.00	21.63	0.5
		September	1.8	28.6	7.74	365.0	0.56	15.98	0.5
Potters D	8600	June	2.7	29.9	8.47	347.9	0.61	2.88	0.8
		September	1.7	27.1	7.10	349.6	0.20	2.90	2.6
BR 1 Cove G	2459	June	3.5	28.4	8.11	369.2	0.23	2.51	1.1
		September	2.5	29.2	8.41	353.6	0.56	22.74	3.6
BR 1 H *	2293	June	3.5	28.8	8.07	377.0	0.00	1.85	0.0
		September	2.4	29.0	8.20	370.6	0.43	6.76	1.2

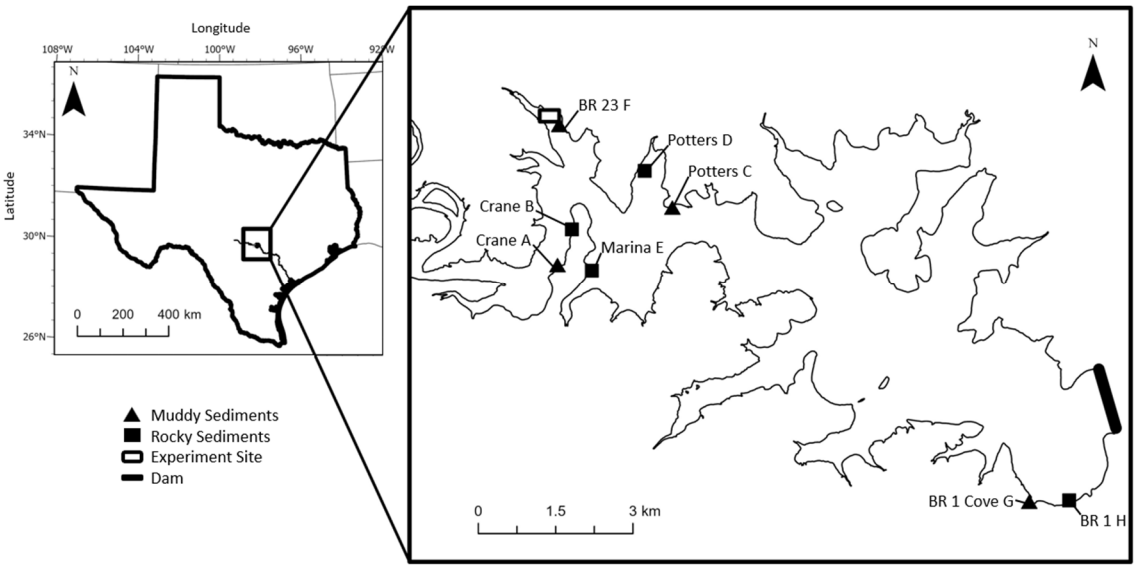

Figure 1. The location of Canyon Lake in Texas, USA, and the dive survey sites for mud- and rock-dominant sediments (triangles and squares). The experiment site (white rectangle) and dam (black line) are also marked on the map.

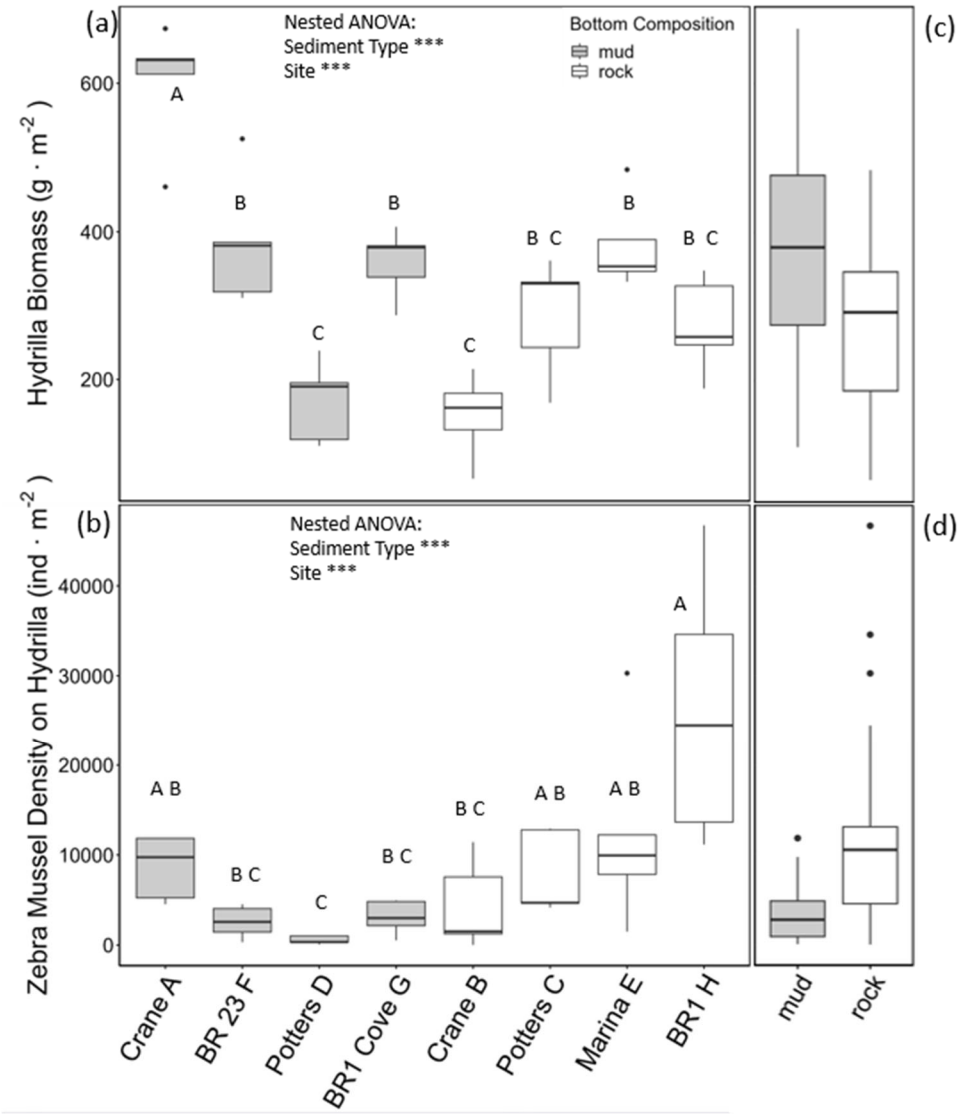

Figure 2. Variation in biomass of Hydrilla (a) and density of zebra mussels attached to Hydrilla (b) at each location surveyed in June 2022. Pooled boxplots for Hydrilla biomass (c) and zebra mussel density (d) are also included to display sediment effects. The lower (25th) and upper (75th) lines in the diagram represent respective percentiles. The diagram also shows the median values (thick bold line). Data falling outside the percentile range are plotted as outliers. Letters above the diagram denote significant differences (Tukey HSD pairwise comparison, p-values<0.05). The factor site was nested within sediment type in ANOVA and significant effects are indicated with asterisks: *** $P < 0.001$.

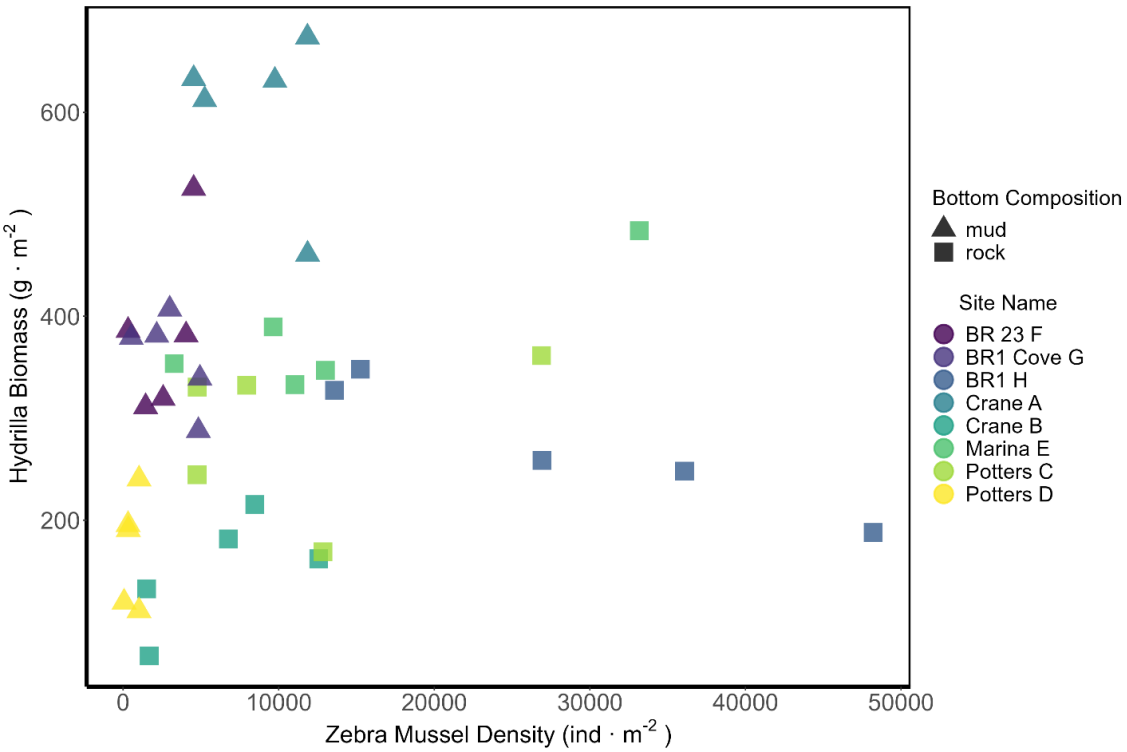

Figure 3. Visual display of relationship between total densities of zebra mussels (benthic and Hydrilla attached) and biomass of Hydrilla for each quadrat in June 2022. Shapes denote difference in bottom composition and colors differentiate site.

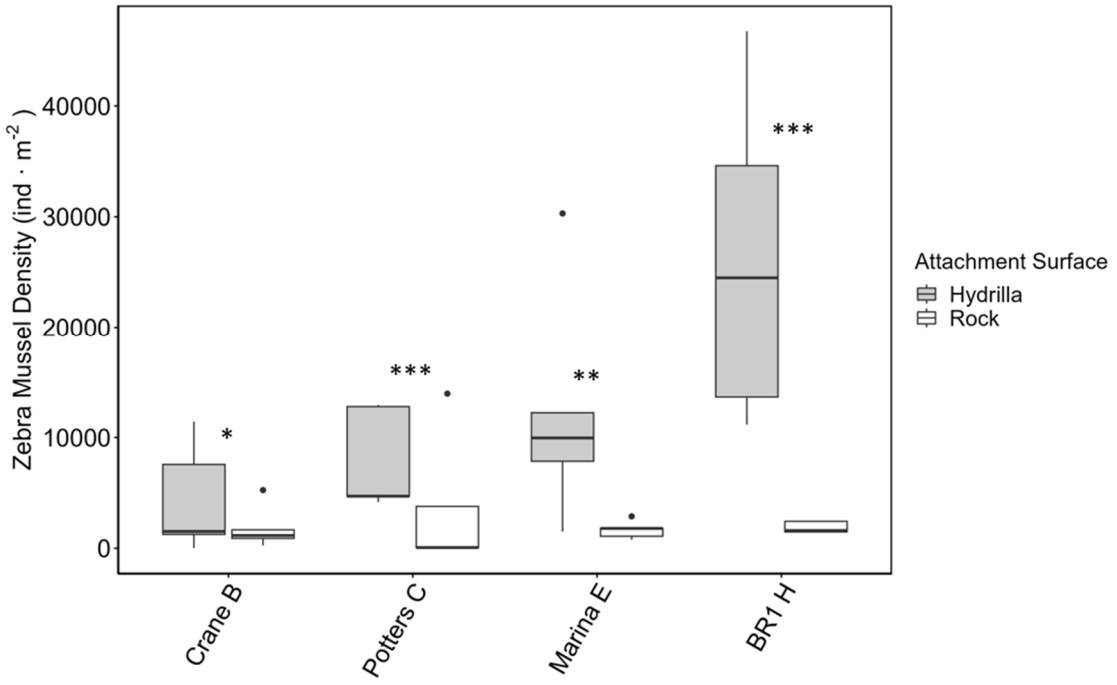

Figure 4. Zebra mussel density on rocks and Hydrilla in June 2022 for sites where rocky substrates were dominant. The lower (25th) and upper (75th) lines in the boxplots represent respective percentiles. The diagram also shows the median values (thick bold line). Data falling outside the percentile range are plotted as outliers. Stars above the diagram denote significant differences for differences between attachment surface at each site (paired student's t-test, *p-values< 0.05, **p-value<0.01, ***p-value<0.001).

*

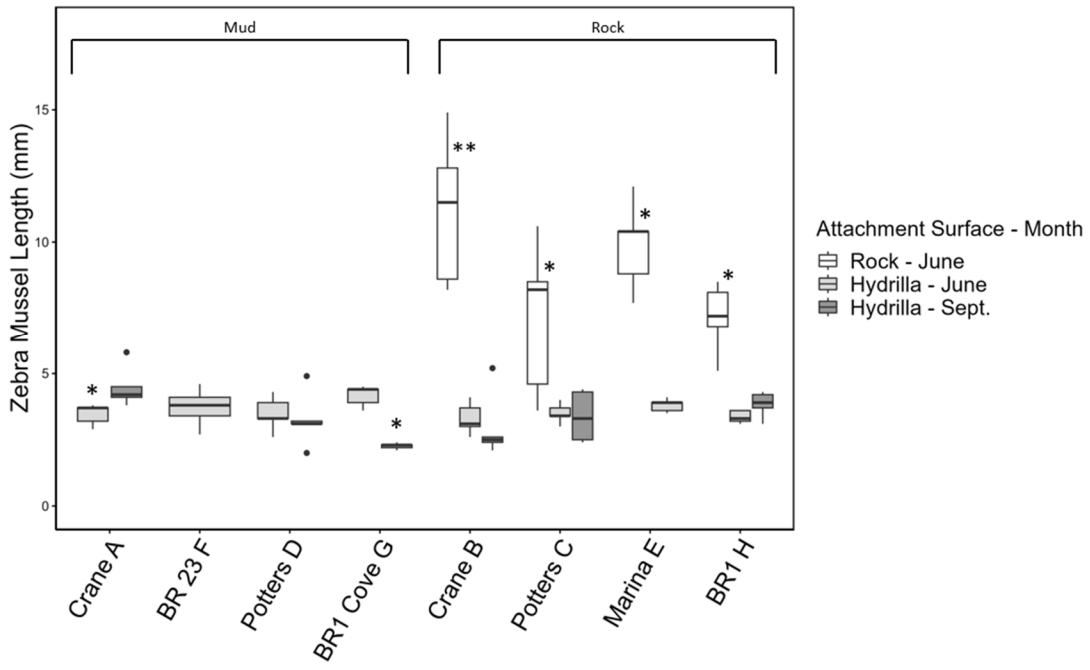

Figure 5. Length of zebra mussels attached to rocks in June (white boxplots) and mussels attached to Hydrilla in June (light grey) and September (dark grey) across sites. The lower (25th) and upper (75th) lines in the boxplots represent respective percentiles. The boxplots also show the median values (thick bold line). Data falling outside the percentile range are plotted as outliers. Stars above the boxplots denote significant differences for tests between attachment surfaces in June and tests for Hydrilla attached mussels between sampling months at each site (Mann Whitney U tests, * p-value< 0.05, **p-value< 0.01).

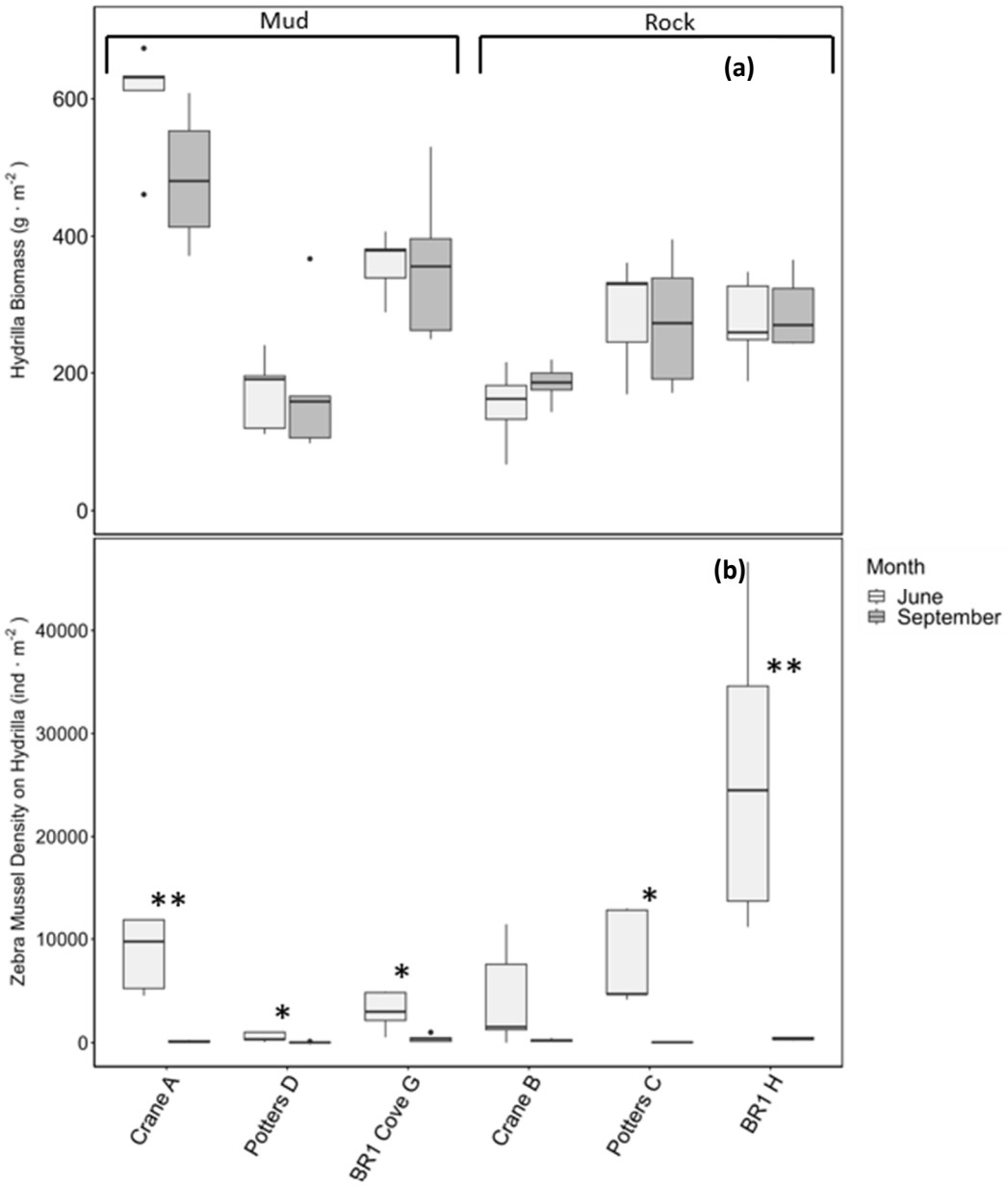

Figure 6. Biomass of Hydrilla (a) and zebra mussel density on Hydrilla (b) at each location surveyed in both June and September 2022. The lower (25th) and upper (75th) lines in the diagram represent respective percentiles. The boxplots also show the median values (thick bold line). Data falling outside the percentile range are plotted as outliers. Stars above the boxplots denote significant differences between sampling month at each site (Mann Whitney U tests, *p-values<0.05 and **p-value<0.001).

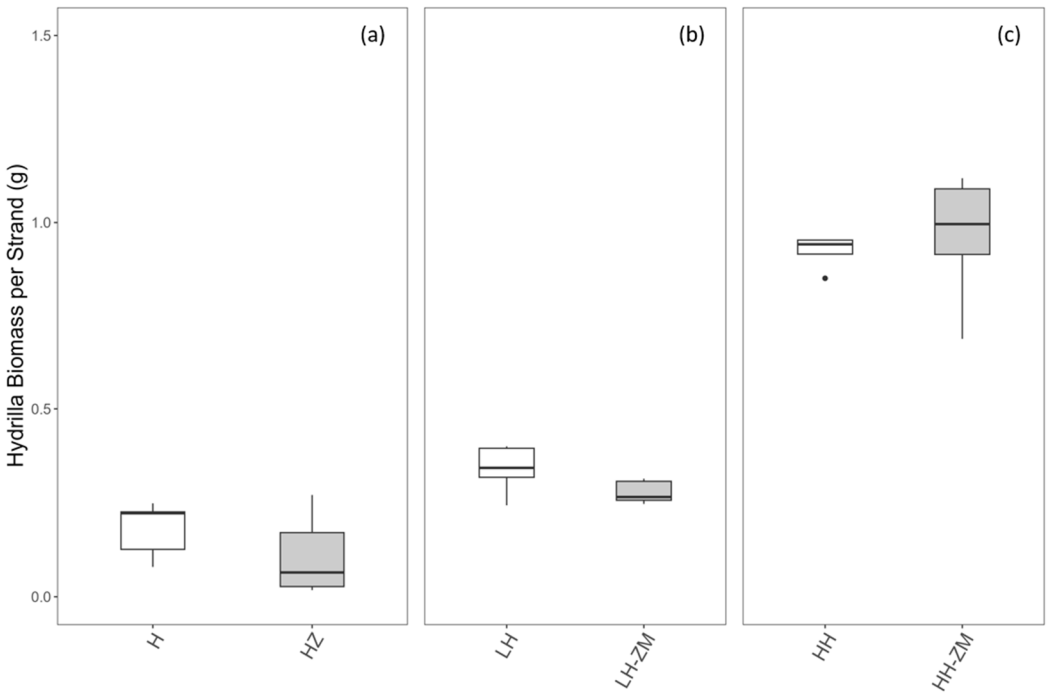

Figure 7. Boxplots summarize the results of two experiments examining the impact of zebra mussel treatments Hydrilla biomass. In the field experiment (a), Hydrilla was tested with (HZ) and without zebra mussels (H), and in the lab experiment low densities of Hydrilla (b) were tested with zebra mussels (LH-ZM) against controls (LH), and high densities of Hydrilla (c) were also tested with zebra mussels (HH-ZM), against controls (HH). Pairwise T-test (Field Experiment) and Mann-Whitney U-tests (Lab experiment) were all insignificant, $p>0.05$.

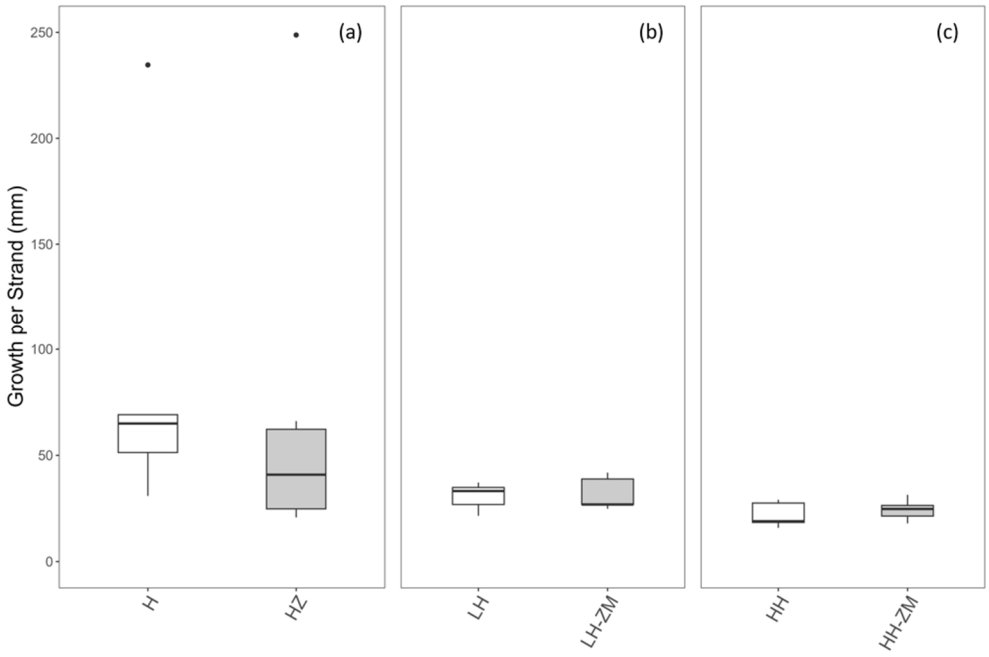

Figure 8. Boxplots summarize the results of two experiments examining the impact of zebra mussel treatments on Hydrilla growth. In the field experiment (a), Hydrilla was tested with (HZ) and without zebra mussels (H), and in the lab experiment low densities of Hydrilla (b) were tested with zebra mussels (LH-ZM) against controls (LH), and high densities of Hydrilla (c) were also tested with zebra mussels (HH-ZM), against controls (HH). Pairwise T-test (Field Experiment) and Mann-Whitney U-tests (Lab experiment) were all insignificant, $p>0.05$.

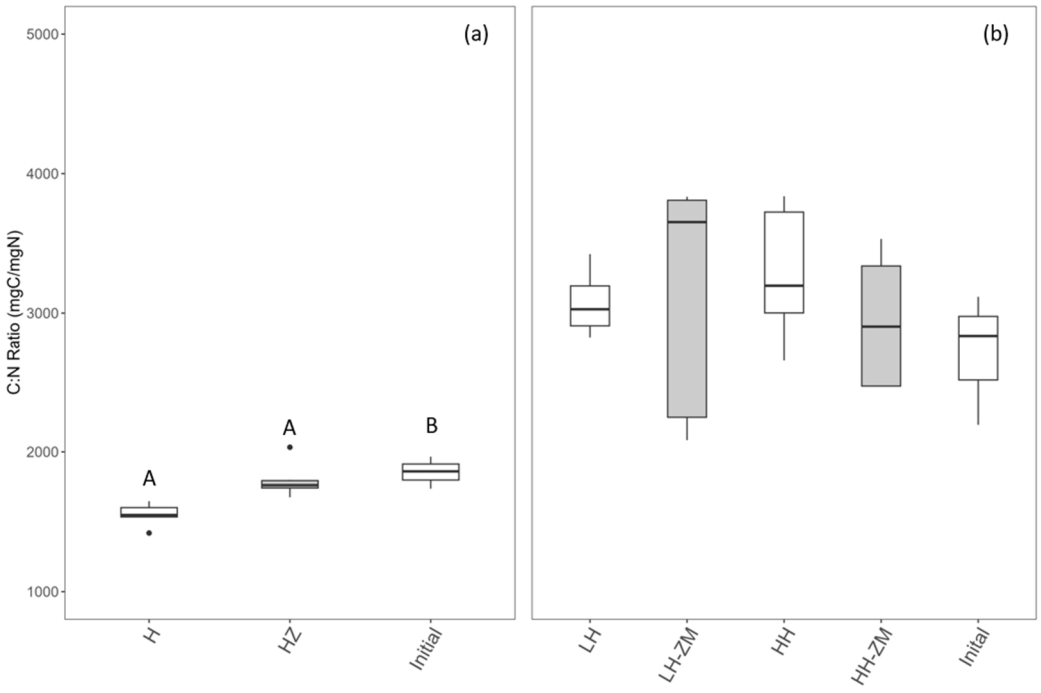

Figure 9. Boxplots summarize C/N concentrations for field experiment (a) controls (H) and treatments with zebra mussels (HZ). Lab experiment C/N concentrations of tissues are shown in panel (b) where low density of Hydrilla (LH-ZM) treated with zebra mussels is compared with controls (LH) and the same for high density Hydrilla (HH-ZM & HH). Initial C/N concentrations were included to illustrate differences between both treatments over sampling period. ANOVA was insignificant in for field experiment (p-value>0.05). Letters above the diagram denote significant differences (Tukey HSD pairwise comparison, p-values<0.05).

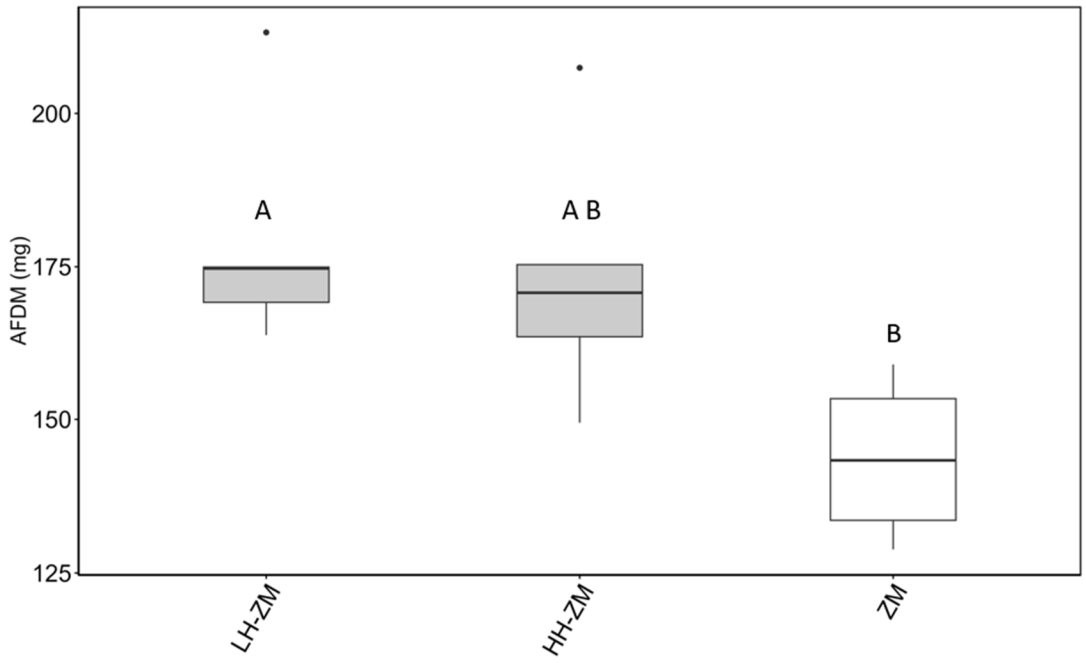

Figure 10. Boxplots summarize zebra mussel biomass (Ash Free Dry Mass, AFDM) for Lab experiment. Treatments include High and Low density Hydrilla (HH-ZM, LH-ZM and controls (ZM). The lower (25th) and upper (75th) lines in the diagram represent respective percentiles and the thick bold line indicates median values. Letters above the diagram denote significant differences (Tukey HSD pairwise comparison, p-values<0.05).

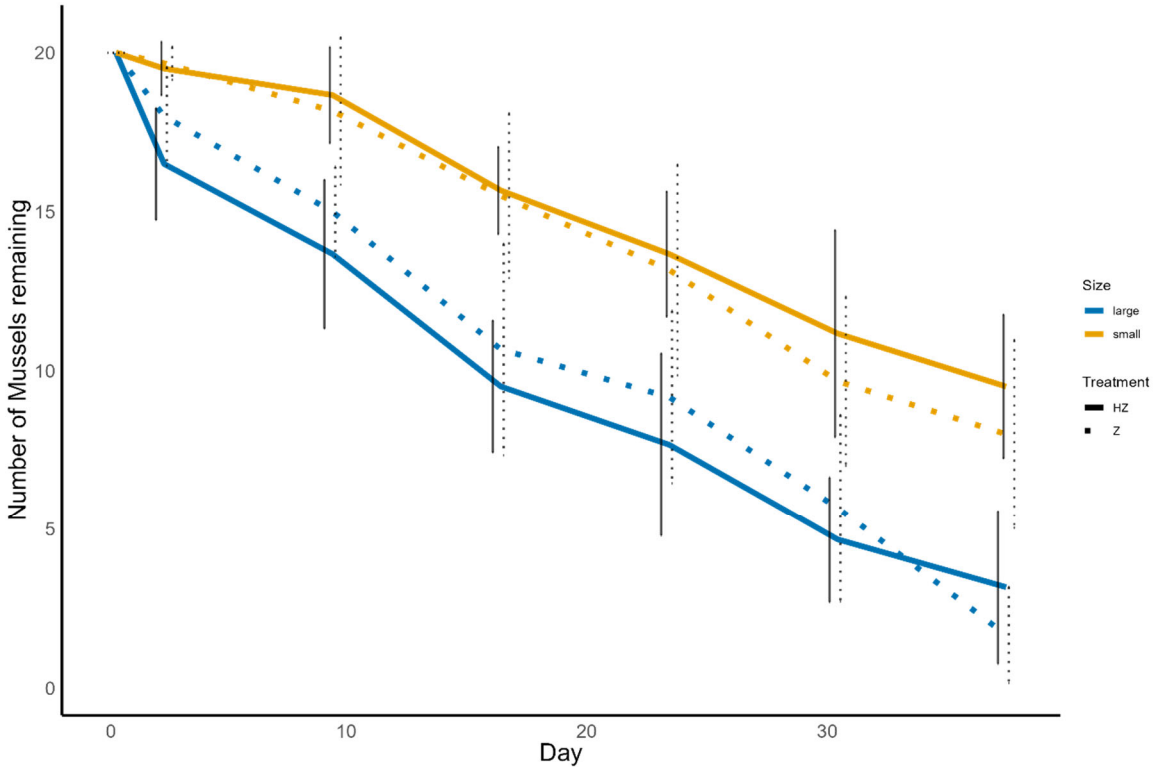

Figure 11. Plot depicts average mussel mortality over lab experiments for large mussels (blue) and small mussels (gold). Treatments and controls are indicated by solid or dotted lines respectively and error bars signify standard deviation.

APPENDIX SECTION

Table A1. Wentworth scale used to characterize substrate composition, modified for visual estimation. (Wentworth, 1922; Hedrick et al. 2013; Londo et al., 2022)

Substrate Type	Particle Diameter (mm)
Boulder	>256
Cobble	>64 to 256
Pebble	>4 to 64
Gravel	>2 to 4
Very Coarse Sand	>1 to 2
Coarse Sand	>0.50 to 1
Medium Sand	>0.125 to 0.25
Fine Sand	>0.0625 to 0.125
Silt	>0.039 to 0.0625
Clay	<0.039

Table A2. Table shows mussel densities and statistical results of pairwise T-tests comparing mean densities for sites with rocky sediments only in June 2022.

Site	Mussels on Hydrilla Mean (ind./m^2) ± SD	Mussels on Rocks Mean (ind./m^2) ± SD	T-Statistic	p-value
Crane B	4355 ± 4918	1843 ± 1969	3.78	1.943e-2
Potters C	7854 ± 4572	3584 ± 6015	11.38	3.396e-4
Marina E	12366 ± 10793	1664 ± 813	6.77	2.483e-3
BR 1 H	26126 ± 14823	1882 ± 505	11.19	3.628e-4

REFERENCES

Armenio, P. M., Mayer, C. M., Heckathorn, S. A., Bridgeman, T. B., & Panek, S. E. (2016). Resource contributions from dreissenid mussels to the benthic algae Lyngbya wollei (Cyanobacteria) and Cladophora glomerata (Chlorophyta). *Hydrobiologi*a, *763*(1), 35-51.

Benson, A. J. 2013. Chronological history of zebra and quagga mussels (Dreissenidae) in North America, 1988–2010, *in* Nalepa, T.F., and Schloesser, D.W., eds., Quagga and zebra mussels—Biology, impacts, and controls: Boca Raton, Fla., CRC Press.

Bianchini, I., Cunha-Santino, M. B. D., Milan, J. A. M., Rodrigues, C. J., & Dias, J. H. P. (2010). Growth of Hydrilla verticillata (Lf) Royle under controlled conditions. *Hydrobiologia*, *644*(1), 301-312.

Bodamer BL, Ostrofsky ML (2010) The use of aquatic plants of the zebra mussel (*Dreissena polymorpha*) (Bivalvia: Dreissenidae) in a small glacial lake. Nautilus 124:100–106

Borcherding, J. (1991). The annual reproductive cycle of the freshwater mussel Dreissena polymorpha Pallas in lakes. *Oecologia*, 87:208-218.

Carlton, J. T. (2008). The zebra mussel Dreissena polymorpha found in North America in 1986 and 1987. *Journal of Great Lakes Research*, *34*(4), 770-773.

Cole, E., Keller, R.P., Garbach, K. (2019). Risk of invasive species spread by recreational boaters remains high despite widespread adoption of conservation behaviors. *Journal of Environmental Management*, 229:112–119.

Crane, K., Coughlan, N. E., Cuthbert, R. N., Dick, J. T., Kregting, L., Ricciardi, A., & Reid, N. (2020). Friends of mine: an invasive freshwater mussel facilitates growth of invasive macrophytes and mediates their competitive interactions. *Freshwater Biology*, *65*(6), 1063-1072.

De Ventura, L., N. Weissert, R. Tobias, K. Kopp, and J. Jokela. (2016). Overland transport of recreational boats as a spreading vector of zebra mussel *Dreissena polymorpha*. Biological Invasions 18:1451-1466.

Drexler, J. Z., Khanna, S., & Lacy, J. R. (2021). Carbon storage and sediment trapping by Egeria densa Planch., a globally invasive, freshwater macrophyte. *Science of the Total Environment*, *755*, 142602.

Drake, D. A. R. (2017). Overland spread of aquatic invasive species among freshwater ecosystems due to recreational boating in Canada. DFO Canadian Science Advisory Secretariat. Research Document 2017/031.

Gantz, C. A., Miller, R., Wells, S., Sytsma, M., & Strecker, A. L. (2022). Mussel squeeze: dissolved oxygen and temperature can "squeeze" zebra mussels out of invaded reservoirs. *Management of Biological Invasions*, *14*.

Hairston, N. G. & C. E. Cáceres, (1996). Distribution of crustacean diapause: micro and macroevolutionary pattern and process. *Hydrobiologia*, 320: 27–44.

Havel, J.E., Kovalenko, K.E., Thomaz, S.M. *et al.* (2015) Aquatic invasive species: challenges for the future. *Hydrobiologia,* 750, 147–170.

Hedrick, L. B., Anderson, J. T., Welsh, S. A., & Lin, L. S. (2013). Sedimentation in mountain streams: a review of methods of measurement. *Natural Resources*, 2013, 4, 92-104.

Heiman, K. W., & Micheli, F. (2010). Non-native ecosystem engineer alters estuarine communities. *Integrative and Comparative Biology*, *50*(2), 226-236.

Higgins, S. N., & Zanden, M. V. (2010). What a difference a species makes: a meta–analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecological monographs*, *80*(2), 179-196.

Horvath, T. G., & Lamberti, G. A. (1997). Drifting macrophytes as a mechanism for zebra mussel (Dreissena polymorpha) invasion of lake-outlet streams. *American Midland Naturalist*, 29-36.

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kuhn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., PyŠek, P., Roques, A., Sol, D., Solarz, W. & Vila, M. (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, 45, 403– 414.

Istvánovics, V., Clement, A., Somlyódy, L., Specziár, A., G.-Tóth, L., & Padisák, J. (2007). Updating water quality targets for shallow Lake Balaton (Hungary), recovering from eutrophication. *Hydrobiologia*, *581*, 305-318.

Jain, M. S., & Kalamdhad, A. S. (2018). A review on management of Hydrilla verticillata and its utilization as potential nitrogen-rich biomass for compost or biogas production. *Bioresource Technology Reports*, *1*, 69-78.

Jeppesen, E., Trolle, D., Davidson, T. A., Bjerring, R., Søndergaard, M., Johansson, L. S., … & Meerhoff, M. (2016). Major changes in CO2 efflux when shallow lakes shift from a turbid to a clear water state. *Hydrobiologia*, *778*(1), 33-44.

Johnson, L. E., A. Ricciardi, and J. T. Carlton. 2001. Overland dispersal of aquatic invasive species: A risk assessment of transient recreational boating. Ecological Applications 11:1789-1799.

Jones, R. C. (1990). The effect of submersed aquatic vegetation on phytoplankton and water quality in the tidal freshwater Potomac River. *Journal of Freshwater Ecology*, *5*(3), 279-288.

Kalff, J. (2001). *Limnology: Inland water ecosystems*. Upper Saddle River, NJ: Prentice Hall.

McMahon, R.F. (1996). The physiological ecology of the zebra mussel, Dreissena polymorpha, in North America and Europe. American Zoologist 36:339-363.

Muskó, I. B., & Bakó, B. (2005). The density and biomass of Dreissena polymorpha living on submerged macrophytes in Lake Balaton (Hungary). *Archiv für Hydrobiologie*, 229-251.

Nichols, S.A. (1992). Depth, substrate, and turbidity relationships of some Wisconsin lake plants. Transactions of the Wisconsin Academy of Sciences, Arts and Letters 80:97-118.

Langeland, K. A. (1996). Hydrilla verticillata (LF) Royle ("Hydrocharitacea"),"The perfect aquatic weed". *Castanea*, 293-304.

Li, F., Zhu, L., Xie, Y., Liang, S., Hu, C., Chen, X., & Deng, Z. (2016). Fragment growth performance of the invasive submerged macrophyte Myriophyllum spicatum under conditions of different water depths and sediment types. *Aquatic Ecology*, *50*, 727-734.

Londo, A. R., Fisher, S. J., Krenz, J. D., & Collison, R. M. (2022). Assessment of organic substrates as sites for zebra mussel (Dreissena polymorpha) attachment in four West-Central Minnesota Lakes. *Journal of Freshwater Ecology*, *37*(1), 71-83.

Madsen, J. D., & Owens, C. S. (1998). Seasonal biomass and carbohydrate allocation in dioecious hydrilla. *Journal of Aquatic Plant Management*, *36*, 138-144.

Martel A (1993) Dispersal and recruitment of zebra mussel (Dreissena polymorpha) in a nearshore area in west-central Lake Erie: the signifcance of postmetamorphic drifting. Can J Fish Aquat Sci 50:3–12

Meza-Lopez, M. M., & Siemann, E. (2015). Experimental test of the Invasional Meltdown Hypothesis: an exotic herbivore facilitates an exotic plant, but the plant does not reciprocally facilitate the herbivore. *Freshwater Biology*, *60*(7), 1475-1482.

Michelan, T. S., Silveira, M. J., Petsch, D. K., Pinha, G. D., & Thomaz, S. M. (2014). The invasive aquatic macrophyte Hydrilla verticillata facilitates the establishment of the invasive mussel Limnoper45ortunenei in Neotropical reservoirs. *Journal of Limnology*, *73*(3).

Mielecki, M., & Pieczyńska, E. (2005). The influence of fragmentation on the growth of Elodea canadensis Michx. in different light conditions.Polish Journal of Ecology, 53, 155–164.

Miller, M. M., Sherman, T. D., & Major, K. M. (2022). Competitive success of the invasive species, Hydrilla verticillata, over Vallisneria neotropicalis in nature could be linked to differences in nitrogen metabolism. *AQUATIC BOTANY*, *182*.

Miranda, L. E., & Hodges, K. B. (2000). Role of aquatic vegetation coverage on hypoxia and sunfish abundance in bays of a eutrophic reservoir. *Hydrobiologia*, *427*(1), 51-57.

Olson, J., Robertson, J., Swannack, T. M., McMahon, R. F., Nowlin, W. H., Schwalb, A. N. (2018). Dispersal of zebra mussels (*Dreissena polymorpha*) downstream of an invaded reservoir. *Aquatic Invasions*, 13, 199-209.

Patrick, A. E., & Florentine, S. (2021). Factors affecting the global distribution of Hydrilla verticillata (L. fil.) Royle: A review. *Weed Research*, *61*(4), 253-271.

Ricciardi, A. (2007). Are modern biological invasions an unprecedented form of global change? Conservation Biology 21: 329–336.

Ricciardi, A., & Hill, J. M. (2023). Passive transport of a zebra mussel attached to a freshwater fish: A novel Dreissena dispersal mechanism?. *Biological Invasions*, 1-4.

Rothlisberger, J. D., Chadderton, W. L., McNulty, J., & Lodge, D. M. (2010). Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. *Fisheries*, *35*(3), 121-132.

Robertson, J. J., and A. Schwalb. 2019. Dispersal, survival, and growth of zebra mussels (Dressenia polymorpha) downstream of invaded Central Texas reservoirs. Final report prepared for Texas Parks and Wildlife in October 2019.

Rooney, N., Kalff, J., & Habel, C. (2003). The role of submerged macrophyte beds in phosphorus and sediment accumulation in Lake Memphremagog, Quebec, Canada. *Limnology and Oceanography*, *48*(5), 1927-1937.

Sand-Jensen, K. A. J. (1989). Environmental variables and their effect on photosynthesis of aquatic plant communities. *Aquatic Botany*, *34*(1-3), 5-25.Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in ecology & evolution*, *8*(8), 275-279.

Schwalb, A. N., Swearingen, D., Robertson, J. J., Locklin, J. L., Moore, J. S., & McGarrity, M. (2023). Living on the edge: thermal limitations of zebra mussels (Dreissena polymorpha) in Central Texas. *Biological Invasions*, *25*(3), 847-861.

Silverman, H., Lynn, J. W., Achberger, E. C., & Dietz, T. H. (1996). Gill structure in zebra mussels: bacterial-sized particle filtration. *American Zoologist*, *36*(3), 373-384.

Simberloff, D., & Von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown?. *Biological invasions*, *1*(1), 21-32.

Sousa R., Gutierrez J. L. and D. C. Aldridge. (2009). Non-indigenous invasive bivalves as ecosystem engineers. Biological Invasions 11: 2367–2385.

Sousa, W. T. Z. (2011). Hydrilla verticillata (Hydrocharitaceae), a recent invader threatening Brazil's freshwater environments: a review of the extent of the problem. *Hydrobiologia*, *669*(1), 1-20.

Spencer, W. E., J. Teeri & R. G. Wetzel, (1994). Acclimation of photosynthetic phenotype to environmental heterogeneity. *Ecology* 75: 301–314.

Strayer, D. (2009), Twenty years of zebra mussels: Lessons from the mollusk that made headlines. *Frontiers in Ecology and the Environment* 7: 135-141.

Tang, Y., Fu, B., Zhang, X., & Liu, Z. (2019). Nutrient addition delivers growth advantage to Hydrilla verticillata over Vallisneria natans: a mesocosm study. *Knowledge & Management of Aquatic Ecosystems*, (420), 12.

Texas Commission on Environmental Quality. (2022). 2022 Texas Integrated Report for Surface Water Quality: Trophic State Index. Retrieved from https://www.tceq.texas.gov/downloads/water-quality/assessment/integrated-report-2022/2022-trophic.pdf

True-Meadows, S., Haug, E. J., & Richardson, R. J. (2016). Monoecious hydrilla—A review of the literature. *J Aquat Plant Manage*, *54*, 1-11.

Ussery, T. A. and R. F. McMahon. (1994). Comparative study of the desiccation resistance of zebra mussels *(Dreissena polymorpha)* and quagga mussels *(Dreissena bugensis). Proceedings, 4th International Zebra Mussel Conference,* Madison, WI. pp. 351-370.

Ventura LD, Sarpe D, Kopp K, Jokela J (2016) Variability in phenotypic tolerance to low oxygen in invasive populations of quagga and zebra mussels. Aquat Invasions 11(3):267–276

Vitousek, PM, Mooney HA, Lubchenco J, Melillo JM. (1997). Human domination of earth's ecosystems. *Science* 277:494–499.

Waters, M. N., Golladay, S. W., Patrick, C. H., Smoak, J. M., & Shivers, S. D. (2015). The potential effects of river regulation and watershed land use on sediment characteristics and lake primary producers in a large reservoir. *Hydrobiologia*, *749*, 15-30.

Watkins II, C. E., & Shireman, J. V. (1983). The Influence of Aquatic Vegetation Upon Zooplankton and. *J. Aquat. Plant Manage*, *21*, 78-83.

Wegner, B., Kronsbein, A. L., Gillefalk, M., Van de Weyer, K., Köhler, J., Funke, E., ... & Hilt, S. (2019). Mutual facilitation among invading Nuttall's waterweed and quagga mussels. *Frontiers in plant science*, *10*, 789.

Wentworth, K. C. (1922). A scale of grade and class terms for clastic sediments. The Journal of Geology 30(5): 377-392.

Zhang, X., Liu, X., & Ding, Q. (2013). Morphological responses to water-level fluctuations of two submerged macrophytes, Myriophyllum spicatum and Hydrilla verticillata. *Journal of Plant Ecology*, *6*(1), 64-70.

Zhu, B., Fitzgerald, D. G., Mayer, C. M., Rudstam, L. G., & Mills, E. L. (2006). Alteration of ecosystem function by zebra mussels in Oneida Lake: impacts on submerged macrophytes. *Ecosystems*, *9*(6), 1017-1028.

Zhu, M., Zhu, G., Nurminen, L., Wu, T., Deng, J., Zhang, Y., ... & Ventelä, A. M. (2015). The influence of macrophytes on sediment resuspension and the effect of associated nutrients in a shallow and large lake (Lake Taihu, China). *PLoS One*, *10*(6), e0127915.

Texas Parks and Wildlife (TPWD) Vegetation Report, Canyon Lake, TX. (2020, 2021). Personal correspondence: TPWD, San Marcos District Supervisor, Patrick Ireland.

Nonindigenous Aquatic Species. (2022). United States Geological Service. (http://nas.er.usgs.gov/)

Drought Conditions, NOAA (2022).

(https://www.drought.gov/states/texas#:~:text=The%20U.S.%20Drought%20Monitor%2 0(USDM,to%20Exceptional%20Drought%20(D4)