Assessing the risk of dreissenid mussel invasion in Texas based on lake physical characteristics and potential for downstream dispersal

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Abstract

Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*) were likely introduced from Ponto-Caspian Eurasia to the Laurentian Great Lakes inadvertently via ballast water release in the 1980s and have since spread across the US, including Texas. Their spread into the state, including reservoirs in both Brazos River and Colorado River basins, has resulted in a need to delimit suitable dreissenid habitat and dispersal potential in Texas. The objective of our research was to assess invasion risk in Texas by 1) predicting distribution of suitable habitat of zebra and quagga mussels using Maxent models; 2) refining lake-specific predictions for present zebra mussels via collection of physicochemical data; and 3) assessing the potential for downstream spread of zebra mussels by applying environmental DNA (eDNA) methods in the Leon and Lampasas Rivers downstream from the invaded Lakes Belton and Stillhouse Hollow, respectively.

Maxent models did not predict the occurrence of suitable habitat for quagga mussels within Texas. However, our models accurately identified global zebra mussel habitat (AUC = 0.919), and Bioclim layers representing temperature and precipitation data both strongly influenced predictions. Predicted "hotspots" of suitable zebra mussel habitat in Texas occurred along the Red and Sabine Rivers of north and east Texas, as well as patches of suitable habitat in central Texas between the Colorado and Brazos Rivers and extending inland along the Gulf Coast. Most of the Texas panhandle, west Texas extending toward El Paso, and the Rio Grande valley were predicted to provide poor habitat suitability.

Collection of physicochemical data (dissolved oxygen, pH, specific conductance, and temperature on-site as well as laboratory analysis for Ca, N, and P) from zebra mussel invaded lakes and a subset of identified high-risk lakes of North and Central Texas, did not aid predictions. Visual inspection of biplots of the first three components of a principle component analysis, which together accounted for ~80% of data variability, did not reveal separation between invaded and uninvaded lakes, and logistic regression analysis also failed to identify predictive relationships between measured variables and invasion status.

Using eDNA analysis, we detected the presence of zebra mussel eDNA at 11 of 12 sites and up to at least 90.7 river km downstream from a pair of infested reservoirs. Rate of positive detection among water samples at each site ranged from 1/5 to 5/5, and within positive water samples, rate of detection among technical replicates ranged from 1/8 to 8/8, suggesting considerable heterogeneity in the zebra mussel eDNA signal in both rivers. Furthermore, no clear spatial pattern in detection rate occurred.

Thus, a monitoring strategy that combines traditional sampling (e.g. settlement substrate samplers and microscopy) at sites immediately below a dam, and transitioning to more sensitive eDNA analysis at distances further from the dam may represent the most successful strategy for detection of dreissenid mussel downstream dispersal. Overall, we have demonstrated that while quagga mussels do not appear to represent an invasive threat in Texas, suitable habitat for continuing zebra mussel invasion exists within Texas, and stream and river connections may contribute to their spread. The threat of continued expansion of this poster-child for negative invasive species impacts warrants further prevention efforts, management, and research.

Background

Zebra mussels (*Dreissena polymorpha*) were likely introduced from Ponto-Caspian Eurasia to the Laurentian Great Lakes inadvertently via ballast water release around 1986 (Hebert et al. 1989), and closely related quagga mussels (*Dreissena bugensis*) followed within the next several years (Mills et al. 1996). Both dreissenid mussel species spread rapidly throughout the Great Lakes basin due to high fecundity (Keller et al. 2007) as well as considerable natural and anthropogenic dispersal potential of their free-floating larval life stages (Bossenbroek et al. 2001, Sieracki et al. 2014). The Great Lakes have served as a beachhead for dreissenid mussel invasion throughout much of North America (Drake and Bossenbroek 2004), including the Hudson River and Mississippi River basins (Strayer et al. 1996, Cope et al. 1997). Today, both species have extended their range throughout the United States (Fig. 1). The initial incursion of zebra mussels into Texas occurred in Lake Texoma around 2009 (TPWD 2017). Given recent spread of zebra mussels further into the state in reservoirs such as Belton Lake and Lake Waco on the Brazos River, delimiting suitable dreissenid habitat and dispersal potential within Texas represents a critical management need.

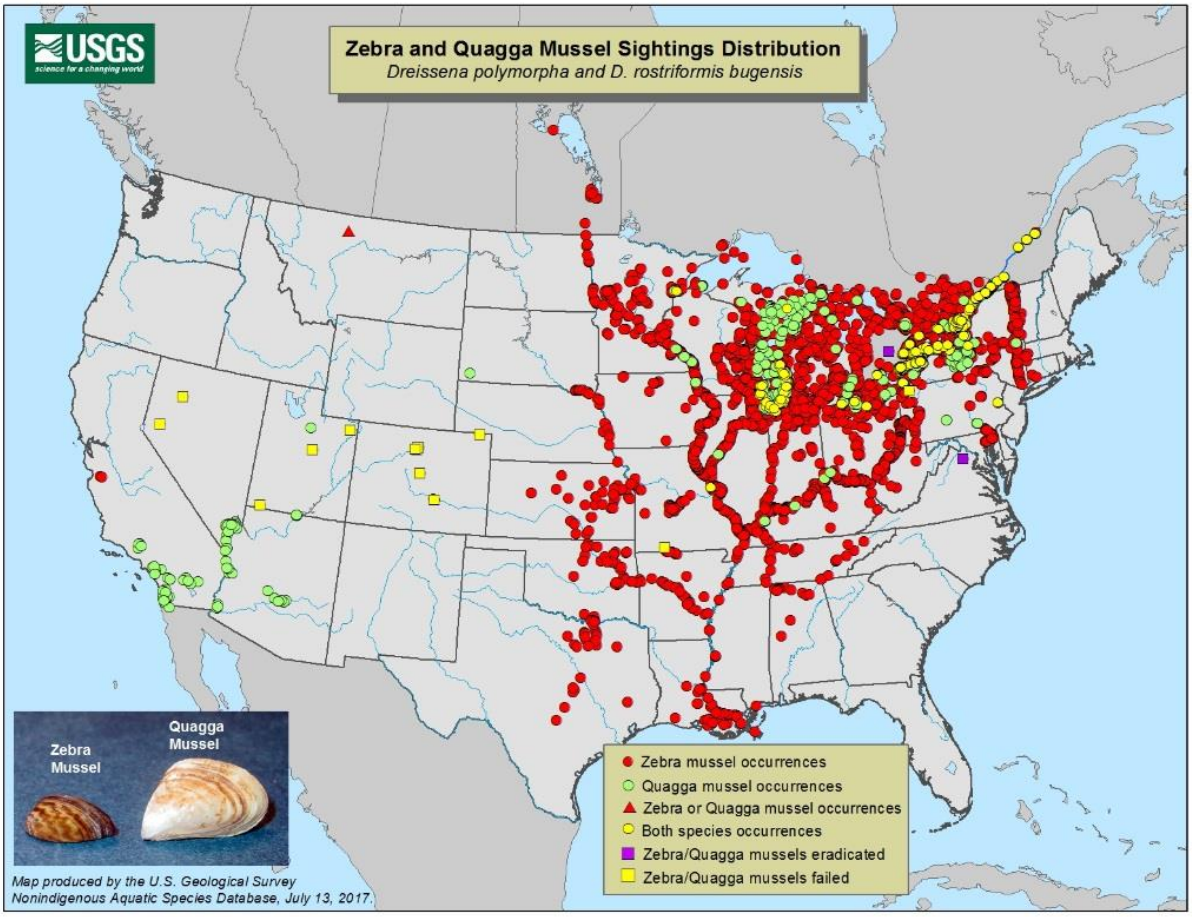

Figure 1. Occurrences of the zebra mussel *Dreissena polymorpha* and quagga mussel *Dreissena bugensis* in the United States.

Management concern about dreissenid mussels stems from their ability to act as ecosystem engineers, manipulating physical habitat by forming dense colonies attached to hard substrates (including native mussels) as well as transforming turbid, eutrophic systems into clear waters through voracious filter feeding behavior, which can contribute to increases in aquatic vegetation and profound shifts in native communities (reviewed in Nakano and Strayer 2014). Additional consequences of zebra mussel invasion include expense of physical removal of colonies from recreational equipment, power plants, municipal water facilities, dams, and other human infrastructure, as well as the application of pesticides to prevent or slow their reintroduction and recolonization of such structures (Aldridge et al. 2006). Overall, the economic burden of zebra mussel invasion approaches hundreds of millions of dollars annually (Strayer et al. 1996, Caraco et al. 1997). Surveillance and rapid response efforts such as the 100th Meridian Initiative (http://www.100thmeridian.org/) have emphasized the ongoing need for large scale management of the dreissenid invasion.

High dissolved calcium requirements for dreissenid shell development presents a potentially important constraining factor for dreissenid mussel colonization (Koutnik and Padilla 1994). Indeed, a risk assessment conducted by Whittier et al. (2008) defined risk based solely on calcium concentrations, distinguishing risk categories of "very low" (< 12 mg/L), "low" (12–20 mg/L), "moderate" (20–28 mg/L), and "high" (> 28 mg/L). Within Texas, the Brazos River basin and its neighboring basins to the southwest (Colorado River and Rio Grande) have naturally high levels of dissolved calcium, especially over the Permian plateau (VanLandeghem et al. 2012, Israël et al. 2014, Sharma et al. 2014). More recent assessment of quagga mussel survival, growth, and reproductive potential in waters of the western United States by Davis et al. (2015) noted that a single indicator such as calcium may oversimplify risk considerations and advocated considering additional factors and scales (e.g. whole lake vs. microhabitat). Therefore, we conducted research to refine understanding of potential dreissenid mussel distribution in Texas at multiple scales through both a state-wide distribution modeling approach using globally available climatic predictors as well as physicochemical data collection at single-lake scales.

Furthermore, we applied state of the art environmental DNA (eDNA) detection methods to study the potential for downstream dispersal from infested lakes. Increasing sophistication of genetic methodologies and decreasing costs have contributed to the recent emergence of techniques which apply genetic tools to identify the source of biological material left in the environment, such as sloughed cells, mucous, and feces, to provide clues regarding species presence (Beja-Pereira et al. 2009). One particularly promising technique is the use of environmental DNA (eDNA), which refers to genetic material collected not through targeted methods such as collecting fresh scats, but extracted from bulk environmental samples such as soil or water (Barnes and Turner 2016). eDNA has previously been applied to the detection of zebra mussels in inland waters (Egan et al. 2013). Bobeldyk et al. (2005) cautioned that streams could enable rapid secondary spread of zebra mussels, so we applied eDNA methods to quantify downstream dispersal distance of zebra mussels from an invaded lake.

Overall, the goal of our research was to assess risk of dreissenid mussel invasion in Texas by increasing our understanding of the availability of suitable habitat as well as dispersal capabilities. Our efforts aimed at predicting suitable habitat were able to focus on both zebra and quagga mussels based on the availability of global occurrence data for each species. Our efforts to refine models at lake-specific scales and study dispersal in Texas were limited to zebra mussels only, since they are the only species which occurs in Texas. Specific objectives of our research included: 1) predicting the general distribution of suitable habitat in Texas using Maxent models; 2) refining lake-specific zebra mussel predictions via collection of physicochemical data from identified high-risk lakes; and 3) assessing the potential for downstream spread of zebra mussels with environmental DNA.

Methods

Objective 1: Predict the general distribution of suitable dreissenid mussel habitat in Texas using Maxent models

Species distribution models for zebra and quagga mussels were created with Maxent, a machine learning tool that compares the probability distributions of species presence and local environmental data to create a model that can be projected in geographic space (Phillips et al. 2006). In other words, Maxent combines species occurrence data and environmental covariates to produce a heat map that can be interpreted as a visual representation of habitat suitability. Among its strengths, Maxent is particularly amenable to datasets consisting exclusively of presence-only data (Elith et al. 2011), and it has been praised for its strong performance compared to other species distribution modeling methods (Elith et al. 2006).

Separate models were developed for zebra and quagga mussels. To collect data for Maxent modeling, we accessed global zebra and quagga mussel occurrence data through the Global Biodiversity Information Facility online database (http://www.gbif.org), and we supplemented this information with known zebra mussel occurrences in Texas (N = 11 from Fig. 2; https://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml). No records for quagga mussel occur within Texas. We used global occurrence data (rather than limiting our data to Texas only) to ensure that the most complete representation of the zebra and quagga mussel niches were represented in model development. Overall, we compiled 13297 total global occurrences of zebra mussels (Appendix 1; Fig. 1A) and 1069 global quagga mussel occurrences (Appendix 1; Fig. 2A). To reduce bias that may be generated by uneven sampling effort, we rarified occurrence data before moving on to model implementation by converting the occurrence points into a raster file with the same cell size as our environmental data (10 arcminute, approximately 340 km^2), then back to a points file, resulting in a maximum of one point per cell (McDowell et al. 2014). Data conversion and all further mentioned visualizations were performed in ArcGIS 10.2.2 (Environmental Systems Research Institute, Redlands, California, USA). As a result of rarefication, the working zebra mussel occurrence dataset included 2080 occurrences, and the working quagga mussel occurrence dataset included 318 occurrences.

Environmental data used in the model included the 19 Bioclim layers (http://www.worldclim.org/bioclim), biologically meaningful and globally continuous layers generated from annual trends in temperature and precipitation (Hijmans et al. 2005), with 10-acrminute (~340 km^2) resolution. Although other predictor variables (e.g. calcium availability) would have obvious appeal for modeling dreissenid mussel habitat suitability, their unavailability as continuous global data layers precluded their incorporation into our modeling effort. (We did, however, seek to refine our model predictions using *in situ* measurements of certain physicochemical data as part of Objective 2).

Because the purpose of our model development was to generate predictions rather than evaluate overall Maxent performance, we generally opted for default Maxent software settings (Phillips and Dudík 2008). However, we did increase the maximum allowable model iterations to 5000 based on pilot model runs in which models didn't appear to converge on optimal solutions within the default 500 iterations. Overall, we produced 100 replicate models for each mussel species, each trained with a randomly selected 80% of rarefied occurrence data and evaluated with the remaining 20%. The results reported represent the average of the 100 models produced for each mussel species. We interpreted the Maxent logistic output as the probability of mussel habitat suitability found around the globe. In addition to visual inspection and description of the Maxent output, we assessed model performance using area under the receiver operating characteristic curve (AUC), where AUC = 0.5 indicates the model predicts outcomes no better than random, and AUC ≥ 0.7 indicates strong predictive power (Hosmer and Lemeshow 2000). Because our models were global in scale, multivariate environmental similarity surfaces (MESS; Elith et al. 2010) and Mobility-oriented parity (MOP; Owens et al. 2013) outputs generated automatically during Maxent implementation were expected to indicate no environmental extrapolation. Model uncertainty as a result of our 80% subsampling routine was assessed using visual inspection of standard deviations of 100 replicate models for each species.

Objective 2: Refine lake-specific predictions based on collection of physicochemical data from identified high-risk lakes of North and Central Texas.

Quagga mussels do not occur in Texas, but to build upon the predictions of suitable zebra mussel habitat produced through Maxent modeling associated with Objective 1, we collected physicochemical data from invaded lakes and a subset of identified high-risk lakes of North and Central Texas (N = 27). Because movement of recreational boats and other anthropogenic vectors represent a primary means of zebra mussel dispersal (Bossenbroek et al. 2001), we prioritized lakes based on an index of human use, number of public boat launches, as well as proximity to lakes already known to be invaded. We further refined our list based on consultations with Monica McGarrity at TPWD, and data availability from previous physicochemical data compilations (VanLandeghem et al. 2012, Dawson et al. 2015).

Surveyed lakes included eight lakes classified as fully infested with zebra mussels by TPWD (meaning the water body has an established, reproducing population): Belton, Bridgeport, Dean Gilbert, Lewisville, Ray Roberts, Texoma, Stillhouse Hollow, and Travis. We also

surveyed Lakes Lavon, Waco, and Austin, where zebra mussels or their larvae have been detected on more than one occasion despite lack of evidence of a fully established, reproducing population (termed "positive" by TPWD). Finally, we surveyed a suite of sixteen negative sites across the Brazos and Colorado River basins, including Lakes Aquilla, Buchanan, Georgetown, Granbury, Granger, Hubbard Creek, Inks, Lady Bird, LBJ, Limestone, Marble Falls, Palo Pinto, Pflugerville, Possum Kingdom, Proctor, and Whitney. Survey sites are depicted in Fig. 2.

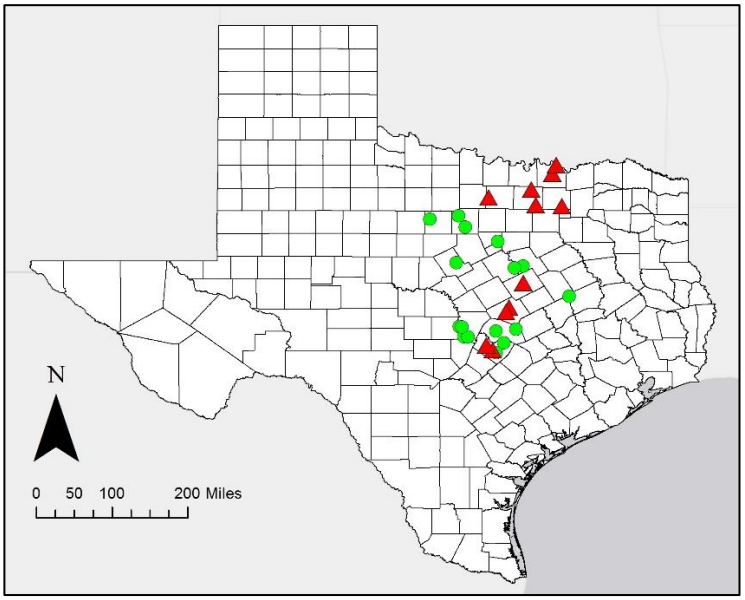

Figure 2: Physicochemical data survey lakes. Sites categorized by TPWD as "infested" (the water body has an established, reproducing population) or "positive" (zebra mussels or their larvae have been detected on more than one occasion despite lack of evidence of a fully established, reproducing population) are indicated by red triangles and included: Lakes Austin, Belton, Bridgeport, Dean Gilbert, Lavon, Lewisville, Ray Roberts, Stillhouse Hollow, Texoma, Travis, and Waco. Sites categorized by TPWD as zebra mussel "negative" are indicated by green circles and included: Lakes Aquilla, Buchanan, Georgetown, Granbury, Granger, Hubbard Creek, Inks, Lady Bird, LBJ, Limestone, Marble Falls, Palo Pinto, Pflugerville, Possum Kingdom, Proctor, and Whitney.

Surveys occurred October 12-16 and 20-21, 2016. At 1-3 public access points (depending upon availability) at each lake, we recorded water chemistry conditions including dissolved oxygen (DO), pH, specific conductance, and temperature using a Hach HQd water chemistry probe or YSI 556 multiparameter probe. The Hach instrument reports actual conductivity, which was converted to specific conductance according to the formula,

$$SC = AC / (1 + r(T-25))$$

where SC is specific conductance at 25°C, AC is actual conductivity, T is the sample temperature, and r (=0.0191) is the temperature correction coefficient (Miller et al. 1988).

Additionally, at each sampled location within a lake, we collected two 500-mL water samples for laboratory determination of total nitrogen (total N), total Kjeldahl nitrogen (TKN), and total phosphorus (total P) using a Hach DR3900 spectrophotometer, and calcium-hardness (Ca) using Hach Digital Titrator, all according to manufacturer instructions. We estimated inorganic N in each sample by subtracting TKN from total N

(and assuming that ammonia-N is negligible). To manage the threat of transporting invasive species between invaded and uninvaded inland waters, extra precaution was taken to prevent the movement of these species on research equipment. All research equipment was soaked in 10% bleach solution for a minimum of 10 minutes between sites. Due to the sensitivity of water chemistry meters, they could not be soaked in bleach, but were instead sprayed with 10% bleach solution, then immediately rinsed with clean water.

Replicate samples within each lake were averaged for each water quality variable, and the average value was used for analysis. We applied Kolmogorov-Smirnov tests to examine normality of distributions of each measured factor, and log transformations were applied when necessary (see Results). Pearson correlation analysis was then applied to identify and eliminate redundant variables ($r \geq \left| 0.7 \right|$). After a priori elimination of correlation between predictor variables, principal component analysis (PCA) was used to determine whether any of the remaining variables were related to zebra mussel presence/absence. Specifically, biplot of principal components 1 and 2 and principal components 1 and 3 were visually inspected to assess patterns of water quality distributions across our survey lakes and identify differences between lakes with and without zebra mussels. In a separate analysis, logistic regression was used to examine the ability to use water quality variables to predict zebra mussel presence. Regressor variables were standardized (mean = 0, standard deviation = 1) prior to analysis to allow direct comparisons of their influence on the presence or absence of mussels. All analyses were conducted with Dell Statistica, version 13 (Dell Inc., Tulsa, Oklahoma, USA).

Objective 3: Assess the potential for downstream spread of zebra mussels based on environmental DNA survey results.

In addition to combining Maxent and lake-specific physicochemical modeling to understand the distribution of suitable habitat in Objectives 1 and 2, we also sought to understand mussel dispersal within Texas. Just as in Objective 2, since quagga mussels do not occur in Texas, our analysis was generally limited to zebra mussels. Specifically, we measured eDNA abundance in two stream habitats to assess the potential for downstream dispersal of zebra mussels from invaded reservoirs. On June 12-13, 2017, we collected water samples in the Leon and Lampasas Rivers, downstream from the invaded reservoirs Lake Belton and Stillhouse Hollow Lake, respectively (Table 1). Water samples were collected moving upstream from the most distant downstream site in each river to reduce potential for cross-site contamination. Concurrent with our sampling, Dr. Astrid Schwalb and PhD student Josi Robertson (Texas State University) conducted plankton and substrate sampling at each site, and future analyses will combine additional ground-truthing (i.e. physical zebra mussel and larvae detection) with eDNA analyses reported here.

Table 1. eDNA collection sites in the Lampasas and Leon Rivers.

River	Site Name	Coordinates	Distance from Dam (rkm)
Lampasas	FM 1915 Crossing	30.821374°N, -97.142001°W	90.7
Lampasas	Reed Cemetery Road	30.896633 N, -97.319732 W	54.7
Lampasas	Dice Grove, Lampasas side	30.983803 N, -97.405615 W	27
Lampasas	Dice Grove, Leon side	30.984970 N, -97.401744 W	27.3
Lampasas	FM 1123 Crossing	30.989977 N, -97.445147 W	19
Lampasas	I-35 Frontage Crossing	31.004260 N, -97.490770 W	5.4
Lampasas	Hamlet Drive Crossing	31.021019 N, -97.510898 W	2.1
Lampasas	Stillhouse Hollow Marina	30.038523 N, -97.532666 W	0
Leon	East 6th Ave Crossing	31.045753 N, -97.432505 W	13.1
Leon	Waco Road Crossing	31.066411 N, -97.442555 W	6
Leon	Hwy 317 Crossing	31.096413 N, -97.453393 W	2.5
Leon	Miller Springs Park	31.103899 N, -97.469524 W	0.4

Following the methods of Egan et al. (2013), we collected 1 L surface water samples (N = 5) at each site using a sterile bottle and gloved hand. In the field, we subsampled 15 mL of each sample and combined with 1.5 mL sodium acetate and 33.5 mL absolute ethanol following the methods of Ficetola et al. (2008). The remainder of each water sample was stored on ice and filtered within 48 hours of collection using 1 µm polycarbonate membrane filters. Genetic material was precipitated from 15 mL water samples following the centrifugation method described by Ficetola et al. (2008). Total genomic DNA from the pellets produced during precipitation as well as the water sample filters was extracted using protocols described by Barnes et al. (2014a). The results of analysis of precipitation samples are presented in the current report (see "Results, Objective 3"). During extraction, three samples were lost accidentally.

Assay for zebra mussel eDNA in each sample was achieved via qPCR analysis using the primers described by Ram et al. (2011). Briefly, for each sample, 8 technical replicate reactions were run on an Applied Biosystems QuantStudio 3 Real-Time PCR System with the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Fluorescence data were collected at each 60°C step. Each 25 µL reaction included 12.5 µL PowerUp SYBR Green Master Mix (Applied Biosystems), forward and reverse primer concentrations of 200 nM, and 4 µL extracted sample DNA. Triplicate negative controls featuring ultrapure H_2O in place of DNA extract were included on each plate of reactions as well as duplicate positive control reactions using genomic DNA derived from tissue from zebra mussel adults collected from Stillhouse Hollow.

Finally, following the same qPCR procedure, but using quagga mussel specific primers instead of zebra mussel primers also designed by Ram et al. (2011), we assayed samples from Stillhouse Hollow Marina and Miller Springs Park (i.e. our closest sites to source populations and site of putative highest eDNA concentrations) for the presence of quagga mussels, which are not known to occur in Texas.

Results

Objective 1: Predict the general distribution of suitable dreissenid mussel habitat in Texas using Maxent models

We produced a Maxent model using the Bioclim environmental layers (i.e. reflections of global atmospheric temperature and precipitation trends) and global zebra mussel occurrence data to predict the extent of suitable zebra mussel habitat in Texas. The result of this analysis is a heat map in which shading indicates the logistic output of the Maxent model; warmer colors are interpreted as relatively suitable habitat, and cooler colors are interpreted as less suitable habitat (Fig. 3; Global model output available in Appendix 1 Fig. A3). The average area under the receiver operating characteristic curve of 0.919 indicated that the model accurately distinguishes global zebra mussel occurrences and provides confidence in our ability to make predictions into uninvaded sites in Texas. The most important environmental layers (i.e. >10% contribution to Maxent model predictions) included annual mean temperature, isothermality, precipitation of the driest month, precipitation coefficient of variation, and precipitation of the driest quarter. Predicted climatic "hotspots" of suitable zebra mussel habitat are concentrated along the Red and Sabine Rivers of the northern and eastern Texas borders, and conspicuous patches of predicted suitable habitat also occur in central Texas between the Colorado and Brazos Rivers as well as extending inland along the Gulf Coast. Most of the Texas panhandle, west Texas extending toward El Paso, and the Rio Grande valley are predicted to provide poor zebra mussel habitat suitability. A list of HUC08 watersheds which contain habitat with suitability of 0.30 or higher (i.e. approximately the upper "half" of model predicted values) is presented in Appendix 1, Table A1.

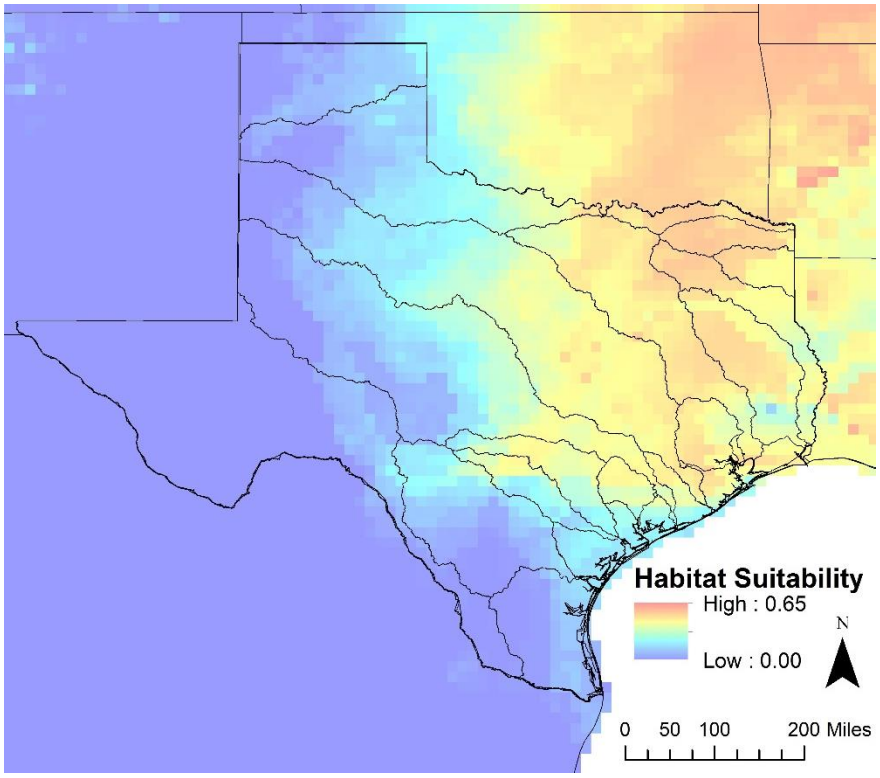

Figure 3: Maxent predictions of suitable *Dreissena polymorpha* habitat in Texas. Shading indicates the logistic output of the Maxent model; warmer colors are interpreted as relatively suitable habitat, and cooler colors are interpreted as less suitable habitat. Polygons represent state and national borders as well as major river basins within Texas.

We also produced a Maxent model using the Bioclim environmental layers and global quagga mussel occurrence data to predict the extent of suitable zebra mussel habitat in Texas. The average area under the receiver operating characteristic curve of 0.976 indicated that the model accurately distinguishes global quagga mussel occurrences. The most important environmental layers (i.e. >10% contribution to Maxent model predictions) included mean temperature of the coldest quarter, precipitation of the wettest month, precipitation coefficient of variation, and precipitation of the driest quarter. No suitable quagga mussel habitat was identified in Texas (i.e. all Maxent scores <0.5; Fig. 4; Global model output available in Appendix 1 Fig. A4).

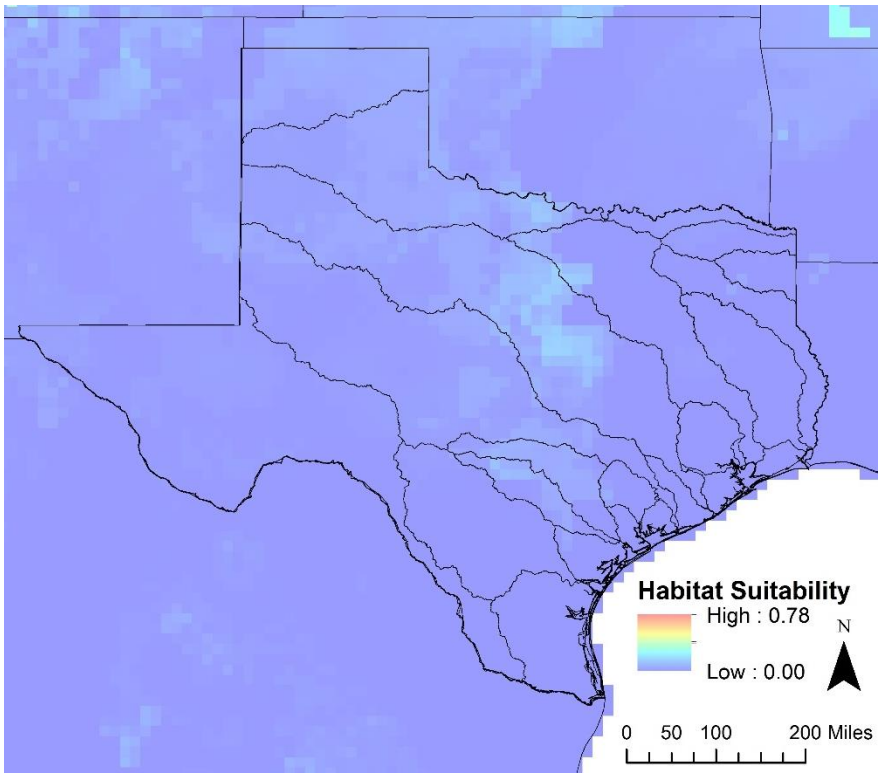

Figure 4: Maxent predictions of suitable *Dreissena bugensis* habitat in Texas. Shading indicates the logistic output of the Maxent model; warmer colors are interpreted as relatively suitable habitat, and cooler colors are interpreted as less suitable habitat. Polygons represent state and national borders as well as major river basins within Texas.

Because we produced Maxent models using global zebra and quagga mussel occurrence data, multivariate environmental similarity surfaces (MESS) and mobility-oriented parity (MOP) outputs generated automatically during Maxent implementation confirmed no environmental extrapolation occurred, as expected, indicating little intrinsic model uncertainty due to transferability. However, the 80% subsampling routine employed during the production of 100 replicate models for each species did result in some variation between models, which can be displayed visually to highlight regions of model uncertainty (Figs. A5-A6). Overall, extremely low variation occurred between quagga mussel models in Texas, and standard deviations <5 points on the logistic scale of Maxent output occurred within Texas among zebra mussel models, suggesting low levels of uncertainty in overall model predictions for both species.

Objective 2: Refine lake-specific predictions based on collection of physicochemical data from identified high-risk lakes of North and Central Texas.

We collected physicochemical data from zebra mussel invaded and uninvaded lakes in North and Central Texas to analyze habitat suitability on a fine scale and build upon the distribution model produced in Objective 1. Notably, every calcium measurement recorded in this study exceeded the "high risk" categorization (i.e. > 28 mg/L) of Whittier et al. (2008). Of the 9 water quality variables measured or estimated, 3 were normally distributed (temperature, pH, DO). Other variables (specific conductance, Ca, total N, inorganic N, TKN, and total P) were log-transformed to improve normality. Correlation analysis showed that total N was highly correlated with TKN (r = 0.98) and Ca was highly

correlated with specific conductance (r = 0.91); thus, TKN and specific conductance were not used in further analyses.

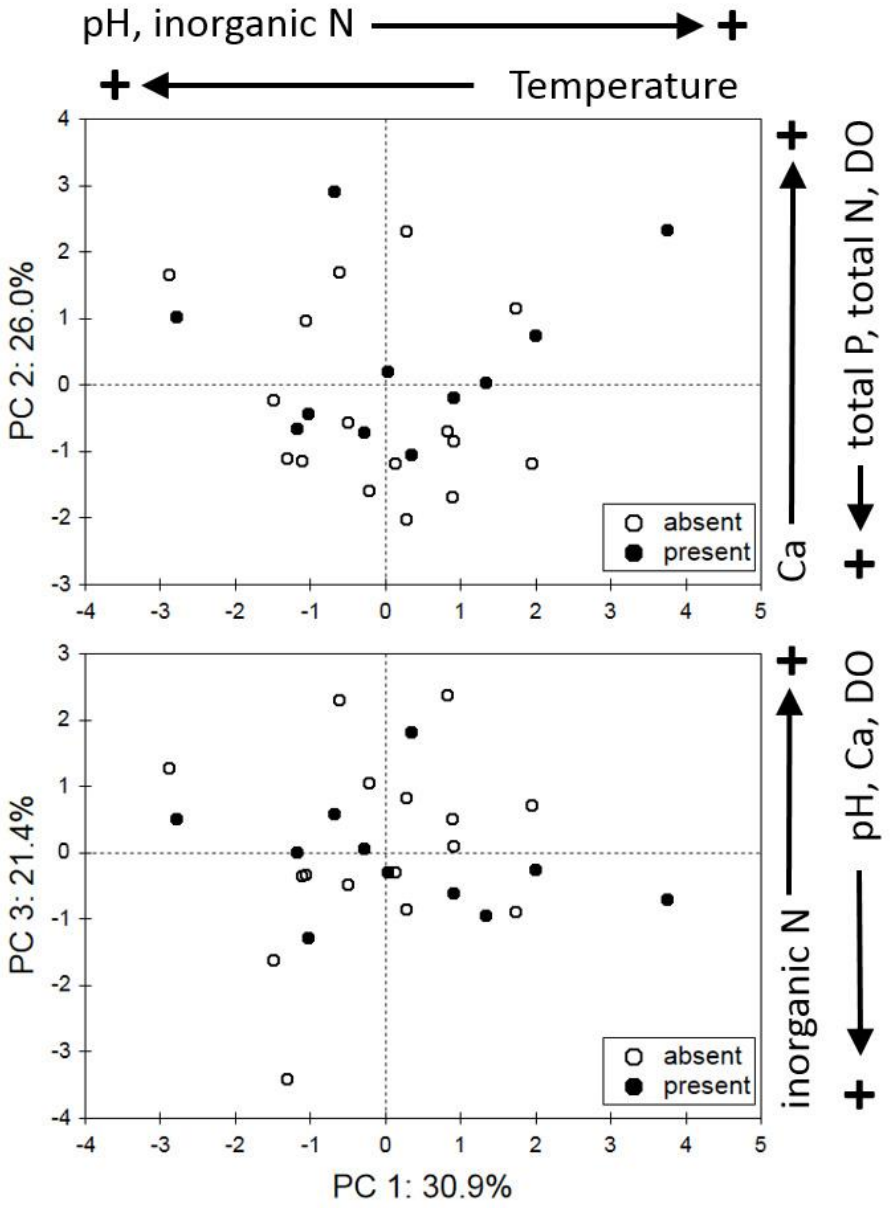

Figure 5. Biplot of components 1 and 2 (A) and 1 and 3 (B) from Principal Component Analysis of water quality variables in 27 study lakes. Variables that predominated in each component (factor loading ≥ |0.50|) are shown on the appropriate axes. Individual lake data are represented by symbols, with open circles representing lakes without previously reported incidences of zebra mussels (16 lakes), and solid circles for those known to harbor the invasive species (11 lakes). No clear separation between the two groups of lakes is evident in either of the biplots.

Visual inspection of biplots of the first three components of a principle component analysis, which together accounted for ~80% of data variability, did not reveal any clear separation between zebra mussel positive and negative lakes (Fig. 5). A multivariate test of

significance on the first three components using presence or absence of zebra mussels as grouping factor also failed to show differences between positive and negative lakes (Wilks λ= 0.934; F(3,23) = 0.539, p = 0.661). In addition, logistic regression analysis also failed to identify any predictive relationships between measured variables and zebra mussel invasion status (Table 3). Compared to other water quality variables, Ca is relatively stable throughout the year and is less affected by sampling date. However, logistic regression using only Ca as regressor variable also did not reveal an association with the presence or absence of zebra mussels (data not shown).

Table 3. Parameter estimates from logistic regression analysis. This analysis was used to model the effects of water quality on zebra mussel presence using grab water samples collected from 27 study lakes, 11 of which have been previously reported to harbor the mussels. No parameter estimates were significant.

Parameter	Estimate	Standard error	Wald statistic	*p*-value
Intercept	0.45839	0.440826	1.081262	0.298415
Temperature	-1.92906	1.595929	1.461045	0.226764
pH	-1.63252	1.380451	1.398540	0.236968
DO	0.63140	0.882041	0.512426	0.474091
Log_(total P)	-0.80549	0.609081	1.748942	0.186010
Log_(total N)	1.06073	0.894822	1.405185	0.235857
Log_(inorganic N)	-0.37564	0.667607	0.316594	0.573662
Log_(Ca)	0.22266	0.519567	0.183647	0.668258

Objective 3: Assess the potential for downstream spread of zebra mussels based on environmental DNA survey results.

The most effective invasive species management strategies recognize that invasion occurs as a stepwise process, beginning with species transport from outside the system, followed by successful introduction and establishment in a novel habitat, and finally secondary spread and accumulation of negative impacts; effective management strategies recognize that different actions (e.g. control, slow-the-spread, adaptation) are relevant at different stages in the invasion process (Lodge et al. 2006). Therefore, in addition to combining Maxent and lake-specific physicochemical modeling to understand the distribution of suitable habitat and predict dreissenid mussel establishment in Objectives 1 and 2, we also sought to understand mussel dispersal within Texas. Because quagga mussels are not present in Texas, our analyses focused primarily on zebra mussels.

Across 12 sites sampled in the Leon and Lampasas Rivers, downstream from the invaded Lakes Belton and Stillhouse Hollow, respectively, qPCR detected the presence of zebra mussel eDNA at 11 sites (Fig. 6). Rate of positive detection among water samples at each site ranged from 1/5 to 5/5, and within water samples, rate of detection among technical replicates ranged from 1/8 to 8/8, suggesting considerable heterogeneity in the zebra mussel eDNA signal in both rivers. No clear spatial pattern in detection rate occurred, with the sites closest to each reservoir (i.e. putative eDNA sources) each yielding 3/5 water samples with positive detections, generally followed by several singleton detections

moving away from each reservoir, and the most distant two sites each had 5/5 water samples test positive for zebra mussel eDNA (up to at least 90.7 river km downstream from invaded reservoirs).

Finally, in addition to testing for the presence of zebra mussel eDNA, we did assay samples from Stillhouse Hollow Marina and Miller Springs Park (i.e. closest sites to source populations and site of putative highest eDNA concentrations) for the presence of quagga mussel eDNA. No quagga mussel eDNA was detected at either site.

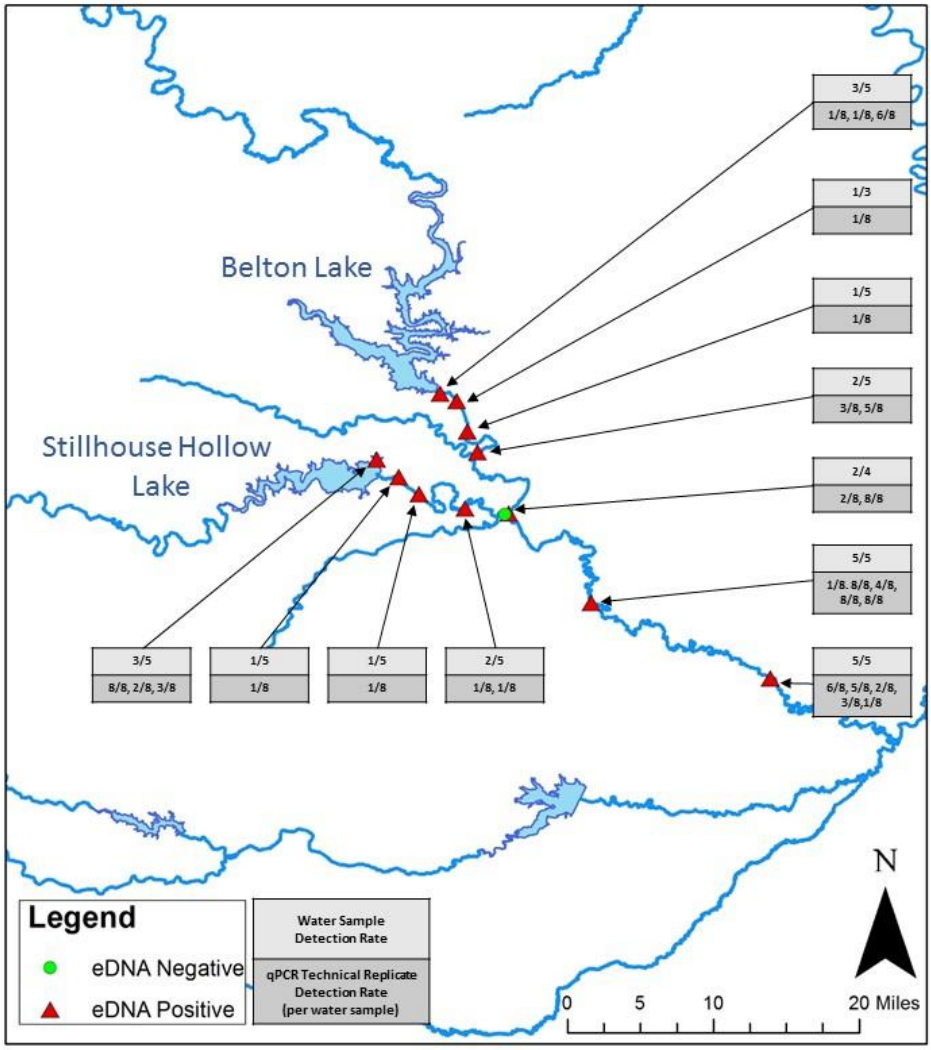

Figure 6. Map of zebra mussel eDNA detections in the Leon and Lampasas Rivers. Callout boxes from each positive detection site indicate number of positive water samples (top box) and number of positive qPCR technical replicates per positive water sample (bottom box). qPCR technical replicates numbered 8 for all samples; the numbers in the bottom boxes report the number of technical replicates that were positive for each positive water sample in the top box. (e.g. if 1/5 water samples tested positive, only 1 batch of 8 technical replicates is reported. If 3/5 water samples were positive, then 3 batches of 8 technical replicates are reported.)

Discussion

We have combined global species distribution modeling with local data collection and eDNA experiments to characterize the potential habitat and downstream dispersal capabilities of nonindigenous invasive dreissenid mussels in Texas. Using global occurrence data and the only continuous environmental layers available at a global scale (i.e. temperature and precipitation based variables), we produced a Maxent species distribution model that predicts that much of north and central Texas contains suitable habitat for zebra mussels (Fig. 3). Therefore, these results suggest that much of Texas is climatically at risk for invasion by zebra mussels, with potential invasion hotspots occurring around the Red, Sabine, Neches, Trinity, Brazos, and Colorado Rivers and their surrounding reservoirs. The model suggested that west Texas (i.e. Rio Grande, Pecos, upper Brazos and upper Colorado Rivers) has a low risk of zebra mussel establishment based on our estimate of habitat suitability. The model predicted that annual mean temperature and isothermality are important drivers of zebra mussel habitat suitability, so west Texas may become too cool in the winter or experience temperature fluctuations over the course of the year that are too large to support zebra mussel populations. Additionally, the model indicated that precipitation of the driest month, precipitation coefficient of variation, and precipitation of the driest quarter all represent important drivers of zebra mussel habitat, which may simply reflect the fact that zebra mussels are obligately aquatic organisms; increased precipitation likely corresponds to more availability of aquatic habitat, and west Texas may appear climatically to have less available habitat. Nevertheless, the aquatic habitats that are available in west Texas are characterized by relatively high calcium concentrations (VanLandeghem et al. 2012, Israël et al. 2014, Sharma et al. 2014), a known predictor of zebra mussel success (Cohen 2005). Furthermore, because zebra mussels can withstand salinities up to 10 ppt (Ludyanskiy et al. 1993), the relatively high salinities of west Texas watersheds are not likely to serve as barriers against expansion. On the contrary, these conditions seem favorable for further zebra mussel expansion. Environmental data used to generate model outputs did not incorporate water quality information, which is also key to understand aquatic species distributions. Clearly, the susceptibility of the western regions of the state to zebra mussel invasion deserves further study.

Texas does not appear to contain habitat suitable for the related quagga mussel (Fig. 4). The most important environmental layers in our Maxent model included mean temperature of the coldest quarter, which could suggest that temperature extremes prevent Texas from providing suitable habitat for quagga mussels. Again, the model also emphasized the importance of precipitation factors, including precipitation of the wettest month, precipitation coefficient of variation, and precipitation of the driest quarter. This could suggest that climatically, Texas is not expected to have enough aquatic habitat to support populations of the obligately aquatic quagga mussel. However, the use of natural indicators of water availability as predictors within species distribution modeling efforts focusing on aquatic organisms has been questioned previously (Barnes et al. 2014b) because this may result in misleading model outputs; although Texas does not naturally have large lake ecosystems, the presence of reservoirs, canals, and even small recreational

and aesthetic ponds could provide pockets of suitable habitat that are unanticipated by the model.

It is also important to note the caveat that distribution models assume that modeled organisms are at equilibrium with their environment (i.e. not demonstrating range expansion or contraction). Given recent, rapid spread of zebra mussels in Texas, this assumption has likely been violated. Previous modeling experiments by Vaclavik and Meentemeyer (2012) demonstrated that the predictions of distribution models may change as an invasion progresses and new sites are colonized. Therefore, while our model represents an accurate (AUC = 0.919) prediction of the distribution of zebra mussel habitat based on current knowledge of zebra mussel occurrences, predictions could change as zebra mussel invasion continues. Similarly, quagga mussel habitat predictions may yet expand into the state. The lack of equilibrium in both zebra mussel and quagga mussel distributions may also help explain why habitat suitability maximums below 1 were predicted (i.e. 0.65 for zebra mussels; 0.78 for quagga mussels). Non-equilibrium conditions could inhibit the ability of the Maxent models to confidently identify habitat of maximum suitability. This phenomenon could also indicate that the two mussel species act as habitat generalists with wide environmental niches. Distribution models may provide some insight into the relative population success that zebra and quagga mussels would experience in various parts of the state if introduced (e.g. Wittmann et al. 2016), but further research into the relationship between dreissenid mussel performance and distribution model results would strengthen this claim.

When we surveyed data in invaded and uninvaded reservoirs of Texas, both PCA and logistic regression failed to identify any association between the physicochemical variables measured in this study and zebra mussel presence (Fig. 5, Table 3). A possible explanation for this finding is that we failed to measure the variables that are most important for zebra mussel establishment. However, this seems unlikely because our variable choices were based on a priori knowledge of zebra mussel habitat requirements, especially the concentration of environmental calcium (Cohen 2005). Another explanation for the failure to distinguish between zebra mussel positive and negative lakes could be that, in fact, no difference exists between the sites we selected in terms of suitability for zebra mussel habitat. This explanation seems parsimonious with our Maxent model findings, and again suggests that much of the water of north and central Texas are at risk for zebra mussel invasion.

We used eDNA methods to demonstrate that zebra mussel genetic material can be found in lotic waters up to at least 90.7 river km downstream from a pair of infested reservoirs (Fig. 6). We cannot say with certainty that eDNA results reflect the presence of living individuals at each site. Indeed, eDNA detection could simply be the result of biological materials such as mucous and feces flowing downstream, and our detection distances are within the range of other eDNA dispersal experiments in streams (Deiner et al. 2016). If eDNA is interpreted as evidence of zebra mussel presence, then it represents a far more sensitive and time efficient tool for zebra mussel detection, especially at downstream sites, compared to visual inspection for colonization and microscopic surveillance for veligers (Robertson, Texas State University, personal communication).

Environmental DNA demonstrates complex relationships in which it is influenced by and influences its surrounding environment, termed "the ecology of eDNA" (Barnes and Turner 2016). Recent work by Jerde et al. (2016) and Shogren et al. (2016, 2017) has demonstrated that eDNA transport in lotic systems is not easily characterized, as eDNA interacts with inorganic substrates and local biota rather than flowing like a conservative tracer. Indeed, we observed some unintuitive spatial patterns in our eDNA survey. Rather than existing in high concentrations at each source (i.e. below each invaded reservoir) and demonstrating decreasing concentrations with distance downstream due to dilution and degradation, we observed moderate eDNA detection rates below each dam, followed by low detection rates at intermediate distances, and our highest rates of detection at our most distant sites. This pattern could indicate that high flow rates or some other source of interference is high near the dam, resulting in lower detection rates, and that detection rates increase downstream as the rivers spread out and slow down, or other (unknown) inhibitors released by the dam become diluted. This pattern may also be indicative of "source-sink" dynamics (e.g. eDNA being produced by isolated colonies of zebra mussels within an overall inhospitable stream environment) similar to the colonization process described by Bobeldyk et al. (2005). Better understanding of zebra mussel population and eDNA dynamics in lotic environments could aid efforts to use eDNA methodologies understand and manage dispersal of invasive zebra mussels or other species of interest through such environments, and more research is warranted to determine the nature of this phenomenon. Future experiments should include studies similar to ours at a wider range of sites across Texas or larger spatial scales to determine the generality of the pattern we have observed. Furthermore, studies of the "ecology of eDNA" immediately downstream of dams, including studies of degradation, size fractionation, interaction with other particles or substances in the environment, and quantification of PCR inhibition could further benefit understanding of eDNA analysis in these environments. For the immediate future, a monitoring strategy that combines traditional sampling (e.g. settlement substrate samplers and microscopy) at sites immediately below a dam, and transitioning to more sensitive eDNA analysis at distances further from the dam may represent the most successful strategy for detection of dreissenid mussel downstream dispersal.

Overall, we have demonstrated that suitable habitat for continuing zebra mussel invasion exists within Texas and that stream and river connections may represent one method for their dispersal. The threat of continued expansion of this poster child for negative invasive species impacts warrants further prevention efforts, management, and research.

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purposes only and does not imply endorsement by the U.S. Government. All raw data were provided to TPWD upon submission of this report and are available by request.

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Appendix 1 Global Occurrences and Model Outputs

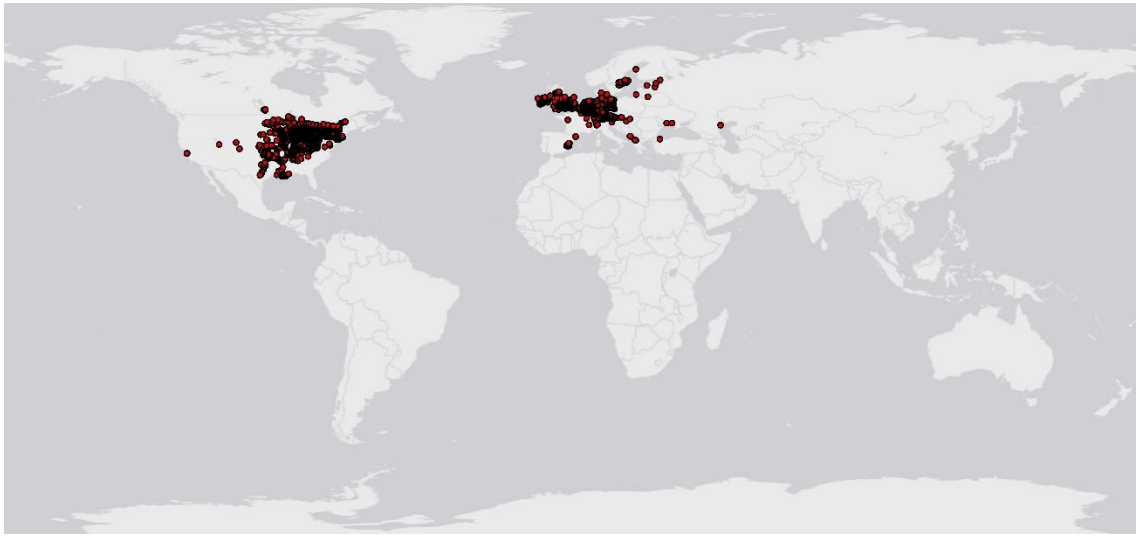

Figure A1. Global zebra mussel occurrence data

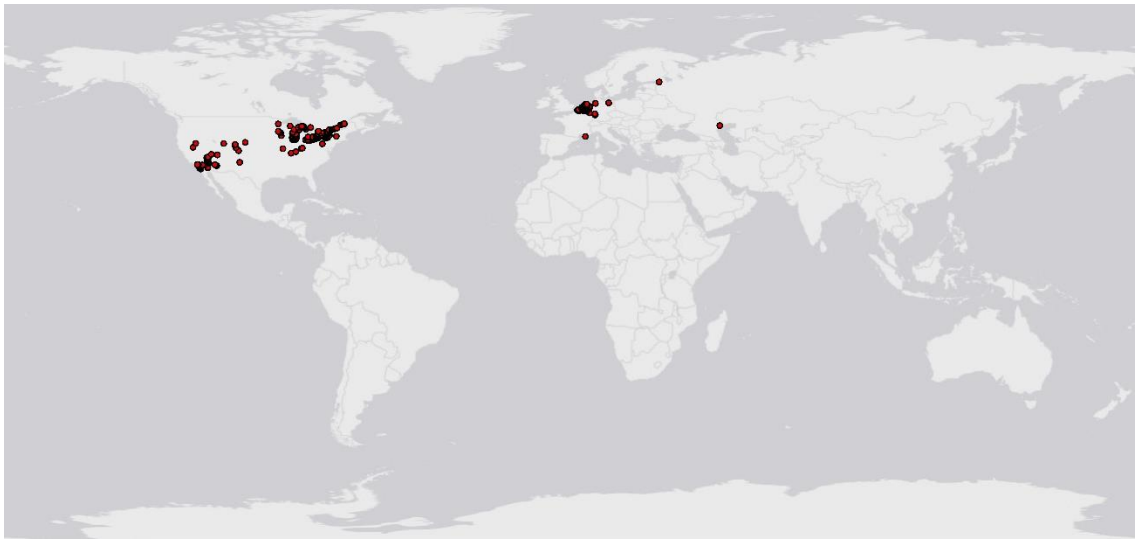

Figure A2. Global quagga mussel occurrence data

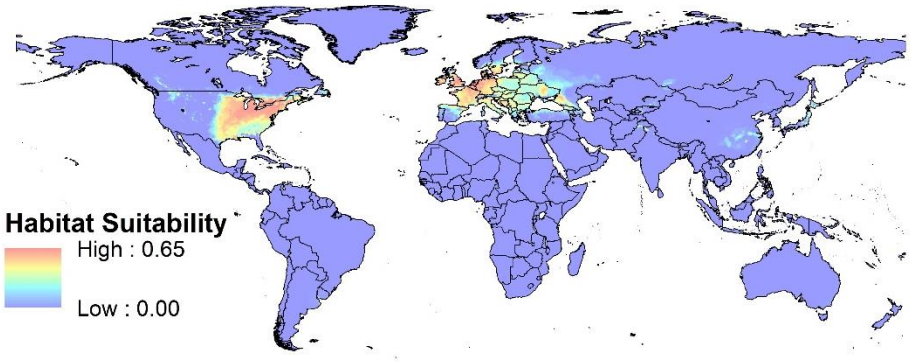

Figure A3. Global zebra mussel predictions represent the average of 100 Maxent predictions of suitable Dreissena polymorpha habitat. Shading indicates the logistic output of the Maxent model; warmer colors are interpreted as relatively suitable habitat, and cooler colors are interpreted as less suitable habitat.

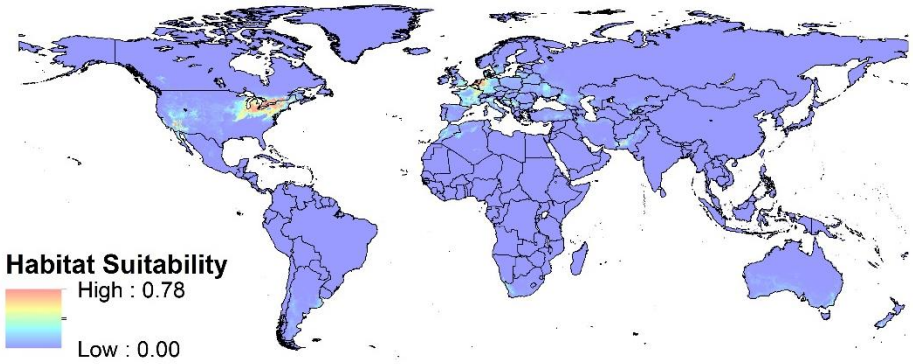

Figure A4. Global quagga mussel predictions represent the average of 100 Maxent predictions of suitable Dreissena bugensis habitat. Shading indicates the logistic output of the Maxent model; warmer colors are interpreted as relatively suitable habitat, and cooler colors are interpreted as less suitable habitat.

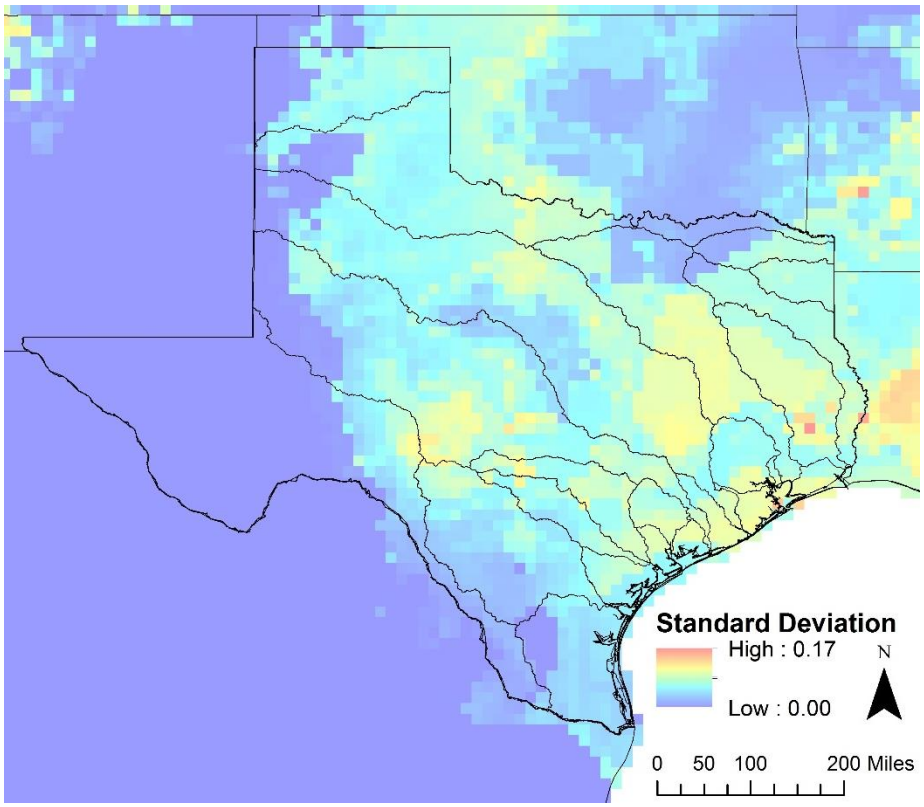

Figure A5 Standard deviation of 100 Maxent predictions of suitable Dreissena polymorpha habitat in Texas. Shading indicates the standard deviation of 100 replicate Maxent models; warmer colors indicate higher levels of disagreement between replicate models, and cooler colors represent more consistent predictions. Polygons represent state and national borders as well as major river basins within Texas.

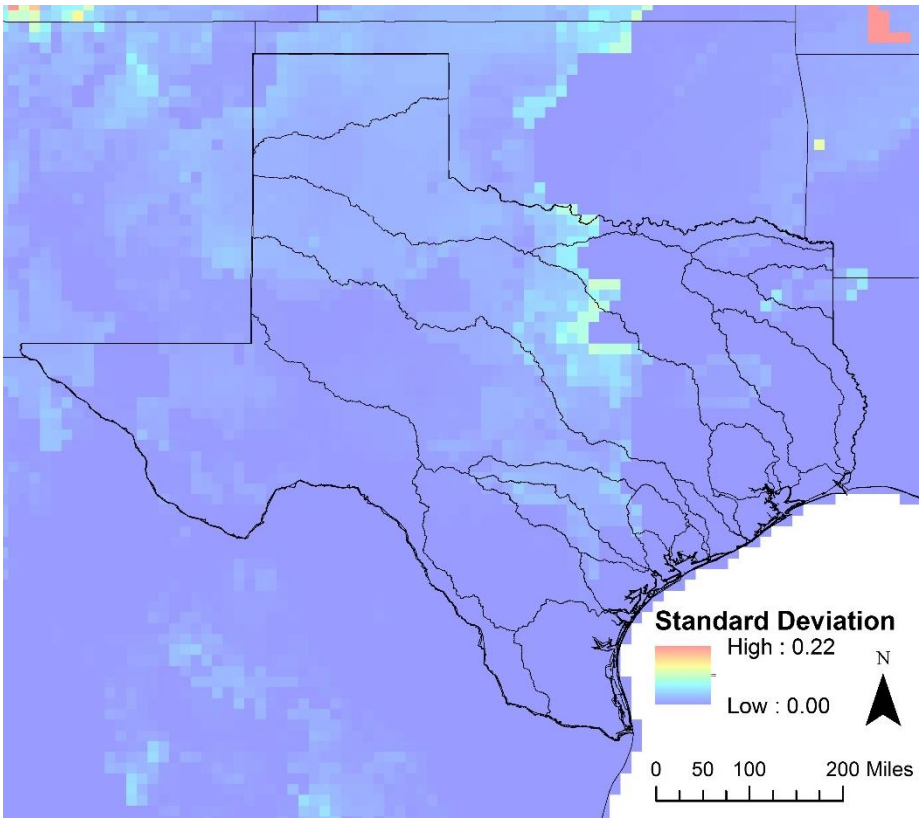

Figure A6 Standard deviation of 100 Maxent predictions of suitable Dreissena bugensis habitat in Texas. Shading indicates the standard deviation of 100 replicate Maxent models; warmer colors indicate higher levels of disagreement between replicate models, and cooler colors represent more consistent predictions. Polygons represent state and national borders as well as major river basins within Texas.

Table A1. HUC08 watersheds which contain habitat with suitability of 0.30 or higher (i.e. approximately the upper "half" of model predicted values)

HUC8	Watershed Name
11130102	Blue-China
11130201	Farmers-Mud
11140201	McKinney-Posten Bayous
11130206	Wichita
11130207	Southern Beaver
11130209	Little Wichita
11140106	Pecan-Waterhole
11140301	Sulphur Headwaters
11140303	White Oak Bayou
11140305	Lake O'the Pines
11140306	Caddo Lake
11140307	Little Cypress
12010001	Upper Sabine
12010002	Middle Sabine
12010003	Lake Fork
12020001	Upper Neches
12020002	Middle Neches
12020003	Lower Neches
12020004	Upper Angelina
12020005	Lower Angelina
12020006	Village
12020007	Pine Island Bayou
12030101	Upper West Fork Trinity
12030102	Lower West Fork Trinity
12030103	Elm Fork Trinity
12030104	Denton
12030105	Upper Trinity
12030106	East Fork Trinity
12030107	Cedar
12030108	Richland
12030109	Chambers
12030201	Lower Trinity-Tehuacana
12030202	Lower Trinity-Kickapoo
12030203	Lower Trinity
12040101	West Fork San Jacinto
12040102	Spring
12040103	East Fork San Jacinto
12040104	Buffalo-San Jacinto

12040202	East Galveston Bay
12040203	North Galveston Bay
12040204	West Galveston Bay
12040205	Austin-Oyster
12060101	Middle Brazos-Millers
12060102	Upper Clear Fork Brazos
12060103	Paint
12060104	Lower Clear Fork Brazos
12060105	Hubbard
12060201	Middle Brazos-Palo Pinto
12060202	Middle Brazos-Lake Whitney
12060203	Bosque
12060204	North Bosque
12070101	Lower Brazos-Little Brazos
12070102	Yegua
12070103	Navasota
12070104	Lower Brazos
12070201	Leon
12070202	Cowhouse
12070203	Lampasas
12070204	Little
12070205	San Gabriel
12090106	Middle Colorado
12090107	Pecan Bayou
12090108	Jim Ned
12090109	San Saba
12090110	Brady
12090201	Buchanan-Lyndon B. Johnson Lakes
12090204	Llano
12090205	Austin-Travis Lakes
12090206	Pedernales
12090301	Lower Colorado-Cummins
12090302	Lower Colorado
12090401	San Bernard
12090402	East Matagorda Bay
12100102	Navidad
12100201	Upper Guadalupe
12100202	Middle Guadalupe
12100203	San Marcos
12100204	Lower Guadalupe
12100301	Upper San Antonio
12100303	Lower San Antonio

12100304	Cibolo
12100401	East Matagorda Bay
12100402	West Matagorda Bay
12110106	Upper Frio
12110107	Hondo
12110109	San Miguel
11140302	Lower Sulpher
12010004	Toledo Bend Reservoir
12040201	Sabine Lake
11130210	Lake Texoma
11140101	Bois D'arc-Island
12100101	Lavaca
12100302	Medina
11140206	Bayou Pierre
12010005	Lower Sabine
11140304	Cross Bayou