Received: 12 August 2022 | Revised: 27 December 2022 | Accepted: 10 January 2023

DOI: 10.1002/eap.2826

ECOLOGICAL APPLICATIONS
ECOLOGICAL SOCIETY OF AMERICA

ARTICLE

Special Feature: Management of biological invasions in China

eDNA-based detection reveals invasion risks of a biofouling bivalve in the world's largest water diversion project

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Funding information
Canada Research Chairs; Natural Science Foundation of China, Grant/Award Number: 31622011

Handling Editor: Xian Liu

Abstract

Environmental DNA (eDNA) has increasingly been used to detect rare species (e.g., newly introduced nonindigenous species) in both terrestrial and aquatic ecosystems, often with distinct advantages over traditional methods. However, whether water eDNA signals can be used to inform invasion risks remains debatable owing to inherent uncertainties associated with the methods used and the varying conditions among study systems. Here, we sampled eDNA from canals of the central route of the South-to-North Water Diversion Project (hereafter SNWDP) in China to investigate eDNA distribution and efficacy to inform invasion risks in a unique lotic system. We first conducted a total of 16 monthly surveys in this system (two sites in the source reservoir and four sites in the main canal) to test if eDNA could be applied to detect an invasive, biofouling bivalve, the golden mussel *Limnoperna fortunei*. Second, we initiated a one-time survey in a sub-canal of the SNWDP using refined sampling (12 sites in ~22 km canal) and considered a few environmental predictors. We found that detection of target eDNA in the main canal was achieved up to 1100 km from the putative source population but was restricted to the warmer months (May–November). Detection probability exhibited a significant positive relationship with average daily minimum air temperature and with water temperature, consistent with the expected spawning season. eDNA concentration in the main canal generally fluctuated across months and sites and was generally higher in warmer months. Golden mussel eDNA concentration in the sub-canal decreased significantly with distance from the source and with increasing water temperature and became almost undetectable at ~22 km distance. Given the enormity of the SNWDP, golden mussels may eventually expand their distribution in the main canal, with established "bridgehead" populations facilitating further spread. Our findings suggest an elevated invasion risk of golden mussels in the SNWDP in warm months, highlighting the critical period for spread and, possibly, management.

KEYWORDS
biological invasions, early detection, invasion management, *Limnoperna fortunei*, seasonal effect, temperature, water canal

INTRODUCTION

Invasive species cause ecological and economic damage and even threaten human health (Holle & Simberloff, 2005; Lockwood et al., 2005). Management of invasive species is often most effective at the prevention stage (Leung et al., 2002). Preventing invasions can be successful by focusing on elimination or dramatically reducing the frequency of introduction events and inoculum size in each event (collectively, propagule pressure) (Holle & Simberloff, 2005; Lockwood et al., 2005). Early detection refers to the ability to detect an introduced species at a very low population size—ideally just after the initial introduction of a population with low propagule pressure—and is critical to the success of subsequent management measures (e.g., prevention, eradication, containment).

Traditional detection methods rely on some variant of "catch and examine" of target organisms and are often challenged in detecting newly introduced species, mainly due to sampling difficulty (Harvey et al., 2009). In addition, immature individuals or species possessing cryptic morphological life stages may be exceptionally difficult to identify even for trained taxonomists, raising the possibility of misidentification (Hebert et al., 2003). The advent of DNA barcoding provided an opportunity to identify species based on unique nucleotide sequences instead of morphological traits, often with enhanced accuracy (Hebert et al., 2003). The ubiquitous nature of environmental DNA (eDNA) in bulk environmental samples provides a cost-efficient way to detect target species by analyzing DNA organisms shed into the surrounding environment rather than isolating the target organisms themselves (de Paula et al., 2020; Thomsen et al., 2012; Xia, Zhan, et al., 2018). The combination of DNA barcoding and eDNA sampling has proven very effective in detecting species at very low abundance (Fukumoto et al., 2015; Thomsen et al., 2012; Wilcox et al., 2013; Xia, Zhan, et al., 2018). Besides presence/absence determination, eDNA methods have also been used to estimate species abundance (Bayer et al., 2019; Pilliod et al., 2013; Thomsen et al., 2012; Tillotson et al., 2018), although with conflicting results (Rice et al., 2018).

The golden mussel *Limnoperna fortunei* (Dunker 1857) is a freshwater bivalve native to the Pearl River basin in south China and several neighboring countries. It has spread widely in East Asia including north to the Yangtze River Basin in China, Japan, and South Korea, and distant locales in South America (e.g., Argentina, Brazil) (Boltovskoy, 2015). In invaded environments, mussel distributions typically expand quickly (Boltovskoy & Correa, 2015). The golden mussel is considered an ecosystem engineer that poses profound impacts on invaded ecosystems (Boltovskoy, 2015). The life cycle of golden mussels comprises a free-swimming, planktonic larval stage and a predominantly sessile adult stage. When environmental conditions (e.g., temperature) permit, the golden mussel can reproduce multiple times or even continuously throughout the year (Dei Tos et al., 2016; Xu et al., 2013). These characteristics greatly enhance the species' opportunity for transport elsewhere. For example, the transcontinental invasions to South America likely occurred via the transfer of planktonic juveniles through ballast water discharge, while inland spread likely occurred mainly by transport through hull fouling by (sub)adults on boats (Barbosa et al., 2016; Nakano et al., 2015). Other anthropogenic activities such as dam construction and water diversion projects also facilitate spread (Nakano et al., 2015; Xu et al., 2014; Zhan et al., 2015). The latter mode may be particularly potent as water diversion projects potentially transport a huge number of planktonic propagules to new locations.

The central route of the South-to-North Water Diversion Project (hereafter SNWDP) in China is a mega artificial project—with a total length of 1432 km, which is mainly comprised of an open concrete canal—designated for transferring water from the water resource-rich south to north where water resources are much more scarce (http://www.csnwd.com.cn/gcjs/zxgc/). The water diversion system connects invaded water bodies in the south with uninvaded, recipient ones in the north; specifically, the golden mussel has been found to inhibit the source reservoir (i.e., Danjiangkou Reservoir in Hubei Province) while the recipient reservoirs (e.g., Miyun Reservoir in Beijing) has no mussels reported before the project begun operation in late 2014 (Zhan et al., 2015). The designated water diversion rate is 350 m^3/s at the canal head, supplying water resources in domestic, industrial, and agricultural (small in quantity) sectors to 19 medium-to-large cities and >100 counties (http://www.csnwd.com.cn/gcjs/zxgc/), involving massive water supply networks and facilities that have been demonstrated to be very sensitive to golden mussels fouling (Boltovskoy, 2015; de Paula et al., 2021; Xu et al., 2013). Therefore, the opening of the SNWDP was suggested to represent a potential "invasion highway" for *L. fortunei* (Zhan et al., 2015), which also poses a strong threat to the water diversion project itself

(e.g., concrete erosion and pipeline clogging). The risk of spread is expected to vary temporally because planktonic larvae production is often seasonal (Nakano et al., 2010). Thus, the postspawning season may present an ideal window of time with which to detect spreading golden mussels because free-living larvae may drift considerable distances from their sites of origin. In the present study, we sought to test if eDNA can be used as a proxy to identify the spawning season of golden mussels in the SNWDP canal and explored environmental factors that may influence eDNA concentration in a sub-canal. We hypothesized that the detection frequency and concentration of golden mussel eDNA in the main canal of SNWDP was consistent with the mussel's spawning season and that the concentration of target eDNA decreased with transport distance.

MATERIALS AND METHODS

Study design

We first sampled multiple sites along the main canal of SNWDP over a duration covering the expected spawning season. Besides thermal conditions (i.e., water or air temperature) and transport distance, we did not consider the impacts of other environmental factors on eDNA occurrence and concentration in the main canal because of sampling difficulty. Instead, we then conducted refined eDNA sampling in a sub-canal of the SNWDP and explored environmental factors that could affect eDNA concentrations in such a running water system.

Main canal sampling

We collected water samples from the central route of the SNWDP from May 2016 through November 2017 in two sampling phases (Figure 1). The first phase comprised 11 campaigns (May 2016–April 2017 with an exemption of February 2017 that was not sampled) covering six sites (two in the source reservoir and four in the main canal; M1–M6 in Figure 1b), and the second (July–November 2017) included five campaigns and covered four sites (two in source reservoir and two in the main canal; M1, M2, M3 and M5 in Figure 1b). Site M1 was located by the Danjiangkou Dam of the source reservoir (Danjiangkou Reservoir, 32°39′0 N, 111°41′15″ E), while site M2 was ~500 m away toward the canal control gate outflow to the SNWDP (i.e., canal head), which was ~30 km from M1 (Figure 1b). Sites M4 and M6 were not included in the second sampling phase due to logistics limitations. Each campaign commenced on approximately the 10th day of the month and finished within 3 days. Water collections were made from upstream to downstream of the canal (i.e., M1 to M6, south to north). At each main canal site, a 1-L surface water sample was collected in a new polyethylene terephthalate (PET) bottle from an overhead bridge of the canal, which was immediately placed in a portable fridge (~4°C) until transport back to the laboratory. For the first sampling phase, the water temperature was measured, although it was not measured in the second sampling phase due to logistics limitations. One bottle filled with distilled water was placed with the samples and served as a sampling control. Water samples were filtered within 48 h of collection at the last site (M6). Each water sample was evenly filtered onto two cellulose acetate microporous membrane filters (0.45 μm pore size) (i.e., 500 mL per filter) with exceptions for samples collected in June 2016 and July and August 2017 that were each filtered onto three filters (i.e., 333 mL per filter) due to filter clogging. Each filter was placed in a 2-mL centrifuge tube and stored at −80°C until extraction.

Sub-canal sampling

The sub-canal is located in Dengzhou, Henan Province, which is the first branch of the main canal after the canal head (Figure 1c). It is used for intermittent irrigation, and the canal side is bare concrete, while the bottom is covered with soft sediment and occupied by submerged macrophytes. Golden mussels have been observed in this canal, but in very limited abundance and rarely attached to secured rocks (Xia, Johansson, et al., 2018). The sub-canal sampling was conducted on 30 May 2018 and covered 12 sites (S1–S12; Figure 1c). This canal was selected because it originates from the main canal and has similar water chemistry to it. Sampling was conducted from downstream to upstream (i.e., S12 to S1, Figure 1c). At each site, three 100-mL water samples were collected by submerging new PET bottles under the surface layer (~20 cm), and each sampling site was ~1 m deep and ~1 m off the side board. Here we collected a smaller volume of water than the main canal because it was known that such an amount of water sample was sufficient to quantify the target eDNA according to our previous study in this system (Xia, Johansson, et al., 2018). A 500-mL bottle was filled and sealed underwater (i.e., no air bubbles were introduced into the bottle) for suspended particle size partitioning. Water temperature and water velocity were measured in situ. Sampling control and sample preservation and transport were performed as above in *Main canal sampling*. Each sample was filtered onto a single filter within 24 h of collection and stored at −80°C until extraction.

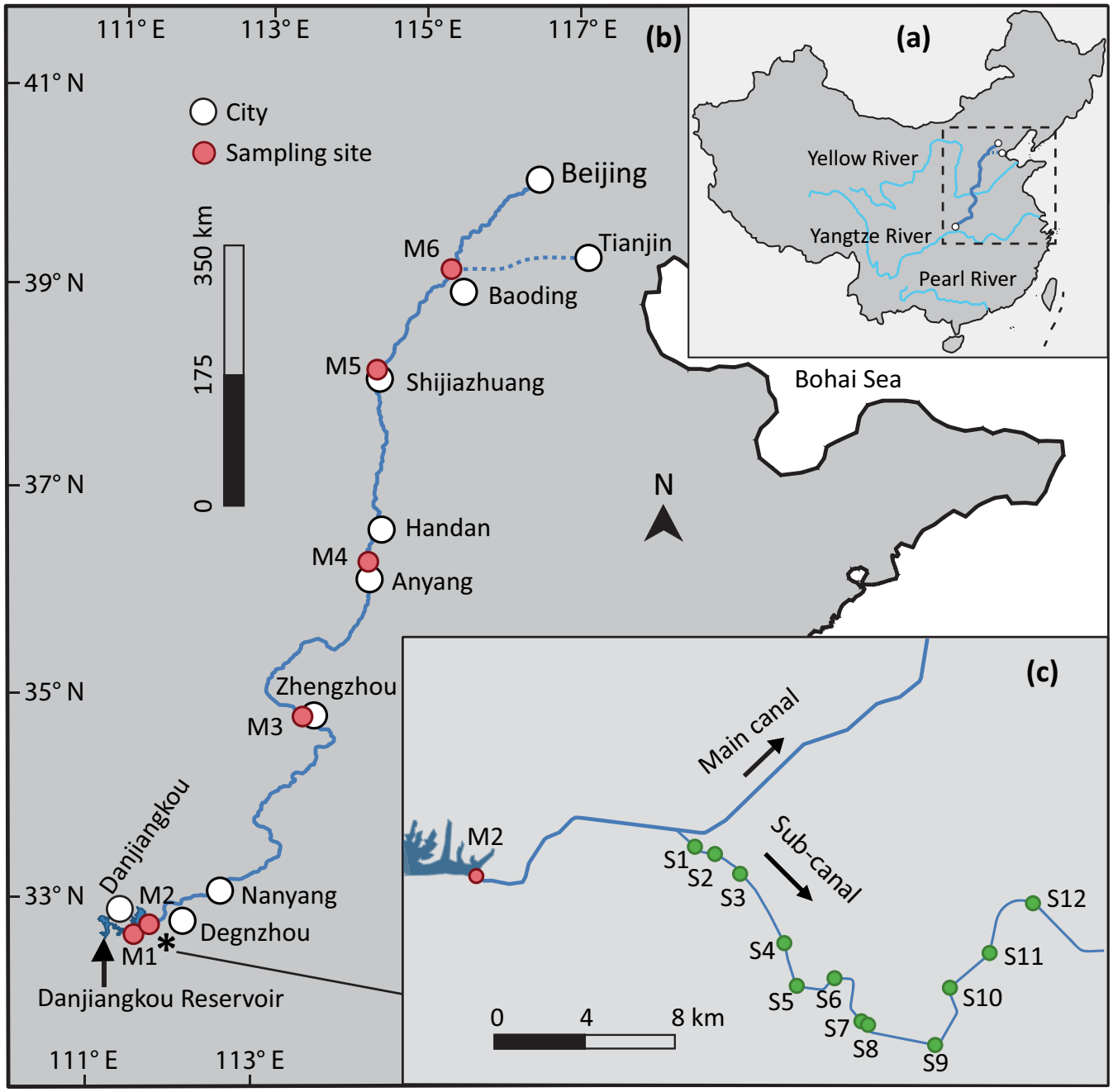

FIGURE 1 Maps of the study area, showing the location of the central route of the South-to-North Water Diversion Project (SNWDP) in China (a, dashed rectangle), sampling sites in the main canal (b, red dots) and sub-canal (c, green dots), respectively. Sites M1 and M2 (~30 km apart) were within the Danjiangkou Reservoir, and the asterisk indicates the proximate location of the subchannel, and arrows in (c) indicate flow directions.

DNA extraction and PCR

We used the phenol–chloroform–isoamyl alcohol (PCI) method of Renshaw et al. (2014) to extract eDNA from filters. For each filter, extracted eDNA was dissolved in 50 µl double distilled water (ddH_2O). We diluted the original eDNA extracts by 1:10 before PCR to reduce possible inhibitors (McKee et al., 2015). For main canal sampling, we performed eDNA extraction for the first and second phases separately. Tissue-derived total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

SYBR Green-based quantitative PCR (qPCR) detailed in Xia, Johansson, et al. (2018) was used to detect and quantify golden mussel eDNA from extracted water

samples. Consistent with eDNA extraction, we conducted PCR for the first and second sampling phases in the main canal separately. For each sample, we ran PCR for the two (or three in a few months as described in *Main canal sampling*) filters separately because of space limitations in each 96-well PCR plate, and we ran each batch twice to generate PCR replicates. Therefore, four (2 PCRs × 2 filters) or six (2 PCRs × 3 filters) replicates were conducted for each sample. To quantify target eDNA, a standard curve using triplicate serial dilutions (i.e., 1:10) of the tissue-derived total genomic DNA was included in each PCR plate. The limit of detection (LoD) of the assay was determined as the lowest concentration of the total genomic DNA that yielded at least a single positive amplification of the replicates. Melting curves of PCR products were generated at the end of qPCR with default settings to confirm the specific amplification of target species (Xia, Johansson, et al., 2018). For a small portion of reactions that yielded mixed signals of no-template-control (NTC) and target species, an adjusted quantity was assigned to the respective sample according to the shape of its melting curve. Specifically, a coefficient of 0.5, 0.25, and 0.125 was assigned to the original quantity yielded by the system for samples with strong, medium, and weak signals of target species (Xia, Johansson, et al., 2018). Also, we sent four qPCR products with high concentrations for Sanger sequencing (Tsingke Biotech Ltd., Beijing, China) to further confirm specificity.

Quality control in the laboratory

To prevent cross-contamination, we rinsed the outside of all sample bottles with distilled water and dried them with paper towels before use. All reusable tools (e.g., glassware, filtration platform, forceps, scissors, etc.) used in sample filtration were submerged in 10% commercial bleach for 20 min, followed by a thorough rinse with distilled water between two uses. Three NTCs using ddH_2O were included in each PCR plate, acting as negative controls.

Data collection and statistical analysis

Standard curves were used to calculate the quantities of samples (in ng total genomic DNA). For filters with ~333 mL water filtered, results were corrected to 500 mL to be consistent with most cases. The average quantity (500 mL for main canal samples and 100 mL for sub-canal samples) was used in the subsequent analysis, and the percentage of replicates detected positive was used to represent the detection probability of a site. The concentration of suspended particles with varying sizes in the sub-canal was partitioned using a particle counter (PAMAS Water Viewer, Germany), which was running with ddH_2O between every two samples to avoid cross-contamination. The mean of three reads of each sample was used in the subsequent analysis. The distance from the source of each sampling site was measured using the default ruler of the path in the Google Earth program. In the case of the main canal, we specified 15 km to sites M1 and M2 as they were located within the donor reservoir. We compiled the daily minimum and maximum air temperature of the neighboring city (see Figure 1) of each sampling site in the main canal (http://www.tianqihoubao.com/; see Appendix S1: Table S1 for detailed access to each city) and calculated the daily averages for each month (Xia, Gu, et al., 2022), which were then used in the analysis.

We conducted binary logistic linear regression of PCR call of target eDNA (i.e., positive or negative) in the main canal against the average daily minimum air temperature of each site to explore the influence of thermal conditions on the occurrence of golden mussel eDNA. We used minimum instead of maximum air temperature in the model as they were strongly correlated ($R^2 = 0.967$, $p < 0.0001$), and the former led to a better model than the latter in a pilot model selection based on the Akaike information criterion (AIC). We used air rather than water temperature because of the following reasons. First, the water temperature for the second phase was unavailable, whereas the air temperature was available. Second, average daily minimum air temperature demonstrated a similar effect on the occurrence of golden mussel eDNA as did water temperature (see *Positive/negative distribution of eDNA in the main canal* for details), but it led to better modeling performance (AIC: 56.9 vs. 67.3) in a pilot test based on the first sampling phase. For golden mussel eDNA concentration in the main canal, we performed multiple linear regressions of eDNA quantity against distance from the source and thermal conditions (either water or minimum air temperature depending on model performance) for June and September, respectively when all six sites were detected positive. In the subcanal, the distance from the source, water velocity, and concentration of suspended particles (0–1 μm) were considered explanatory variables in modeling eDNA concentration. We only included the 0–1 μm suspended particles in the formal analysis as it was the best predictor of target eDNA concentration among all size categories (see *eDNA concentration in the main canal* for more details). Water temperature was excluded from the multiple regression in the subcanal due to a significant collinear relationship with distance from the source and was analyzed with a simple linear regression instead. We standardized and centered the explanatory variables to stabilize the

variance before modeling. The analysis was performed in R 4.2.2 (R Core Team, 2022).

RESULTS

Performance of qPCR assay

The LoD of the assay was identified as 1.2×10^{-7} ng of tissue-derived genomic DNA, which was achieved in two PCR plates (Appendix S1: Table S2). Efficiencies of qPCR reactions ranged between 96% and 102% with R^2 of standard curves between 0.98 and 1.00 (Appendix S1: Table S2), suitable for quantifying water samples. Melting peaks (i.e., temperature) of PCR products of the target species and NTC (if signals were detected) were ~79.5 and ~77.0°C, respectively, and could be clearly distinguished from each other by eye (Appendix S1: Figure S1; Xia et al., 2023), consistent with a previous study using the assay (Xia, Zhan, et al., 2018).

Positive/negative distribution of eDNA in the main canal

Golden mussel eDNA was detected in eight of the 11 campaigns (74%) in the first sampling phase (i.e., May–October, December 2016, and March 2017 in the first sampling phase; July–November 2017 in the second phase) and all five campaigns (100%) in the second (left and right panel, respectively; Figure 2). A total of 95% (42 of 44) of sites had positive hits achieved between May and November. The remaining positives were detected in December and March of the first phase, at low eDNA concentrations (Figure 2). No sampling controls or NTCs tested positive throughout the study indicating no contamination, and sequencing results confirmed the target species (Appendix S1: Figure S2).

For the first sampling phase, positive detections were concentrated in May through October (Figure 2), although their frequency (i.e., no. campaigns detected positive) declined significantly with distance from the source ($F_{1,4} = 11.7$, $R^2 = 0.745$, $p = 0.027$). The northernmost site M6 had the lowest detection frequency (two of 11 campaigns). This pattern was not followed in the second sampling phase among the four sites (Figure 2, right panel). Overall, the detection success of golden mussel eDNA increased significantly with air temperature while the minimum air temperature had better model performance (i.e., lower AIC; Appendix S1: Table S3), with an inflection point (i.e., 50% probability) at 13.2°C ($df = 85$, $p < 0.001$; Figure 3). Water temperature demonstrated a similar effect on the detection success of target eDNA (Appendix S1: Figure S3). The distance from the donor reservoir, however, had no significant relationship with the occurrence of golden mussel eDNA (Appendix S1: Table S3).

eDNA concentration in the main canal

The concentration (mean ± SE) of golden mussel eDNA ranged between $9.8 \pm 9.0 \times 10^{-8}$ and $9.4 \pm 7.8 \times 10^{-2}$ ng per reaction, and varied substantially among sites and months (Figure 2). Only in June and September did all six sites have positive detections, although the eDNA concentration exhibited different relationships with distance from the donor reservoir between the 2 months. Specifically, the concentration of golden mussel eDNA decreased significantly with distance in June ($p = 0.036$) but was unrelated to distance in September (Table 1). Unlike the positive/negative distribution, neither the average daily minimum air temperature nor the water temperature had a statistically significant relationship with the concentration of golden mussel eDNA in the main canal in both campaigns ($p = 0.810$ in June and 0.112 in September; Table 1).

eDNA concentration in the sub-canal

Golden mussel eDNA was detected at all 12 sites in the sub-canal with concentration (mean ± SE) ranging between $1.1 \pm 0.3 \times 10^{-4}$ and $1.2 \pm 0.6 \times 10^{-7}$ ng per reaction (Figure 4). eDNA concentration was significantly related to distance from the source ($p = 0.001$) and its interaction with the concentration of suspended particles (0–1 μm size) ($p = 0.002$), but not the concentration of suspended particles itself ($p = 0.925$) (Table 1). Specifically, eDNA concentration decreased with distance from the source but increased with the interaction term (Table 1, Figure 4a). The eDNA concentration was also negatively related to water temperature ($p = 0.007$) (Table 1, Figure 4b), but not to water velocity (Appendix S1: Figure S4).

DISCUSSION

Water eDNA methods provide a promising advance in the accurate detection of rare species and a reduction in type II errors. One important application of water eDNA methods is to predict spawning (Bayer et al., 2019; Tillotson et al., 2018), which is useful for informing the risk of invader spread. However, detection probability is dependent on the availability of target eDNA and is

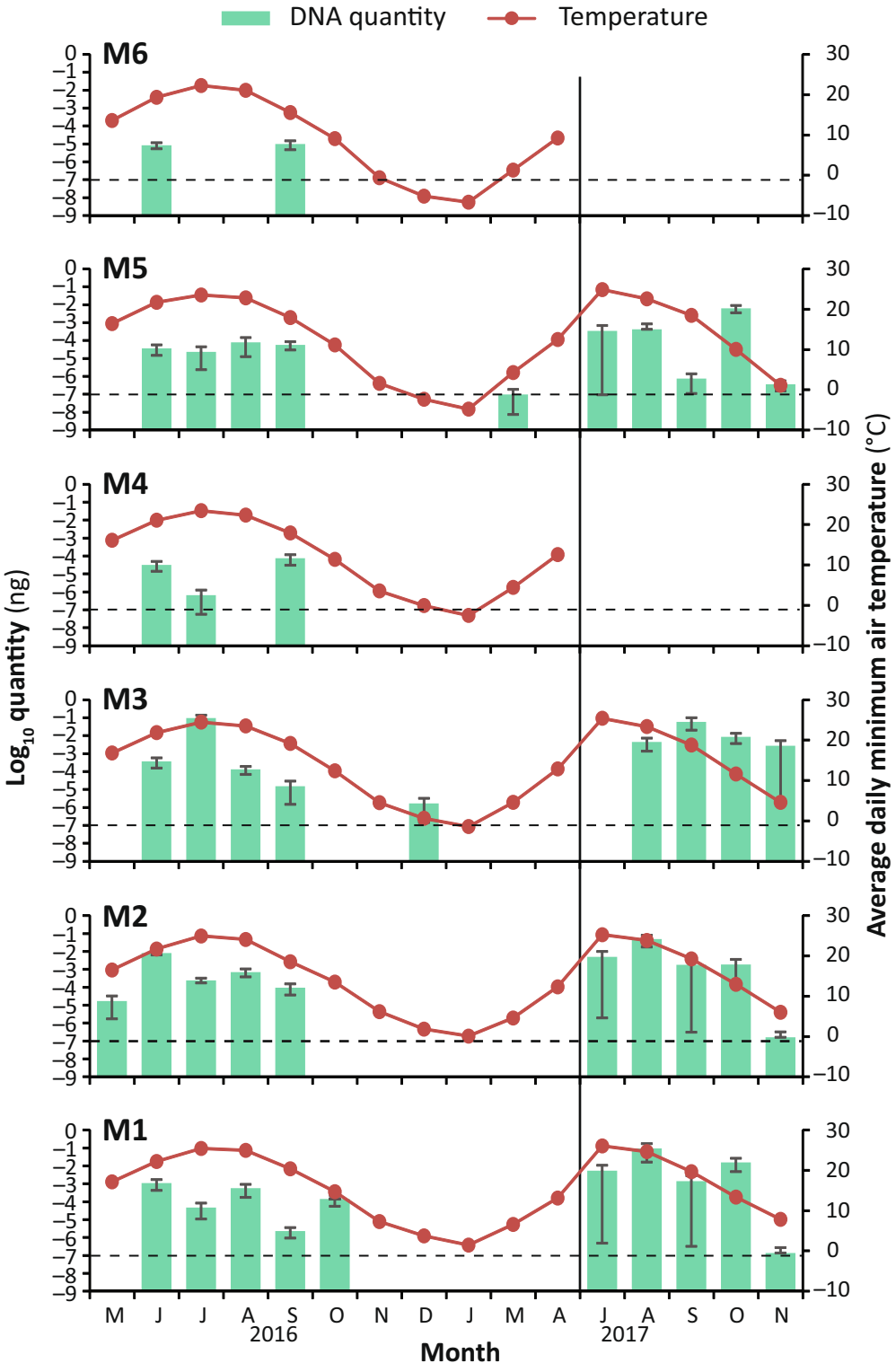

FIGURE 2 Quantity of golden mussel DNA (mean ± standard error) in 500 mL water sample in the main canal of the SNWDP and the average daily minimum low air temperature of the neighboring city at each site. Horizontal dashed lines indicate the limit of detection (1.2×10^{-7} ng), while the vertical solid line separates the first (left panel: sites M1–M6) and second (right panel: sites M1–M3 and M5) phases of sampling. Panels of sampling sites (M1–M6) are ordered from south to north in the SNWDP.

affected by factors such as species reproductive cycles, abundance, and environmental conditions (Buxton et al., 2017; Pilliod et al., 2013; Strickler et al., 2015). Zhan et al. (2015) suggested that the opening of SNWDP could facilitate the invasion of golden mussels from southern China to the north. Here, we conducted eDNA sampling shortly

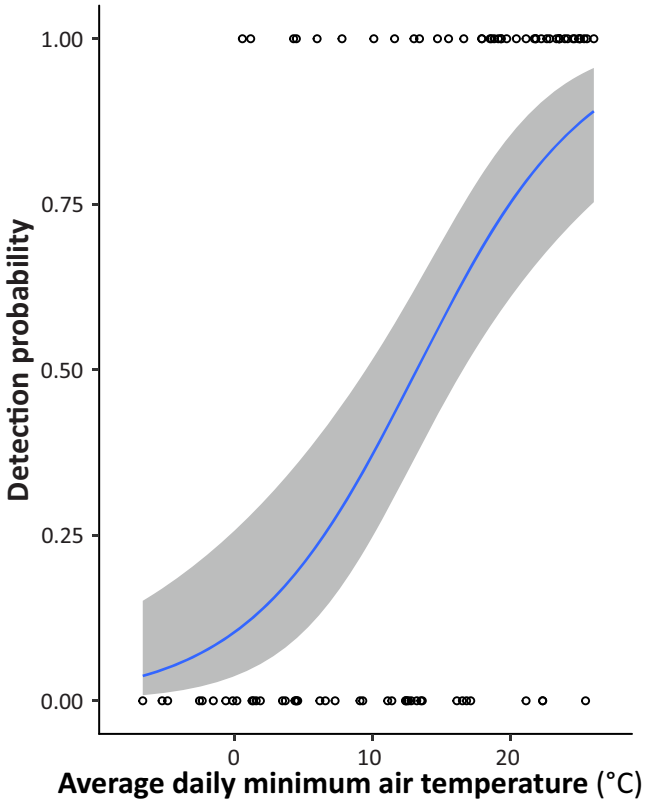

FIGURE 3 Relationship between detection probability of golden mussel DNA from the main canal and the average daily minimum air temperature, (°C) showing the fitted curve of binomial logistic regression and actual detection results (circles). The shaded band indicates ±1 standard error of the prediction.

after (~1.5 years) the SNWDP was opened in December 2014 and detected golden mussel eDNA at sites (M5 and M6) far removed (up to 1100 km) from the putative source in Danjiangkou Reservoir. This marked spread may result from eDNA excreted by adult mussels in Danjiangkou Reservoir or by their adult offspring that had established downstream, by sperm and eggs released by these individuals, or by planktonic larvae produced by these individuals or subsequent generations and subsequently advected downstream. In a low-flow environment, benthic adult mussels may deliver DNA to a limited extent of the surroundings via siphonal jets (Nishizaki & Ackerman, 2017) or weak bottom resuspension (Xia, Depew, et al., 2022), complicating their eDNA detection from surface water (Xia, Zhan, et al., 2018). Also, DNA excreted by sessile animals would almost certainly be highly degraded within a short downstream transport distance. For example, the eDNA of caged fishes became undetectable in as little as <2 km downstream in rivers (Balasingham et al., 2017; Jane et al., 2014). Similarly, sperm and eggs have a relatively short lifespan (e.g., 3.5 h at 26°C; Boltovskoy, 2015), and even with degradation would be highly unlikely to provide eDNA 1100 km downstream. Production of planktonic veligers, whose development and metamorphosis are temperature dependent, could remain in the plankton for up to 20 days before settlement (Boltovskoy, 2015), during which time they could be advected between 346 km (at 0.2 m/s) and 3460 km (at 2 m/s) downstream. The SNWDP open waterway is well-fenced to protect water quality and is not used for recreation or commercial or recreational boating, thus these important vectors of spread elsewhere (Boltovskoy, 2015) are very unlikely to apply here. The concentrations of golden mussel eDNA varied by six orders of magnitude (i.e., 9.8×10^{-8} and 9.4×10^{-2} ng per reaction) among sites and months. Given the consistent sampling schemes, such a large variation is unlikely to be a result of natural fluctuations in larvae-free DNA in the water sample. The most plausible mechanism for the tremendous dispersal of golden mussel DNA is the downstream transport of veligers.

Impact of temperature on eDNA detection in the main canal

Most eDNA detections in the water were observed in the warm months (Figure 2), and the probability of detection increased with air temperature (Figure 3). Such temporally dependent detection of species from eDNA samples has been reported for other rare species including amphibians (Buxton et al., 2017; Spear et al., 2015), fishes (de Souza et al., 2016; Gingera et al., 2016; Turner et al., 2014; Xu et al., 2018) and reptiles (de Souza et al., 2016), which result from spawning (Buxton et al., 2017; Gingera et al., 2016; Xu et al., 2018) and enhanced activities (Souza et al., 2016).

Temperature plays a crucial role in inducing bivalves to spawn and develop (Cataldo et al., 2005; Dei Tos et al., 2016; Philippart et al., 2003). For example, 16–17°C (water temperature) is the approximate lower thermal limit triggering the spawning of introduced golden mussels in Japan (Lake Ohshio and Lake Takenuma, ~36° N, 139° E; Nakano et al., 2010) and Argentina (Paraná de las Palmas river, ~34° S, 59° W; Cataldo & Boltovskoy, 2000). Studies on invasive populations in south China (Hong Kong and Shenzhen: ~22° N, 114° E) demonstrated that golden mussels could spawn several times per year, with multiple peaks in the warm months but limited reproduction in cool seasons (Xu et al., 2013). Populations in our study were located much farther north (M1–M6: ~32–39° N, 112–115° E). This may limit the spawning of the subtropical golden mussels to the warm months only. Records indicated that the water temperature in Danjiangkou Reservoir remained more than ~16–17°C for

TABLE 1 Summary of linear (or multiple linear) regressions of environmental DNA (eDNA) quantity (in Log_{10} scale) as a function of distance from the source (in kilometers) in the main canal, and distance from source, 0–1 μm suspended particles (number of particles per milliliter), and water temperature in the sub-canal, respectively.

Model	Estimate	Standard error	*t*-Value	*p*-value	Deviance explained (%)
Main_Jun					91.1
Intercept	−3.747	0.176	−21.28	<0.001	
Distance	−1.02	0.278	−3.65	**0.036**	
Air.min	0.073	0.278	0.26	0.810	
Main_Sep					50.8
Intercept	−4.643	0.202	−22.98	<0.001	
Water temperature	−0.449	0.221	−2.03	0.112	
Subcanal					
Intercept	−5.24	0.139	−37.8	<0.001	
Water temperature	−0.489	0.145	−3.4	**0.007**	
Subcanal					93.0
Intercept	−5.427	0.073	−73.93	<0.001	
Distance	−0.387	0.079	−4.91	**0.001**	
Particles (0–1 μm)	−0.008	0.081	−0.1	0.925	
Distance × particles	0.368	0.084	4.39	**0.002**	

Note: Main_Jun and Main_Sep represent June and September of the main canal in the first sampling phase, respectively. Air.min: Average daily minimum air temperature. Values in boldface indicate significance at the $p = 0.05$ level.

~6.5 and ~4.5 months in surface and deeper layers, respectively (Duan et al., 2018), consistent with our observations in the main canal (Appendix S1: Figure S5) and also the period when golden mussel eDNA was detected at sites M1 and M2 in this study (May–October; Figure 2). Across the entire SNWDP, an average daily minimum air temperature of 13.2°C signaled a 50% occurrence probability of golden mussel eDNA (Figure 3). We found that, in the first sampling phase, the air temperature profile preceded water temperature by ~1 month (all cross-correlation $r > 0.9$ for six sites at time $t - 1$; Appendix S1: Figures S5 and S6), thereby supporting our choice of air temperature. In addition, the average daily air temperature might have been a better indicator because it represents the integrated thermal condition of the entire month, and such integrated information is often suggested to be a better indicator than the discrete, one-time records in field studies (e.g., Xia, Depew, et al., 2022).

Increased water temperature may increase filtering activity in bivalves (e.g., Xia, MacIsaac, et al., 2021). For golden mussels, enhanced filtering of suspended particles occurs in warm waters (Sylvester et al., 2005). Enhanced filtering at elevated temperatures will allow golden mussels to shed more DNA via the production of feces and pseudofeces into the environment (Freitas et al., 2022). However, this may add very limited DNA to the environment relative to spawning and larval production. Also, high temperatures will increase the degradation of feces-associated eDNA (Strickler et al., 2015) but enhance the growth and activities of free-living individuals. Thus, we propose that the spawning and larval production that occurs at elevated temperatures substantially improves detection probability from water samples. This effect might be augmented by enhanced filtering activities of golden mussels at the main canal sites in warmer months.

Factors influencing eDNA concentration in canals

Golden mussel eDNA in the sub-canal declined significantly with distance from the source (Table 1; Figure 4), while the same pattern was identified in the main canal in June of the first sampling phase (Table 1, Figure 2). These findings are consistent with existing studies in natural rivers (Balasingham et al., 2017; Jane et al., 2014), and with a previous study in the same system (Xia, Johansson, et al., 2018). In flowing rivers and artificial waterways, many factors affect eDNA concentration in water. eDNA originating from a point source such as caged animals (Balasingham et al., 2017; Jane et al., 2014) exhibited attenuation with transport distance owing to a combination of degradation, dilution, and sedimentation, the latter of which has been suggested as a

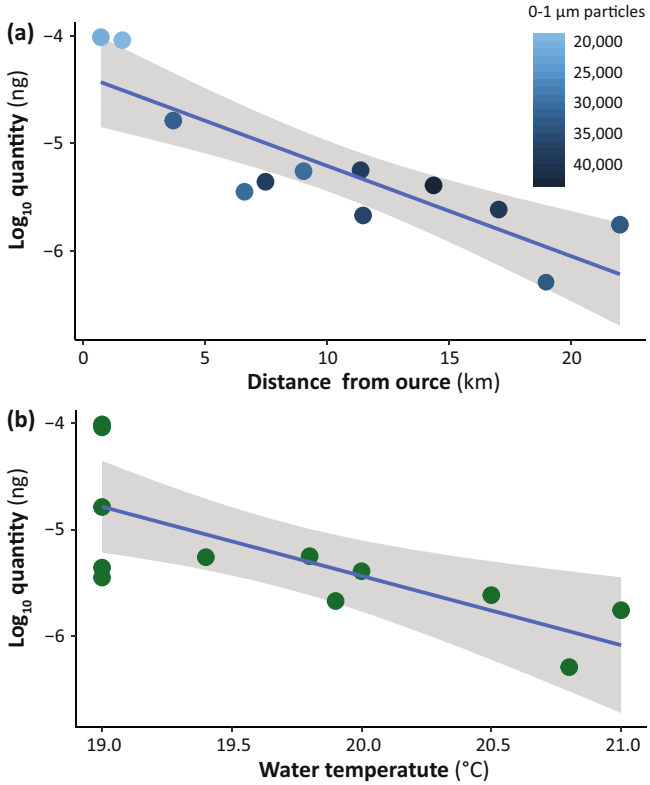

FIGURE 4 Relationship between concentration of golden mussel DNA and (a) distance from the source, concentration of suspended particles (0–1 μm); and (b) water temperature. Unit of 0–1 μm suspended particles is the number of particles per milliliter. The solid line indicates fitted prediction and the shaded band indicates ±1 standard error.

dominant carrier of eDNA in natural environments (Balasingham et al., 2017; Jane et al., 2014; Seymour et al., 2018; Turner et al., 2015). The mortality of planktonic golden mussel larvae is often very high (~90%; Boltovskoy et al., 2022), and live veligers may experience a sharp numerical decline (Horvath & Lamberti, 1999). Despite the low abundance, the presence of adult golden mussels might have also contributed to the loss of live veligers due to filter-feeding by adult mussels (da Silva Bertão et al., 2021). These losses, combined with settling by metamorphosing individuals, could account for the diminution of eDNA in surface waters.

In the sub-canal, golden mussel eDNA concentration dropped sharply after the first three sites (Figure 4a), suggesting rapid sedimentation or degradation of particles carrying enriched eDNA. However, the concentration of fine particles demonstrated a marginally significant increase with distance ($p = 0.062$) and water velocity ($p = 0.068$), suggesting resuspension of sediments in the subcanal. Turner et al. (2014) found that common carp eDNA in a lake and a pond were most abundant in particles between 1–10 μm, and the concentrations of golden mussel eDNA in our study were adversely associated with fine suspended particles <15 μm (estimates <0 for all size categories, $p = 0.0504 \sim 0.0308$; Appendix S1: Table S4). However, flow distance had a much stronger effect on golden mussel eDNA than suspended particles in the sub-canal, revealed by the much greater absolute standardized coefficient for the former (i.e., −0.387 vs. −0.008; Table 1). This suggests that the slightly increased suspended particles did not compensate for eDNA loss during downstream transport. In addition, water temperature significantly increased with distance in the sub-canal (Appendix S1: Table S5; $p < 0.001$), suggesting a higher degradation rate downstream compared with upstream sites (Strickler et al., 2015).

In a pilot test, the estimated coefficient of eDNA concentration (i.e., slope in linear regression) against transport distance in the main canal (June) was substantially lower than in the sub-canal (i.e., −0.002 vs. −0.3), suggesting a lower attenuation rate of target eDNA in the former system. This may have occurred because of the higher water velocity in the main canal (1–2 m/s), which reduced both the retention time and sedimentation rate of suspended particles. Also, established animals in the main canal may have a lagged spawning time relative to the source population in the south (Dei Tos et al., 2016; Xu et al., 2013), allowing them to add newly released larvae to the water and compensate for eDNA losses. Further, eDNA is likely to be preserved better from south to north in the main canal than in the sub-canal because of their opposite water temperature distribution. These conditions could collectively reduce the attenuation rate of target eDNA in the main canal.

Implications for managing golden mussels in the SNWDP

Water eDNA can provide much earlier signals for the presence of aquatic invasive species than traditional methods (Jerde, 2021), triggering earlier management intervention. In the main canal, initial detection and subsequently elevated concentration of golden mussel eDNA in warm months should be considered signals of likely invasions. The central route of the SNWDP had been in operation for ~1.5 years before our study was initiated. Golden mussels had probably established in, at least part of, the canal during the first spawning season following its inception. The canal acts as a "corridor-like" extension of the Danjiangkou Reservoir, and any environment connected to the main canal via water flow would be vulnerable to invasion. In such a system, suitable downstream habitats will be successively invaded in turn by propagules produced in upstream bridgehead

populations. In November 2018 and March 2019, ~2.5 years following our study, Liu et al. (2019) found that the golden mussels inhabiting the inner surface of the underground duct of the main canal extended to Tianjin (a location close to M6 in Figure 1; 10–156 mussels per square meter, up to 30 mm in size). This provides validation for our hypothesis (Zhan et al., 2015). Xia, Barker, et al. (2021) found that golden mussels could survive the cold winter in areas covering the entire SNWDP. This suggests that established populations can produce propagules during the warm months that then may disperse to new locations. Management of invasive species in systems like SNWDP must focus on the introduction of propagules. This objective will be challenging in upstream locations that receive a massive annual infusion of larval propagules. Downstream sites would at first seem better protected given propagule attenuation with distance from the source. However, if the same dynamics of reproduction and spread play out across multiple years, we expect that the species will utilize prior bridgehead populations to facilitate gradual spread during subsequent years. Over the long term, we expect much or all of the hydraulically-connected SNWDP to be invaded by golden mussels. Since implementation, the central route of SNWDP has largely improved water resource conditions in recipient areas (http://nsbd.mwr.gov.cn/). We call for management measures integrating multiple sectors to control the propagule pressure of golden mussels (especially in warm months) to new areas, and removal of established populations (where possible) to reduce potential adverse effects, thus guarding the sustainable functioning of this mega project.

CONCLUSIONS

We employed a qPCR assay to detect golden mussel eDNA from water in the main canal of the SNWDP (central route), with detection success varying both spatially and temporally. Increased temperature induces the spawning of golden mussels and the production of free-living larvae, elevating detection success and eDNA concentration. Target eDNA concentration decreases with transport distance downstream possibly because of larvae and suspended particles settlement and potential eDNA degradation. Invasion of golden mussels throughout this water diversion project seems inevitable, while eDNA could provide an early detection tool to alert against their invasions. Our findings validate the prediction of Zhan et al. (2015) regarding the spread of the species and highlight the importance of warm months as a window of opportunity for effective propagule pressure control. We call for immediate management measures to reduce the spread of golden mussels through the world's largest water diversion project.

AUTHOR CONTRIBUTIONS

Aibin Zhan and Hugh J. MacIsaac conceived this study. Zhiqiang Xia, Junnong Gu, Ying Wen, and Xinkai Cao collected the water samples. Zhiqiang Xia collected and analyzed the data and wrote the first draft. Hugh J. MacIsaac and Aibin Zhan revised the draft; Yangchun Gao and Shiguo Li assisted in laboratory analysis. All authors commented on and approved the submission.

ACKNOWLEDGMENTS

We are grateful to two anonymous reviewers for their constructive comments on the previous version of this work. Funding was provided by the China Scholarship Council to Zhiqiang Xia, the Natural Science Foundation of China (31622011) to Aibin Zhan, NSERC Discovery grants and Canada Research Chairs to G. Douglas Haffner and Hugh J. MacIsaac, and a Yunnan University research chair to Hugh J. MacIsaac.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data for the daily air temperature at sites in the main canal were downloaded from "天气后报" (http://www.tianqihoubao.com/) by searching for cities using the details provided in Appendix S1: Table S1 of this paper. The calculated daily average air temperature data (Xia, Gu, et al., 2022) are available in Science Data Bank at https://doi.org/10.57760/sciencedb.06981. Sequences of golden mussels (Xia et al., 2023) are available in figshare at https://doi.org/10.6084/m9.figshare.21971672.v2.

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REFERENCES

Balasingham, K. D., R. P. Walter, and D. D. Heath. 2017. "Residual eDNA Detection Sensitivity Assessed by Quantitative Real-Time PCR in a River Ecosystem." *Molecular Ecology Resources* 17: 523–32.

Barbosa, N. P. U., F. A. Silva, M. D. de Oliveira, M. A. dos Santos Neto, M. D. de Carvalho, and A. V. Cardoso. 2016. "*Limnoperna fortunei* (Dunker, 1857) (Mollusca, Bivalve, Mytilidae): First Record in the São Francisco River Basin, Brazil." *Check List* 12: 1846.

Bayer, S. R., P. D. Countway, and R. A. Wahle. 2019. "Developing an eDNA Toolkit to Quantify Broadcast Spawning Events of the Sea Scallop *Placopecten magellanicus*: Moving beyond Fertilization Assays." *Marine Ecology Progress Series* 621: 127–41.

Boltovskoy, D. 2015. *Limnoperna fortunei, the Ecology, Distribution and Control of a Swiftly Spreading Invasive Fouling Mussel* 1–476. Switzerland: Springer.

Boltovskoy, D., and N. Correa. 2015. "Ecosystem Impacts of the Invasive Bivalve *Limnoperna fortunei* (Golden Mussel) in South America." *Hydrobiologia* 746: 81–95.

Boltovskoy, D., E. Paolucci, H. J. MacIsaac, A. Zhan, Z. Xia, and N. Correa. 2022. "What We Know and Don't Know about the Invasive Golden Mussel Limnoperna Fortunei." *Hydrobiologia*. https://doi.org/10.1007/s10750-022-04988-5.

Buxton, A. S., J. J. Groombridge, N. B. Zakaria, and R. A. Griffiths. 2017. "Seasonal Variation in Environmental DNA in Relation to Population Size and Environmental Factors." *Scientific Reports* 7: 46294.

Cataldo, D., D. Boltovskoy, J. L. Hermosa, and C. Canzi. 2005. "Temperature-Dependent Rates of Larval Development in *Limnoperna fortunei* (Bivalvia: Mytilidae)." *Journal of Molluscan Studies* 71(1): 41–6.

Cataldo, D. H., and D. Boltovskoy. 2000. "Yearly Reproductive Activity of *Limnoperna fortunei* (Bivalvia) as Inferred from the Occurrence of its Larvae in the Plankton of the Lower Paraná River and the Río de la Plata Estuary (Argentina)." *Aquatic Ecology* 34: 307–17.

da Silva Bertão, A. P., R. V. V. Leite, A. Horodesky, M. R. Pie, T. L. Zanin, O. S. M. Netto, and A. Ostrensky. 2021. "Ecological Interactions between Invasive and Native Fouling Species in the Reservoir of a Hydroelectric Plant." *Hydrobiologia* 848: 5169–85.

de Paula, R. S., M. d. P. Reis, R. B. de Oliveira Júnior, G. R. Andrade, M. D. de Carvalho, A. V. Cardoso, and E. C. Horge. 2020. "Genetic and Functional Repertoires of *Limnoperna fortunei* (Dunker, 1857) (Mollusca, Mytilidae): A Review on the Use of Molecular Techniques for the Detection and Control of the Golden Mussel." *Hydrobiologia* 847: 2193–202.

de Paula, R. S., M. P. Reis, G. R. Andrade, C. C. e. Souza, A. V. Cardoso, and E. C. Jorge. 2021. "A Case for the Continued Study of the Golden Mussel Invasion of Brazil: Efficient Detection and Containment of *Limnoperna fortunei* (Dunker, 1857) Dispersion Involves Multiple Approaches and Different Actors." *Oceanography Fish Open Access Journal* 13(4): 555868.

de Souza, L. S., J. C. Godwin, M. A. Renshaw, and E. Larson. 2016. "Environmental DNA (eDNA) Detection Probability is Influenced by Seasonal Activity of Organisms." *PLoS One* 11: e0165273.

Dei Tos, C., I. Quagio-Grassiotto, and T. Sarah Mazzoni. 2016. "Cellular Development of the Germinal Epithelium during the Gametogenic Cycle of the Golden Mussel *Limnoperna fortunei* (Bivalvia: Mytilidae)." *Revista de Biologia Tropical* 64(2): 521–36.

Duan, Y., T. Qin, J. Wang, P. Mu, X. Lei, and H. Zheng. 2018. "Research on Three-Dimensional Hydrodynamic Simulation of Danjiangkou Reservoir." *Yellow River* 40: 119–22 (in Chinese with English abstract).

Freitas, E. T. F., A. M. S. Moreira, R. S. de Paula, G. R. Andrade, M. D. de Carvalho, P. S. Assis, E. C. Jorge, and A. V. Cardoso. 2022. "Ultrastructure of the Gill Ciliary Epithelium of *Limnoperna fortunei* (Dunker 1857), the Invasive Golden Mussel." *BMC Zoology* 7: 6.

Fukumoto, S., A. Ushimaru, and T. Minamoto. 2015. "A Basin-Scale Application of Environmental DNA Assessment for Rare Endemic Species and Closely Related Exotic Species in Rivers: A Case Study of Giant Salamanders in Japan." *Journal of Applied Ecology* 52: 358–65.

Gingera, T. D., T. B. Steeves, D. A. Boguski, S. Whyard, W. Li, and M. F. Docker. 2016. "Detection and Identification of Lampreys in Great Lakes Streams Using Environmental DNA." *Journal of Great Lakes Research* 42: 649–59.

Harvey, C. T., S. A. Qureshi, and H. J. MacIsaac. 2009. "Detection of a Colonizing, Aquatic, Non-indigenous Species." *Diversity and Distributions* 15: 429–37.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. de Waard. 2003. "Biological Identifications through DNA Barcodes." *Proceedings of the Royal Society B: Biological Sciences* 270: 313–21.

Holle, B. V., and D. Simberloff. 2005. "Ecological Resistance to Biological Invasion Overwhelmed by Propagule Pressure." *Ecology* 86: 3212–8.

Horvath, T. G., and G. A. Lamberti. 1999. "Mortality of Zebra Mussel, *Dreissena polymorpha*, Veligers during Downstream Transport." *Freshwater Biology* 42: 69–76.

Jane, S. F., T. M. Wilcox, K. S. McKelvey, M. K. Young, M. K. Schwartz, W. H. Lowe, B. H. Letcher, and A. R. Whiteley. 2014. "Distance, Flow, and PCR Inhibition: eDNA Dynamics in Two Headwater Streams." *Molecular Ecology Resources* 15: 216–27.

Jerde, C. L. 2021. "Can We Manage Fisheries with the Inherent Uncertainty from eDNA?" *Journal of Fish Biology* 98: 341–53.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. "An Ounce of Prevention or a Pound of Cure: Bioeconomic Risk Analysis of Invasive Species." *Proceedings of the Royal Society B: Biological Sciences* 269: 2407–13.

Liu, X. Y., X. C. Xu, J. P. Liu, M. D. Huang, J. X. Zhu, J. F. Feng, and L. Zhu. 2019. "Risk Assessment of the Golden Mussel (*Limnoperna fortunei*) in the Tianjin Canal of the South-to-North Water Diversion Project." *Proceedings of the 2019 Annual Academic Conference of the Chinese Hydraulic Engineering Society* 4: 197–203.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. "The Propagule Pressure in Explaining Species Invasions." *Trends in Ecology and Evolution* 20: 223–8.

McKee, A. M., S. F. Spear, and T. W. Pierson. 2015. "The Effect of Dilution and the Use of a Post Extraction Nucleic Acid Purification Column on the Accuracy, Precision, and Inhibition of Environmental DNA Samples." *Biological Conservation* 183: 70–6.

Nakano, D., T. Baba, N. Endo, S. Nagayama, A. Fujinaga, A. Uchida, A. Shiragane, M. Urabe, and T. Kobayashi. 2015. "Invasion, Dispersion, Population Persistence and Ecological Impacts of a Freshwater Mussel (*Limnoperna fortunei*) in the Honshu Island of Japan." *Biological Invasions* 17: 743–59.

Nakano, D., T. Kobayashi, and I. Sakaguchi. 2010. "Differences in Larval Dynamics of Golden Mussel *Limnoperna fortunei* between Dam Reservoirs With and Without an Aeration System." *Landscape and Ecological Engineering* 6: 53–60.

Nishizaki, M., and J. D. Ackerman. 2017. "Mussels Blow Rings: Jet Behavior Affects Local Mixing." *Limnology and Oceanography* 62: 125–36.

Philippart, C. J. M., H. M. van Aken, J. J. Beukema, O. G. Bos, G. C. Cadée, and R. Dekker. 2003. "Climate-Related Changes in

Recruitment of the Bivalve *Macoma balthica*." *Limnology and Oceanography* 48: 2171–85.

Pilliod, D., C. S. Goldberg, R. S. Arkle, and L. P. Waits. 2013. "Estimating Occupancy and Abundance of Stream Amphibians Using Environmental DNA from Filtered Water Samples." *Canadian Journal of Fisheries and Aquatic Sciences* 70: 1123–30.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Renshaw, M. A., B. P. Olds, C. L. Jerde, A. M. McVeigh, and D. M. Lodge. 2014. "The Room Temperature Preservation of Filtered Environmental DNA Samples and Assimilation into a Phenol-Chloroform-Isoamyl Alcohol DNA Extraction." *Molecular Ecology Resources* 15: 168–76.

Rice, C. J., E. R. Larson, and C. A. Taylor. 2018. "Environmental DNA Detects a Rare Large River Crayfish but with Litter Relation to Local Abundance." *Freshwater Biology* 63: 443–55.

Seymour, M., I. Durance, B. J. Cosby, E. Ransom-Jones, K. Deiner, S. J. Ormerod, J. K. Colbourne, et al. 2018. "Acidity Promotes Degradation of Multi-Species Environmental DNA in Lotic Mesocosms." *Communications Biology* 1: 4.

Spear, S. F., J. D. Groves, L. A. Williams, and L. P. Waits. 2015. "Using Environmental DNA Methods to Improve Detectability in a Hellbender (*Cryptobranchus alleganiensis*) Monitoring Program." *Biological Conservation* 183: 38–45.

Strickler, K. M., A. K. Fremier, and C. S. Goldberg. 2015. "Quantifying Effects of UV-B, Temperature, and pH on eDNA Degradation in Aquatic Microcosms." *Biological Conservation* 183: 85–92.

Sylvester, F., J. Dorado, D. Boltovskoy, Á. Juárez, and D. Cataldo. 2005. "Filtration Rates of the Invasive Pest Bivalve *Limnoperna fortunei* as a Function of Size and Temperature." *Hydrobiologia* 534: 71–80.

Thomsen, P. F., J. Kielgast, L. L. Iversen, C. Wiuf, M. Rasmussen, M. T. P. Gilbert, L. Orlando, and E. Willerslev. 2012. "Monitoring Endangered Freshwater Biodiversity Using Environmental DNA." *Molecular Ecology* 21: 2565–73.

Tillotson, M. D., R. P. Kelly, J. J. Duda, M. Hoy, J. Kralj, and T. P. Quinn. 2018. "Concentrations of Environmental DNA (eDNA) Reflect Spawning Salmon Abundance at Fine Spatial and Temporal Scales." *Biological Conservation* 220: 1–11.

Turner, C. R., M. A. Barnes, C. C. Y. Xu, S. E. Jones, C. L. Jerde, and D. M. Lodge. 2014. "Particle Size Distribution and Optimal Capture of Aqueous Macrobial eDNA." *Methods in Ecology and Evolution* 5: 676–84.

Turner, C. R., K. L. Uy, and R. C. Everhart. 2015. "Fish Environmental DNA is More Concentrated in Aquatic Sediments than Surface Water." *Biological Conservation* 183: 93–102.

Wilcox, T. M., K. S. McKelvey, M. K. Young, S. F. Jane, W. H. Lowe, A. R. Whiteley, and M. K. Schwartz. 2013. "Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity." *PLoS One* 8: e59520.

Xia, Z., J. R. Barker, A. Zhan, G. D. Haffner, and H. J. MacIsaac. 2021. "Golden Mussel (*Limnoperna fortunei*) Survival during Winter at the Northern Invasion Front Implies a Potential High-Latitude Distribution." *Diversity and Distributions* 27: 1422–34.

Xia, Z., D. C. Depew, R. Valipour, H. J. MacIsaac, and R. P. Weidman. 2022. "Impacts of Invasive Mussels on a Large Lake: Direct Evidence from in Situ Control-Volume Experiments." *Science of the Total Environment* 849: 157924.

Xia, Z., Y. Gao, and A. Zhan. 2023. "Aligned Mitochondrial COI Sequences of Golden Mussel *Limnoperna fortunei* from Water Samples with a Known Sequence." Figshare. Dataset. https://doi.org/10.6084/m9.figshare.21971672.v2.

Xia, Z., J. Gu, Y. Wen, X. Cao, Y. Gao, S. Li, G. D. Haffner, H. J. MacIsaac, and A. Zhan. 2022. "Golden Mussel eDNA Occurrence vs. Temperature in a Large Water Diversion Canal." Science Data Bank. https://doi.org/10.57760/sciencedb.06981.

Xia, Z., M. L. Johansson, Y. Gao, L. Zhang, G. D. Haffner, H. J. MacIsaac, and A. Zhan. 2018. "Conventional Versus Real-Time Quantitative PCR for Rare Species Detection." *Ecology and Evolution* 8: 11799–807.

Xia, Z., H. J. MacIsaac, R. E. Hecky, D. C. Depew, G. D. Haffner, and R. P. Weidman. 2021. "Multiple Factors Regulate Filtration by Invasive Mussels: Implications for Whole-Lake Ecosystems." *Science of the Total Environment* 765: 144435.

Xia, Z., A. Zhan, Y. Gao, L. Zhang, G. D. Haffner, and H. J. MacIsaac. 2018. "Early Detection of a Highly Invasive Bivalve Based on Environmental DNA (eDNA)." *Biological Invasions* 20: 437–47.

Xu, M., G. Darrigran, Z. Wang, N. Zhao, C. C. Lin, and B. Pan. 2014. "Experimental Study on Control of *Limnoperna fortunei* Biofouling in Water Transfer Tunnels." *Journal of Hydro-Environment Research* 9: 248–58.

Xu, M., Z. Wang, C. C. Lin, B. Pan, and N. Zhao. 2013. "Experimental Study of Invasion and Biofouling of Freshwater Mussel *Limnoperna fortunei*." *International Journal of Geosciences* 4: 1–7.

Xu, N., B. Zhu, F. Shi, K. Shao, Y. Que, W. Li, W. Jiao, H. Tian, and J. Chang. 2018. "Monitoring Seasonal Distribution of an Endangered Anadromous Sturgeon in a Large River Using Environmental DNA." *The Science of Nature* 105: 62.

Zhan, A., L. Zhang, Z. Xia, P. Ni, W. Xiong, Y. Chen, G. D. Haffner, and H. J. MacIsaac. 2015. "Water Diversions Facilitate Spread of Non-native Species." *Biological Invasions* 17: 3073–80.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Xia, Zhiqiang, Junnong Gu, Ying Wen, Xinkai Cao, Yangchun Gao, Shiguo Li, G. Douglas Haffner, Hugh J. MacIsaac, and Aibin Zhan. 2023. "eDNA-Based Detection Reveals Invasion Risks of a Biofouling Bivalve in the World's Largest Water Diversion Project." *Ecological Applications* e2826. https://doi.org/10.1002/eap.2826