**Short Communication**

# Rapid response and eradication of zebra mussels (*Dreissena polymorpha*) from Lake Waco, Texas, USA, using a gas impermeable benthic barrier approach

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## Abstract

Zebra mussels (*Dreissena polymorpha*) are an invasive bivalve that has spread across much of the central and eastern U.S. since their initial introduction to North America. Eradication efforts have largely relied on chemical control methods, which can be costly and not always effective. In 2014, a highly localized introduction of zebra mussels was discovered in the 35.6 $km^2$ Lake Waco in Central Texas, USA. After weighing treatment options, a decision was made to attempt eradication using a novel gas impermeable benthic barrier approach previously only used for bivalves to successfully suppress Asian clam (*Corbicula fluminea*) populations. A polyvinyl chloride benthic barrier was installed to cover an area of approximately 3,900 $m^2$ of the shoreline and lake bottom in the littoral zone and left in place for nearly five months with frequent monitoring and periodic maintenance. Following barrier removal, environmental DNA, plankton sampling for larvae, and shoreline/substrate surveys for settled mussels were implemented to monitor eradication effort success. Although two live mussels were found immediately after barrier removal indicating mortality was not 100%, there were no further detections of zebra mussels suggesting an insufficient number remained post-treatment to develop a population. Following over five years with no detections, the zebra mussel population was declared to be eradicated from the lake in early 2021. This is the first demonstrated use of a benthic barrier approach to successfully eradicate a dreissenid mussel introduction. The use of this technique was facilitated by early detection, the localized nature of the introduction, cooperation in closing the boat ramp in the affected area, the gently sloping bathymetry of the lake's littoral zone, and partnership efforts and contributions by multiple agencies and other entities. Lake Waco remains at risk for future zebra mussel re-introduction and early detection monitoring is ongoing.

**Key words:** aquatic invasive species, aquatic ecosystems, dreissenid, early detection, reservoir

## Introduction

Zebra mussels (*Dreissena polymorpha*) are an invasive bivalve shellfish native to the Black Sea region of Eurasia (Rosenberg and Ludyanskiy 1994). This species was first introduced into North America in the Great Lakes region in 1988 (Hebert et al. 1989) and is believed to have been introduced via ballast water of oceangoing ships. Zebra mussels have since spread to water bodies throughout much of the central and eastern United States. Zebra mussels were first documented in Texas in 2009 in Lake Texoma (TPWD 2009) and, as of April 2024, have spread to 36 water bodies across seven river basins within the state. Spread within the state has occurred via a combination of overland dispersal via watercraft and subsequent downstream dispersal within the state's coupled river-lake systems. Zebra mussels primarily inhabit the littoral zone, above the thermocline where oxygen availability and hard substrate are adequate for survival.

Zebra mussel infestations can cause significant economic damages, primarily related to impacts on water infrastructure, such as municipal water delivery and hydroelectric facilities, with mitigation cost at some facilities estimated in the hundreds of millions of dollars (MacIsaac 1996; Park and Hushak 1999; Connelly et al. 2007; IEAB 2010, 2013; Prescott et al. 2013; Robinson et al. 2013). Furthermore, this species is known to have significant negative impacts on ecological function and native aquatic species including native mussels, many of which are imperiled in Texas (Higgins and Vander Zanden 2010; Lucy et al. 2014). Recreational or quality of life impacts may also occur, as zebra mussels damage boats (i.e., hulls, motors, water intakes/pumps) and litter shorelines with dangerously sharp shells.

Control options for zebra mussel infestations in open water are limited primarily to molluscicide treatments (e.g., EarthTec QZ, copper sulfate pentahydrate, Earth Science Laboratories LLC; Zequanox, killed *Pseudomonas fluorescens*, ProFarm Group, Inc.; potassium chloride). In one instance, however, SCUBA removal alone has seemingly proven effective at eradication – but required eight years of sustained, intensive effort (Wimbush et al. 2009). However, chemical treatments can be costly. Eradication of zebra mussels from the approximately 0.05 $km^2$ Millbrook Quarry (Virginia, USA) using potassium chloride cost $365,069, in addition to requiring an EPA Section 18 Pesticide Emergency Exemption and other state approvals (Fernald and Watson 2013). A low-dose copper-based (cupric ion form) molluscicide, EarthTec QZ, has shown promise for eradicating dreissenid mussels (e.g., quagga mussel, *Dreissena bugensis*) at substantially lower cost, but effective treatment of the approximately 0.12 $km^2$ Billmeyer Quarry (Pennsylvania, USA) cost $109,400 (Hammond and Ferris 2019). Furthermore, efforts to eradicate localized infestations of zebra mussels detected early using localized, reduced-cost molluscicide treatments have proven ineffective in Christmas Lake (Minnesota, USA; Zequanox, EarthTec QZ, and potassium chloride) and Richland-Chambers Reservoir (Texas, USA; EarthTec QZ), with subsequent spread of zebra mussels to other areas of these lakes (Lund et al. 2018, TPWD 2020).

Covering small, experimentally established colonies of zebra mussels with benthic mats (polyvinyl chloride) for 8–9 weeks resulted in mortality rates exceeding 98.8% (Braithwaite 2003), but to our knowledge this method has not been attempted for control of dreissenids at large scales. Additionally, gas impermeable benthic barriers (ethylene propylene diene monomer sheets) have been used for experimental control of another invasive bivalve, Asian clam (*Corbicula fluminea*), in 9 $m^2$–1,950 $m^2$ plots in Lake Tahoe (CA-NV, USA), achieving 98% reduction in abundance of Asian clams immediately following treatment with a > 90% reduction continuing to be seen a year post-treatment (Wittmann et al. 2012). This method has been commonly used with mixed success for control of aquatic macrophytes for decades (Nichols 1974; Engel and Nichols 1984; Boylen et al. 1996), yet to our knowledge its use for control of invasive bivalves has been limited to the one instance in Lake Tahoe (Wittmann et al. 2012).

The gas impermeable benthic barrier control method seeks to achieve control or eradication by reducing dissolved oxygen concentrations beneath the barrier, inducing anoxic conditions that result in bivalve mortality. In small-scale *in situ* experiments, a steep decline in dissolved oxygen (DO) under 16$m^2$ benthic barriers was observed (Braithwaite 2003); in their study, DO levels dropped rapidly to an average of 0.52 ± 0.23 mg $l^{-1}$, persisting at this level for the course of the 8-week experiment. By comparison with regards to zebra mussel mortality, Yu and Culver (1999) found the threshold oxygen level for zebra mussel survival to be between 1.0–1.7 mg $l^{-1}$ at water temperatures 17–18 °C (i.e., similar to Lake Waco temperatures at the time of benthic barrier placement). In the case of dreissenid mussels, which unlike native unionids produce free-floating veliger larvae, the barrier may also serve to hinder successful spawning and prevent veligers from dispersing should mussels under the barrier spawn successfully prior to mortality being achieved.

Lake Waco in Central Texas, USA is an impoundment supplied by the four branches of the Bosque River and Hog Creek, with an outlet to the Bosque River which flows for approximately 6.5 km to its confluence with the Brazos River. Lake Waco provides drinking water to approximately 200,000 people (Conry 2010) and is popular for fishing and other water recreation. This is a eutrophic, polymictic lake that covers approximately 35.3 $km^2$ and has a maximum depth of 26 m and an average depth of 6.8 m (Conry 2010). Lake Waco has been determined to be high risk for zebra mussel invasion, as physicochemical conditions in the lake are highly conducive to zebra mussel establishment, with more than adequate calcium (45.2 ± 11.8 mg/l), moderate pH (8.0 ± 0.3), low salinity (0.16 ± 0.02 PSU), and average summer surface water temperatures below 32 °C (McGarrity and McMahon 2024; Texas Commission on Environmental Quality *unpublished data*). Should zebra mussels become successfully established, they could have both economic impacts on water control and supply infrastructure and ecological impacts. One imperiled native unionid mussel which could be negatively impacted by zebra mussels—the Brazos Heelsplitter (*Potamilus* *streckersoni*; state threatened)—has been documented in Lake Waco along with nine additional non-imperiled unionids (Randklev et al. 2023). Downstream in the Brazos River, which could be invaded by zebra mussels via downstream dispersal if Lake Waco became infested, Brazos Heelsplitter, Texas Fawnsfoot (*Truncilla macrodon*; state threatened, federal candidate), and at least 20 non-imperiled unionid species have been documented (de Moulpied et al. 2022).

Since zebra mussels were first detected in Texas in 2009 (TPWD 2009), the City of Waco was extremely proactive about zebra mussel prevention with outreach signage including installation of highly visible painted outreach stencils with prevention messaging on boat ramp surfaces, volunteers at boat ramps to inspect boats and educate boaters during summer months, and monthly shoreline surveys by volunteers for zebra mussels. It was during the course of these surveys that small numbers of zebra mussels were detected in a localized area in Lake Waco at Ridgewood Marina boat ramp in late September of 2014. Additional surveys and checks of boats in the marina identified the presence of a zebra mussel-infested barge in the lake, which was believed to have been present since July and to have dislodged mussels at the boat ramp introduction site during launch. The barge was removed from the lake within two days of the initial detection of zebra mussels at the boat ramp and the owner was cited for illegally introducing zebra mussels, which are a prohibited species in Texas. This early detection of a highly localized introduction in Lake Waco offered a unique opportunity for mounting a rapid response effort.

## Materials and methods

### *Detection and infestation delineation*

#### Shoreline and substrate sampling pre/post detection

Beginning in 2012, monthly shoreline and substrate surveys were conducted at Lake Waco by City of Waco staff and volunteers to search for zebra mussels. Immediately following the detection of zebra mussels at the Ridgewood Marina boat ramp on September 26, 2014, additional surveys were conducted as part of the rapid response effort. Texas Parks and Wildlife Department (TPWD) staff conducted shoreline and substrate surveys, assisted by City of Woodway divers who brought submerged rocks to the surface for inspection for attached zebra mussels by TPWD staff.

#### Plankton sampling for veligers pre/post detection

Plankton samples for analysis for zebra mussel veliger larvae were collected at one nearby site in June 2014 prior to the detection of zebra mussels in the lake and at four sites—two near the detection site and two elsewhere in the lake—in September and October 2014 following zebra mussel detection but prior to placement of the benthic barrier. Plankton samples were collected and analyzed following protocols modified from those of the Bureau of Reclamation (2013a, b, c). A 64 µm mesh plankton net (Aquatic Research Instruments) was used to sample a minimum volume of 1,000 l of water for each sample. Duplicate samples were collected for microscopy and eDNA analysis. Plankton nets are an accepted method for collecting samples for eDNA and have been shown to considerably increase sample volume and spatial coverage (Schabacker et al. 2020; Bucklin et al. 2021). Furthermore, eDNA analysis is a proven method for early detection of dreissenids (Sepulveda et al. 2019). Samples were preserved in 50% non-denatured ethanol, buffered with 0.1g of baking soda per 100 ml of sample volume to prevent veliger degradation, and placed on ice, followed by refrigeration until samples were analyzed. Plankton nets were decontaminated between sites by soaking for one to two hours in vinegar (pH 2.5) such that any remaining veligers would not be detected with cross-polarizing light microscopy (CPLM; Bureau of Reclamation 2013a, d). Nets were then disinfected by a 10-minute soak in 10% household bleach to denature any zebra mussel DNA and then rinsed with tap water to remove any residual chlorine.

Plankton samples were transferred to Imhoff cones, triple rinsing the sample container with deionized (DI) water. Samples were allowed to settle 18–24 hours in covered Imhoff cones. Following settling, approximately 40 ml of sediment and liquid were removed from the Imhoff cone using a 10-ml glass pipette and transferred to glass petri dishes for microscopic analysis. Samples were analyzed using CPLM, and morphology of any organisms showing a Maltese cross was examined using light microscopy. Suspect dreissenid veligers were photographed, measured, and isolated from the sample for DNA verification. Photographs were reviewed by multiple experts for tentative identification prior to DNA verification. All equipment used in sample preparation and microscopy was decontaminated by a one to two-hour soak in vinegar (pH 2.5), disinfected by a 10-minute soak in a 10% household bleach solution, and rinsed in tap water between uses.

### Environmental DNA analysis

Plankton samples collected as described above at one nearby site in June 2014 prior to the detection of zebra mussels in the lake were analyzed for zebra mussel environmental DNA (eDNA). Samples collected in September and October 2014 following zebra mussel detection but prior to placement of the benthic barrier at four sites—two near the detection site and two elsewhere in the lake—were also analyzed for eDNA.

If no suspect zebra mussel veligers were detected in a sample, then approximately 1.5 g of sample contents were collected and eDNA was isolated using a Qiagen DNeasy PowerSoil Pro Kit. The DNA quality and concentration was determined for each sample using a NanoDrop One spectrophotometer. Samples were then subjected in triplicate to a polymerase chain reaction (PCR) assay using zebra mussel-specific primers and protocol described by Rochelle et al. (2010). Briefly, the amplification reaction contains Invitrogen reagents: 10X PCR reaction buffer, 1.5 $MgCl_2$, 0.2 mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 0.25 μM of each forward and reverse primers, 1 unit/rxn Platinum Taq DNA Polymerase, and 2 μL of sample template DNA, for a total volume of 25 μL per sample reaction. Thermal cycling conditions were 95 °C for 5 min to activate the "hot-start" Taq polymerase, followed by 40 cycles of 95 °C for 45 seconds, 59 °C for 45 seconds, and 72 °C for 45 seconds. This was followed by a final incubation at 72 °C for 5 minutes to ensure all amplification products are extended to full length and then cooling to 4 °C until ready for visualization of amplicon band(s). Assay samples were then subjected to separation using gel electrophoresis (Owl electrophoresis rig) using a 0.8% agarose gel with ethidium bromide stain for nucleic acids. UV transillumination (LabNet Enduro Gel Documentation System; GDS) was used for visualization of the gel image for amplicon(s) with size of 386 base pairs as compared with positive controls to confirm the presence of zebra mussel eDNA.

### *Rapid response eradication effort conceptualization*

On October 3rd, 2014, one week after the initial detection of zebra mussels in Lake Waco, representatives from TPWD, City of Waco, and the U.S. Army Corps of Engineers (USACE) met in Waco to discuss potential for a rapid response effort based on review of literature and technical reports on potential control methods and consideration of available funding, staff capacity, and equipment. Chemical and physical methods were considered as potential options for mounting a rapid response. Lake draining was determined to be not feasible, due to logistics and the importance of providing a reliable water supply. Chemical methods were determined to be too costly and less certain to be effective with a localized treatment. Placement of benthic barriers was discussed and identified as the most feasible and cost-effective method for rapid response to attempt eradication of this highly localized introduction. Taking action was determined to be a significantly lower cost alternative to the potential impacts on infrastructure. Subsequent coordination with Ridgewood Marina determined that boat ramp closure during the benthic barrier placement could be achieved to facilitate this effort and the USACE agreed to provide a Nationwide Permit 18 to authorize this activity.

### *Benthic barrier installation and monitoring*

Benthic barrier placement was conducted as quickly as possible following detection in an attempt to have barriers in place to prevent successful spawning and larval dispersal. Spawning in Texas is biannual, occurring primarily in the spring and fall months at water temperatures of 18–28 °C

(Arterburn and McMahon 2022). Nine sheets (~ 46 m × 9 m) of 30-mil polyvinyl chloride (PVC; Colorado Lining International) weighing approximately 430 kg each were purchased with expedited delivery for October 20th. Benthic barrier placement began on October 21st and was completed by October 23rd. The PVC sheets were moved into place and unrolled with the assistance of a crane truck and then manually placed along the shoreline, with the upper edge located approximately 3 m above the water level to account for the possibility of rainfall resulting in lake level rise. Two boats were used to pull the PVC sheets into the water, and American Underwater Services divers assisted with PVC and sandbag placement in deeper water. The PVC sheets were placed such that they overlapped by 1.5–2 m, to ensure water would not pass between abutted sheets, and weighted with sandbags (~ 1,000 total). A total area of approximately 3,900 $m^2$ (i.e., 0.0001% of lake area) including and surrounding the boat ramp where zebra mussels were detected was covered by the PVC sheets (Figure 1). Weekly observations of exposed PVC sheet condition were conducted by City of Waco staff. The PVC sheet placement at the exposed edges along the shoreline was affected by wave and wind action, and PVC sheets and sandbags were repositioned manually by staff several times during deployment. The Ridgewood Marina boat ramp was closed for the duration of the benthic barrier deployment. The PVC sheets were left in place for nearly five months, and were removed on March 17th–19th, 2015 with the assistance of heavy equipment and transported and disposed as landfill waste.

### *Post eradication effort monitoring*

The PVC sheets were inspected upon removal for signs indicative of anoxic conditions being achieved. Approximately 50 submerged rocks were collected by volunteer SCUBA divers from the area where benthic barriers had been placed soon after removal and were searched by staff for live attached mussels. Spring/summer and fall plankton samples (N = 60) for zebra mussel veligers and eDNA were collected and analyzed at multiple sites (Figure 2) each year from July 2015 through November 2020 (i.e., > 5 years) using the methods described above, and several shoreline and substrate surveys were also conducted. Not all sites were sampled each year.

## Results

### *Pre-post detection monitoring*

The plankton sample collected at Lake Waco in June 2014 prior to the zebra mussel detection was negative for the presence of zebra mussel veligers as well as for eDNA. Shoreline and substrate samples in the days after the detection found—and removed—approximately 75 settled zebra mussels, all of which were found in the littoral zone in the immediate vicinity of the Ridgewood Marina boat ramp. Plankton sampling conducted

**Figure 1.** Placement of PVC sheet benthic barrier for zebra mussel eradication efforts. Photograph by Texas Parks and Wildlife Department.

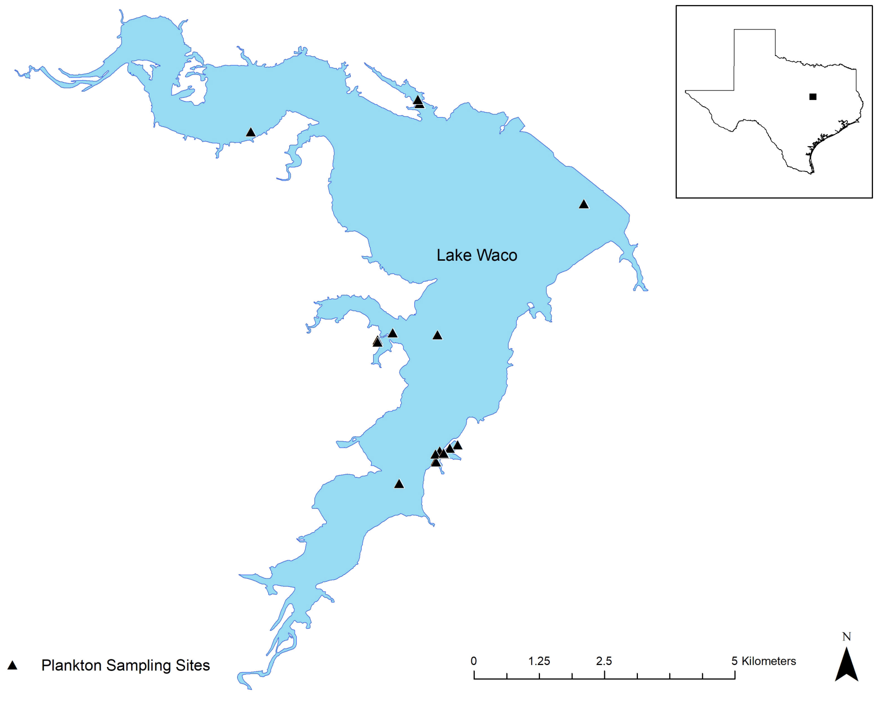


**Figure 2.** Map of Lake Waco showing 2014–2020 plankton sampling sites. Not all sites were sampled each year. Southeastern cluster of sampling sites surrounds location of zebra mussel introduction and treatment area.

at four sites in September and October 2014 prior to benthic barrier placement found low numbers (i.e., < 10) of zebra mussel veligers in September only at the Ridgewood Marina site where settled mussels were detected. Zebra mussel eDNA (1/3 replicates) was detected at the Speegleville Park/Lake Waco Marina site in October 2014; this site is located across the lake arm and slightly downstream approximately 3 km from the initial detection site.

*Post eradication effort monitoring*

Inspection of PVC sheets upon removal found black residue on the underside of the benthic barriers indicative of anoxic conditions being achieved. Inspection of submerged rocks from the treatment area found two live zebra mussels, which were removed and disposed. Plankton sampling for more than five years found no evidence of zebra mussel veligers or eDNA; suspect veligers were tentatively identified through microscopic analysis in July 2015 samples but DNA results for these organisms were negative and they were likely young ostracods. Shoreline and substrate sampling also found no settled mussels. To date, no additional zebra mussel larvae or adults have been documented in Lake Waco, and monitoring for early detection of new invasions is ongoing.

## Discussion

This zebra mussel eradication effort is, to our knowledge, the first successful use of gas impermeable benthic barriers to successfully eradicate zebra mussels or any bivalve. These efforts were further supported by removals of mussels using SCUBA divers prior to benthic barrier placement, which may have aided in enhancing the success of this effort but were not adequate alone to achieve eradication. Although two live zebra mussels were found upon barrier removal indicating mortality was not 100%, there were no future detections indicating remaining zebra mussels, if any, were not sufficient to develop a population. In January 2021, TPWD declared the zebra mussels in Lake Waco to be eradicated (TPWD 2021), following established western criteria requiring at least five years of monitoring without detections post eradication effort to determine successful eradication (WRP 2019).

Although one goal was to install benthic barriers prior to water temperatures declining to within the spawning range for zebra mussels, recorded surface water temperatures on October 20$^{th}$–21$^{st}$ just prior to benthic barrier placement averaged 23.4 °C, below the 28 °C upper threshold at which spawning may begin in Texas (Arterburn and McMahon 2022). However, peak spawning may begin at lower temperatures, and the success of this eradication effort and lack of detection of zebra mussels elsewhere in the lake over the course of more than five years of monitoring suggests that substantial spawning did not occur prior to benthic barrier placement.

Despite evidence of anoxic conditions (i.e., black residue on underside of PVC sheets), two live zebra mussels were found during extensive searches immediately following benthic barrier removal indicating mortality was not 100%. However, it is important to note that oxygen levels under the benthic barrier were not monitored due to lack of appropriate equipment to do so without disturbing the barrier as would be needed, and some oxygenated water could have intruded at areas of overlap between PVC sheets. While the treatment did not achieve 100 percent mortality, the lack of subsequent detections over more than five years indicates that if there were any additional live mussels, they were too few and too dispersed to reproduce successfully. Other benthic invertebrates, including unionid mussels, were not surveyed prior to or monitored following the treatment due to lack of capacity to do so and urgency of installing the benthic barrier prior to zebra mussel spawning to prevent dispersal and these species may have also experienced mortality due to the treatment. Similarly, aquatic plants, which consist primarily of smartweed, may have also experienced mortality or suppression. Additionally, harmful chemicals such as hydrogen sulfide and ammonia produced under anoxic conditions under the barrier may have been released. However, the treatment area was small (3,900 $m^2$; 0.0001% of lake area) and any such impacts were thus presumably negligible at a lake scale. Equipment used for installation was used only on the shoreline on the boat ramp and adjacent mowed or paved areas and did not cause significant disturbance to riparian habitats.

The total direct cost incurred for this effort was approximately $30,000 USD for PVC sheets. However, there were also substantial unquantified costs of in-kind contributions (e.g., equipment and supplies provided by the City of Waco, volunteer diver services, labor costs for all involved agencies) associated with this method that must be considered prior to undertaking the use of the benthic barrier method for dreissenid mussel eradication. These include costs for sandbags for weighting the barrier, rental of crane truck and/or telescoping forklift for barrier placement and removal, trucks or dump truck rental for PVC transport upon removal, boat usage, landfill disposal of PVC, diving services for surveys and sandbag placement, and labor costs. Costs for equipment, supplies, and services are likely to vary considerably over time and geography as well as with the remoteness of the eradication site and warrant consideration.

It is also noteworthy that the littoral bathymetry of Lake Waco played an important role in the success of this effort. Had the littoral zone been steep (e.g., limestone canyon), this method would not have been feasible. Although zebra mussels were successfully eradicated from the lake, it still remains susceptible to invasion and critical early detection monitoring and outreach and prevention efforts are ongoing.

## Authors' contribution

All authors contributed to eradication effort conceptualization and planning. TMC, JET, MSB, BEVZ, HRM, and BWM contributed to eradication effort implementation. JET, MSB, GMS, and MEM contributed to sample collection and analysis. MEM and GMS prepared the draft manuscript with contributions from TMC. All authors except TMC contributed to manuscript revision and editing.

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City of Woodway divers assisted with early detection substrate surveys soon after the initial detection. Benthic barrier installation was facilitated in part by American Underwater Services. Texas State University and the University of Texas at Arlington contributed to post-eradication sampling. Anonymous reviewers provided comments to improve this manuscript.

## Dedication

This manuscript is dedicated to Thomas M. Conry. Tom Conry was a leader in conservation at Lake Waco and the Waco wetlands including research, education, and protection against aquatic invasive species. Tom was also the driving force behind the Lake Waco zebra mussel eradication effort. Tom will be sorely missed by all who knew him.

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