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Environmental DNA (eDNA) Shedding and Decay Rates to Model Freshwater Mussel eDNA Transport in a River

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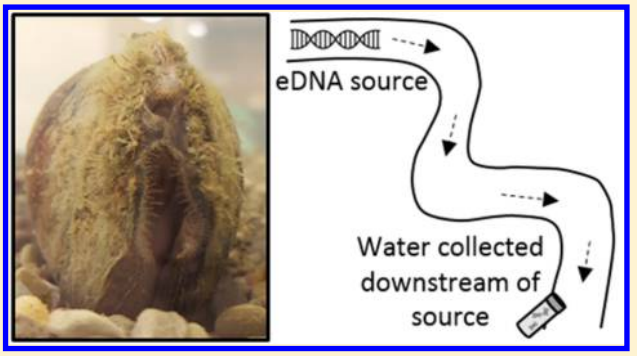
Supporting Information

ABSTRACT: Freshwater mussels are vital components of stream ecosystems, yet remain threatened. Thus, timely and accurate species counts are critical for proper conservation and management. Mussels live in stream sediments and can be challenging to survey given constraints related to water depth, flow, and time of year. The use of environmental DNA (eDNA) to monitor mussel distributions and diversity is a promising tool. Before it can be used as a monitoring tool, however, we need to know how much eDNA mussels shed into their environment and how long the eDNA persists. Here, we present a novel application of eDNA to estimate both the presence/absence and abundance of a freshwater mussel species, *Lampsilis siliquoidea*. The eDNA shedding and decay rates reported within are the first for freshwater mussels. We determined that eDNA shedding was statistically similar across mussel densities, but that first-order decay constants varied between experimental treatments. Finally, we effectively modeled downstream transport of eDNA and present a model that can be used as a complementary tool to estimate mussel density. Our results suggest that eDNA has the potential to be a complementary tool to survey mussels and enhance current efforts to monitor and protect freshwater mussel biodiversity.



INTRODUCTION

Freshwater mussels are a diverse group of long-lived, benthic invertebrates that perform vital functions in freshwater ecosystems.[1,2] As suspension feeders, mussels stimulate a bottom-up trophic cascade[3] by increasing primary[3,4] and secondary production.[5,6] They also help make more nutrients available by shortening nutrient spirals and reducing nutrient loss downstream.[4,7] Unfortunately, mussels remain among the world's most imperiled species. North America contains the richest biodiversity in the world, with nearly 300 freshwater mussel species, but >75% of these species are extinct, endangered, or threatened.[8,9] Freshwater mussel biodiversity remains threatened by anthropogenic stressors including historical over harvesting, habitat fragmentation and degradation, water pollution, and climate change.[8]

Key to understanding the important role mussels play in ecosystem processes and the impact of mussel biodiversity loss are accurate accounts of species diversity and distributions. Monitoring mussel populations, however, can be challenging. Typically, monitoring is done by employing quantitative or qualitative sampling. Quantitative methods include collecting mussels in randomly placed quadrats that are excavated to a depth of ∼15 cm, often using snorkel or SCUBA. Sampling with quadrats provides a means for quantifying population density, size demography, and recruitment.[10] The disadvantage to sampling with quadrats is that it is time-consuming,[11] can underestimate both total number of species and rare species,[12] greatly disturbs sediment habitat,[11] and is expensive.[13] Qualitative methods include timed visual searches using snorkel, SCUBA, or view buckets, brailing, shoreline collections of shells, or midden-pile collections[14] and are more likely to detect rare or endangered species than quantitative surveys.[12,13,15] However, qualitative methods can be size and sculpture selective by underrepresenting small species or juveniles and those with smooth shells.[12,16] Both methods require skilled taxonomic professionals for species identification based on external morphology and are influenced by seasonal fluctuations in water level and temperature. Because of the limitations of current survey methods, alternative methods are needed to complement monitoring efforts.

The detection and quantification of environmental DNA (hereafter eDNA) offers a novel species detection technique that could improve the ability to detect and monitor freshwater mussel populations by removing bias for size, sculpture, or incidence level.[17] The use of eDNA to detect aquatic species has largely focused on fish[18−26] and amphibians.[20,27−29] Other studies examine the use of eDNA to detect invertebrates[20,30,31]

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or parasites,[32,33] while few have considered freshwater mussels.[31,34,35] eDNA has widely been demonstrated to successfully detect the presence/absence of a species.[18,21,36,37] While presence/absence detection informs species richness, quantitative measures are needed to predict biomass or abundance. Recent studies have begun correlating eDNA concentration to organism abundance through mesocosm experiments and water sampling alongside traditional monitoring efforts.[20−25,36,38] Mesocosm studies are preferred to examine the specific mechanisms driving the production and persistence of DNA, but realistic estimates in freshwater[31,38−41] and marine[19,25] systems provide a stronger biomonitoring tool. To fully investigate the use of eDNA as a biomonitoring tool, both mesocosm laboratory experiments and field sampling are needed.

Mussels shed DNA into the environment in the form of sloughed tissue or cells, gametes, or filter excreta,[42,43] and shell material.[44] The concentration of mussel eDNA in a water parcel is controlled by a number of processes including shedding, decay, advection and dispersion, and resuspension (Figure 1). The factors influencing mussel eDNA shedding

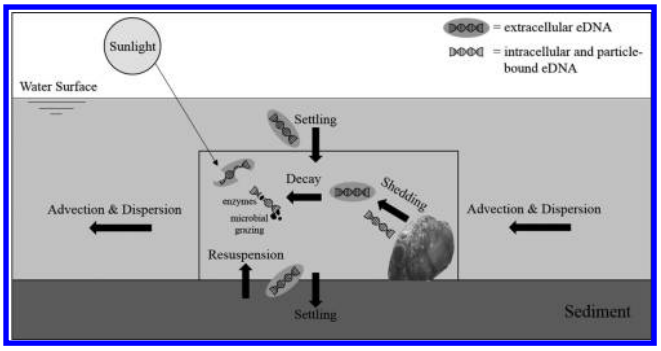

Figure 1. Conceptual model of the source, transport, and fate of eDNA from a freshwater mussel in a stream environment. Processes illustrated here include eDNA shedding, decay (due to sunlight and biological processes such as microbial grazing and enzymatic activity), advection/dispersion, settling (eDNA attached to particles), and resuspension.

rates are not known but likely include species, size, age or life stage, and mussel density. Previous studies show differences in shedding rates due to species,[25] age/life stage,[20,28,45] and number of organisms.[20,21,46] Before eDNA can be used as a tool to estimate and model mussel presence and density, mussel eDNA shedding and decay rates must be quantified and the factors affecting shedding rates need to be better understood.

The goal of the present study was to investigate freshwater mussel eDNA shedding and decay rates to inform the use of eDNA as a biomonitoring tool. We developed a *Lampsilis*-specific SYBR Green qPCR assay for the detection of *Lampsilis siliquoidea*. We then used this assay to quantify mussel eDNA shedding and decay rates in mesocosm experiments. Next, we investigated the effect of mussel density on eDNA shedding and decay rates. Finally, we developed and tested a one-dimensional mass balance model to estimate (1) how far downstream *L. siliquoidea* eDNA can be detected and (2) the density of mussels upstream given a measured eDNA concentration. The results of this study provide a novel biomonitoring tool to enhance our understanding of mussel distributions and inform conservation efforts for this ecologically important group of invertebrates.

MATERIALS AND METHODS

Experimental Design for Laboratory Shedding and Decay Experiments. We performed five freshwater mesocosm experiments to determine the shedding and decay rates of the freshwater mussel species, *Lampsilis siliquoidea*, a mussel common throughout western New York. Mussels were collected from Tonawanda Creek, a local creek known for its mussel biodiversity, in June 2016 and August 2017. Mussels were immediately transported back to the laboratory at the University at Buffalo, scrubbed free of all algae using a mesh cloth, measured for length, height, width, and mass, and housed in freshwater tanks filled with tap water and treated with AmQuel (Kordon, Model-31261) to neutralize chlorine, chloramine, and ammonia in the water. Mussels were fed an algae diet twice per week (Shellfish Diet 1800, Reed Mariculture, Campbell, CA). Mussels were allowed to acclimate for up to 3 weeks in the lab prior to the start of the experiments. For the experiments, an 8 cm gravel bed was added to each mesocosm. Gravel (D_{50} = 0.01 m) was purchased from a local quarry, rinsed with tap water to remove fine sediment, dried, and stored in closed buckets until used. The mesocosms were filled with tap water treated with AmQuel (Kordon, Model-31261). Tap water was used instead of water from Tonawanda Creek to minimize the potential background DNA signal from *L. siliquoidea* in the creek. Mesocosms were continuously aerated with air pumps (ActiveAQUA, Model no. AAPA15L). Water temperature was maintained at room temperature in the laboratory (22 ± 1 °C) for the duration of the experiment. The mesocosms were exposed to natural diurnal light cycles and indirect sunlight through a window in the laboratory.

Experiments were conducted with different densities of mussels to determine the effect of mussel density on eDNA shedding and decay rates. Mussel densities in the three mesocosms were 16, 55, and 110 mussels per m^2, hereafter referred to as low density (2 mussels per tank), moderate density (10 mussels per tank), and high density (20 mussels per tank) treatments. The high density treatment was conducted in August 2016. One replicate of the moderate density treatment was conducted in September 2016 and a second replicate of the moderate density treatment and two replicates of the low density treatment were conducted in August 2017. Mussels remained in the tanks for 72 h and were not fed during the duration of the experiment to control for excess eDNA input resulting from feeding and defecation.

Given eDNA decay is influenced by a number of environmental variables, we performed an additional experiment to measure mussel eDNA decay using water from Tonawanda Creek (hereafter the environmental treatment). Water was collected from Tonawanda Creek in 5-gallon buckets and immediately brought back to the lab. A mesocosm was set up with ∼40 L of Tonawanda Creek water following methods described above. To ensure *L. siliquoidea* eDNA was in the mesocosm, five *L. siliquoidea* mussels were added to the mesocosm for 24 h with no feeding and then removed. The environmental treatment experiment was conducted in September 2016.

Water Sampling Procedure for Shedding and Decay Experiments. Water samples were collected in sterile 120 mL disposable vessels (IDEXX Laboratories Inc., ME) at each time point in duplicate. For the three different mussel density experiments, baseline samples were collected before the

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 1. Genus-Specific Primers Designed to Amplify *Lampsilis siliquoidea* DNA

	primer	gene target	fragment size	final primer concentration (μM)	annealing temperature (°C)	slope	intercept	limit of quantification (copy number)	assay efficiency (%)
forward	383F-5′ TCG AGC CAT AGC TCA AAC CA 3′	NADH Dehydrogenase	147	0.4	60	−3.35	33.1	1	99.0
reverse	529R-5′ GCG AGT GGT AGT GAA AGA GT 3′								

mussels were added. The next samples were collected approximately 12 h after the mussels were added to allow the eDNA concentration in the mesocosms to reach steady state. Steady state was defined as the time period during which eDNA concentration did not change with respect to time. For the environmental treatment, the baseline was collected immediately before the mussels were removed, and the next sample was taken approximately 12 h later. Water was sampled 2−4 times per day for the first 4−6 days (depending on the experiment). After 6 days, water was sampled once per week for up to 4 weeks (Table S1, S2). Each day, 100 mL deionized water was also filtered to test for contamination during the filtration process. New gloves were worn for each tank to prevent contamination. All samples were immediately processed after collection. Two 100 mL water samples were collected at each time point and filtered through 0.4 μm pore size track-etched polycarbonate filters (HTTP04700, EMD Millipore, Germany). Filters were frozen at −20 °C until DNA extraction (approximately 2−8 weeks after the experiment). Filtration funnels were acid-washed (10% hydrochloric acid) and rinsed with DI water between samples to remove DNA contamination.

DNA Extraction. DNA was extracted from mussel tissue and filters using the Qiagen DNeasy Blood and Tissue extraction kit (Qiagen, Valencia, CA) with minor modifications. The volume of Buffer ATL and proteinase K was doubled and samples (mussel tissue and filters) were incubated with Buffer ATL and proteinase K for 12−14 h (filters) and 24 h (tissue) at 56 °C to ensure tissue degradation. Following incubation, 400 μL of Buffer AL and 400 μL of 100% molecular grade ethanol was added for a 1:1:1 volume ratio (Buffer ATL plus proteinase K: Buffer AL: ethanol). Two washes of 500 μL of AW1 and AW2 were performed and DNA was eluted in two steps with warmed Buffer AE for a total of 100 μL DNA extract. One filter was analyzed for each time point, while duplicate filters were analyzed for every other time point. A DNA extraction blank was extracted with each set of samples to check for contamination during the extraction process.

Genus-Specific Assay Design and qPCR Optimization. Genus-specific primers were designed to amplify DNA from *L. siliquoidea*. Primers were designed using PrimerBlast (see SI).[47] Primers targeting multiple mitochondrial genes were tested and the best performing assay targeted the *L. siliquoidea* NADH dehydrogenase gene (ascension no. HM852927). The specificity of primers was assessed in silico using PrimerBlast.[47] Primer sequences showing specificity to *L. siliquoidea* were synthesized by Integrated DNA Technologies (IDT, Coralville, Iowa) and tested for cross-reactivity using genomic DNA extracted from freshwater mussels found in similar habitats as those of *L. siliquoidea*. Genomic DNA from *Amblema plicata* (13.08 ng), *Ligumia recta* (1.47 ng), and *Lampsilis cardium* (3.72 ng) was tested against each primer set. Genomic DNA was extracted from tissue samples collected from mussels in September 2016 (all mussels were immediately returned to Tonawanda Creek after tissue sampling). Genomic DNA was extracted from the tissue samples using the Qiagen DNeasy Blood and Tissue extraction kit with minor modifications described above (Qiagen, Valencia, CA). Primer sets were considered specific to *L. siliquoidea* if no amplification (40 cycles) was observed for any of the nontarget genomic DNA. The final primer sequences and optimized primer concentrations and annealing temperature are shown in Table 1.

Quantitative Polymerase Chain Reaction (qPCR) standards were constructed using gBlock Gene Fragments synthesized by IDT (IDT, Coralville, IA). Standard curves consisted of 1:10 serial dilutions of the gBlock oligo from 1 to 10000 copies and were run in triplicate alongside samples in each 96-well plate (BioRad, Hercules, CA). Standard curves were pooled across plates to calculate concentrations of unknown samples.[48] Each plate contained triplicate no template controls (NTCs). All samples were diluted 1:10 to reduce inhibition and amplified in triplicate 20 μL reactions. The qPCR contained final concentrations of 1X SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), and 0.4 μM forward and reverse primers. Cycle quantification (Cq) thresholds were set at 150 for all qPCR plates. A sample was considered quantifiable if two out of three qPCR triplicates were amplified below 33 cycles and if the melt temperature peaked at 76.5 ± 0.5 °C. The lowest detectable standard (i.e., 1 copy per uL) amplified at Cq = 33.1 ± 1.0. Thus, samples that amplified at a Cq greater than 33 cycles were considered below level of quantification (LOQ). If no amplification was observed or if only 1 out of the 3 qPCR triplicates amplified the sample was a nondetect (ND).

Data Analysis. Each mesocosm tank was modeled as a completely mixed batch reactor to calculate the shedding and decay rates:

$$V\frac{dC}{dt} = S - kCV \quad (1)$$

where V is the volume of the tank (mL), C is the concentration of eDNA (copies/mL), t is the time since the start of the experiment (h), S is the eDNA shedding rate (copies/h), and k is the first-order decay rate (h^{-1}). eq 1 assumes that the tank is well mixed and that the decay is first order. Although mussels are sedentary, aerators supplied to each tank provided constant mixing, thus we assumed each tank was completely mixed. Steady state was reached within the first 12 h after the mussels were added and lasted until the mussels were removed from the mesocosm. At steady state, $dC/dt = 0$, therefore $S = kCV$. The error associated with the shedding rate was determined by propagating errors associated with k, C, and V (see SI). A z-test ($p \leq 0.05$) was used to test the null hypothesis that the shedding rates did not differ between each mesocosm.

Once the mussels were removed, $S = 0$ and $dC/dt = -kC$. A first-order decay rate, k, was calculated for each mesocosm tank using the data collected after the mussels were removed. Assuming first-order decay, the first-order decay rate constant

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

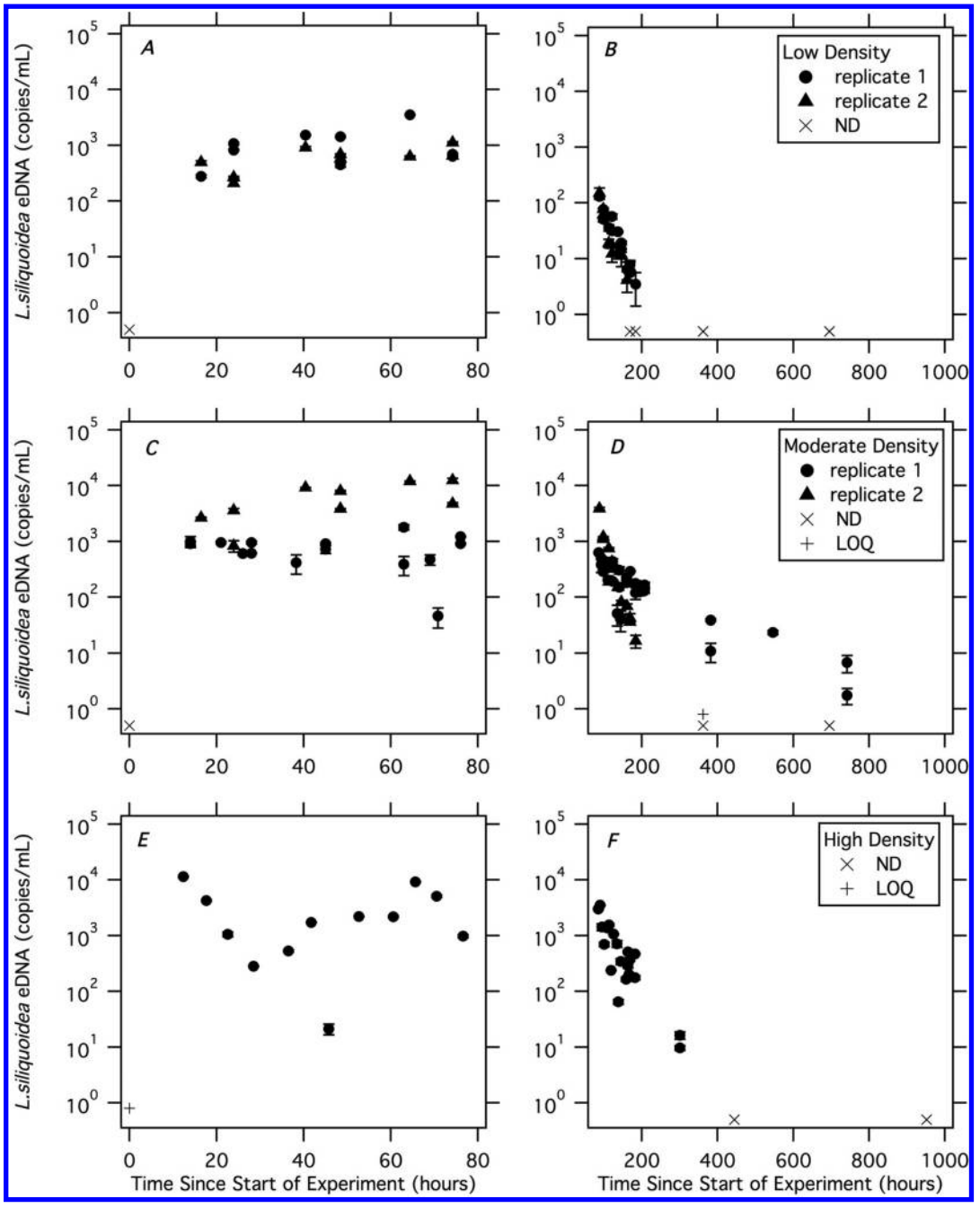

Figure 2. eDNA concentrations in the different mussel density mesocosm experiments, separated by shedding (panels A, C, and E) and decay (panels B, D, and F) analysis: low density (top panel), moderate density (center panel), and high density (bottom panel). The *y* axis is the concentration of DNA (copies/mL) determined with synthesized standards. The *x* axis is the time since the start of the experiment in hours. Experimental replicates (replicates 1 and 2) are represented as separate symbols and the error bars on each panel represent ±1 standard error of triplicate qPCR measurements. "+" symbols represent samples below the level of quantification (LOQ) while "x" represents nondetect (ND) samples.

and its standard error were calculated by fitting a straight line to $\ln(C/C_o)$ versus time using linear regression in *R*.[49] For C_o, we used the mean mussel eDNA concentration in the tank at steady state. Decay rate constants for the different experimental treatments were compared using an analysis of covariance (ANCOVA) with a Tukey's honestly significant difference (HSD) posthoc to test the null hypothesis that decay rate constants were not significantly different between mesocosms.[50] Statistical significance was determined by $p < 0.05$.

Modeling eDNA in Tonawanda Creek. A simplified, one-dimensional plug-flow reactor model was developed to model *L. siliquoidea* eDNA as a function of distance from a mussel bed

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial x} = -kC \tag{2}$$

where C represents the eDNA concentration (copies/mL), t represents time in hours, u is the water velocity (m/s) in the streamwise direction (x-direction), and k is the first-order decay constant. Because the shedding rate was not significantly

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 2. Shedding Rates and Decay Rate Constants for *Lampsilis siliquoidea* at Different Mussel Densities in Experimental Tanks[a]

		shedding rates			
treatment	mussel density (mussels/m^2)	copies/hour ± propagated standard deviation	copies/h/mussel	copies/h/g	decay rate constant/hr ± standard error[b]
low (replicate 1)	16	$1.5 \times 10^6 \pm 1.3 \times 10^6$	7.6×10^5	5.5	$3.9 \times 10^{-2} \pm 6.5 \times 10^{-3}$ c
low (replicate 2)	16	$8.7 \times 10^5 \pm 4.3 \times 10^5$	4.4×10^5	2.5	$3.8 \times 10^{-2} \pm 8.0 \times 10^{-3}$ bc
moderate (replicate 1)	56	$5.4 \times 10^5 \pm 3.7 \times 10^5$	5.4×10^4	0.7	$9.7 \times 10^{-3} \pm 3.9 \times 10^{-3}$ a
moderate (replicate 2)	56	$2.4 \times 10^7 \pm 1.6 \times 10^7$	2.4×10^6	6.9	$5.3 \times 10^{-2} \pm 6.4 \times 10^{-3}$ bc
high	111	$5.6 \times 10^6 \pm 6.1 \times 10^6$	2.8×10^5	1.5	$2.4 \times 10^{-2} \pm 5.1 \times 10^{-3}$ a
environmental					$2.9 \times 10^{-2} \pm 9.3 \times 10^{-3}$ b

[a]The errors for the decay rate constants represent the standard error. The shedding rate (in copies/h) has a propagated standard deviation based on the error associated with the average eDNA concentration at steady state, the tank volume, and the decay rate constant. [b]Letters denote posthoc group differences determined by Tukey's honestly significant difference method (HSD) at $p < 0.05$.

different between the three mussel densities (see below), the mean shedding rate of the three mussel densities was used to model the transport and fate of *L. siliquoidea* eDNA in Tonawanda Creek. For the application of this model, we assumed steady state and solved for eq 2

$$C = C_{\text{bed}} e^{-kx/u} \tag{3}$$

where C_{bed} represents the concentration of *L. siliquoidea* eDNA being shed from a mussel bed and was determined as

$$C_{\text{bed}} = \overline{S} \times M \times t \tag{4}$$

where $\overline{S}$ is the mean *L. siliquoidea* eDNA shedding rate in copies per hour per mussel determined in the laboratory shedding and decay experiments, M is the number of mussels per cubic meter of water inside the mussel bed, and t is time in hours.

To test the efficacy of the simplified, one-dimensional model, we collected water samples (hereafter, field samples) along a portion of Tonawanda Creek downstream of a known mussel bed[51] (Sansom et al. unpublished data). Water samples were collected in acid washed (10% hydrochloric acid) 500 mL polypropylene bottles at the downstream edge of the mussel bed, and 10-, 25-, 100-, 300-, and 1000-m downstream the mussel bed. No mussels were observed between the mussel bed and the most downstream sample location. Triplicate biological samples were filtered (400−500 mL), and DNA was extracted using the methods outlined above. The samples (n = 18) collected from Tonawanda Creek were tested for inhibition by comparing 1:1, 1:10, and 1:100 dilutions. Assuming a 100% efficiency, we expected a Cq change of 3.32 cycles ($\log_2(10)$ = 3.32) if there was no inhibition. All environmental samples were diluted 1:10 to address inhibition and amplified in triplicate 20 μL reactions. A standard curve, as described above, was run in triplicate and triplicate NTCs were run on each plate of samples. In addition to the qPCR Mastermix described above, a final concentration of 0.2 mg/mL bovine serum album (BSA) was added to address inhibition.[52] The Cq, LOQ, and ND thresholds were classified as described above based on the lowest detectable standard. For the field samples, the lowest detectable standard (i.e., 1 copy per microliter) amplified at Cq = 35 ± 0.4.

RESULTS AND DISCUSSION

Genus-Specific Assay Design and qPCR Optimization. The primers developed for this study amplified *L. siliquoidea* eDNA in environmental waters. The assay was sensitive with a limit of quantification of 1 copy of the gene target/mL. The assay efficiency based on pooled standard curves with a slope = −3.35 and an intercept = 33.1 was 99.0% (Table 1; Figure S1; r^2 = 0.93; linear dynamic range = 0−4 log copies).[53] All filtration and extraction blanks showed no evidence of contamination, and all qPCR NTCs showed no amplification. The assay showed minor cross reactivity within the *Lampsilis* genera. Amplification was observed after 33 cycles with 3.72 ng per reaction for *Lampsilis cardium* DNA. The primers cross reacted with one species outside the *Lampsilis* genera, *Ligumia recta,* but the reaction was below the limit of quantification (amplification was observed at 38 cycles for 1.47 ng DNA per reaction). No cross reactivity was observed with *A. plicata* tissue (13.08 ng DNA per reaction).

eDNA Shedding for Varying Mussel Densities. After the mussels were added to the different density treatment tanks, the concentration of eDNA in the tank water increased and remained at steady state (Figure 2). At steady state, shedding balanced decay, and thus the concentration at steady state was used to calculate the shedding rates using eq 1. To account for differences in mussel biomass, the shedding rates are presented as copies of DNA/hour, copies of DNA/hour/mussel, and copies of DNA/hour/gram of mussel (Table 2). The shedding rates ranged from 5.4×10^4 to 2.4×10^6 copies/h/mussel (Table 2) and were not statistically different on a per hour, per mussel, or per gram basis ($p < 0.05$ for each). The eDNA shedding rates are among the first reported for freshwater mussels, and the pattern of eDNA production (i.e., initial increase in eDNA concentration followed by a prolonged period of steady concentration where shedding equaled decay) is similar to previous studies.[25,54] Shedding rates have been previously reported for marine fish (1.4×10^5 to 1.1×10^7 pg/hour[25]) and freshwater salamanders (4.5×10^4 pg/hour[54]), but making comparisons between species, systems (i.e., freshwater vs marine; lotic vs lentic), and units (e.g., mass of DNA vs copies of DNA; eDNA/hour vs eDNA/mass vs eDNA/organism) is difficult.

Previous studies suggest the size or biomass of an organism, such as fish[19,21,25] or salamanders,[54] influences the amount of eDNA shed. However, Pilliod et al.[54] observed that differences in shedding rates for salamander biomass disappeared once eDNA production balanced decay. Further, unlike the organisms listed in the aforementioned studies in which soft tissue is continuously exposed to the environment and therefore the amount of DNA being shed likely increases as body surface area increases, mussels are enclosed in a calcified shell that protects the soft tissue anatomy. Mussel shells are extremely robust, do not readily breakdown,[55,56] and although

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

shell material does contain DNA,[44] the amount of DNA sloughed off from shell material is likely to be minimal. Likewise, mussels remain relatively sedentary throughout their life and partially to fully buried in the substratum.[2] Such a lifestyle likely reduces the effect of organism size or biomass on DNA shedding rates.

Biological activity such as filter feeding[42,43] or burrowing likely drives shedding rates in mussels. As mussels burrow, they extend their foot which helps anchor the shell in the sediment. Through burrowing, DNA could be shed from the soft tissue of the foot that is exposed to the sediment or through friction forces acting on the shell material. In our experiments, mussels were initially buried 3 to 5 cm into the gravel bed. In all of the experiments, we observed that mussels repositioned during the first 12−24 h but did not move after this initial acclimation. This movement was minimal, and we do not believe that burrowing or horizontal movement contributed to a significant amount of shedding activity.

Instead, filter feeding, and the feces or pseudofeces produced as a result of filter feeding, or sloughed tissue cells leaving the body cavity of the mussel through the excurrent aperture, was likely the main driver of eDNA shedding in our experiments.[42,43] Mussels are powerful filter feeders and the cumulative filtration of a relatively high density mussel bed can equal or exceed the stream's flow rate.[1,57] Although we fasted the mussels during the experiments, we visually observed mussels with both the incurrent and excurrent aperture open and attempting to feed. Few to no feces or pseudofeces were observed in the tanks after the mussels were removed, and we therefore believe that the physical action of filter feeding alone was the largest source of eDNA in our experiments. Additional research on the impact of biological activity including both burrowing and filter feeding, and whether intra- or interspecies variation influence the concentration of eDNA shed into the environment would be useful to better understand the main factors contributing to eDNA shedding rates of freshwater mussels.

eDNA Decay for varying mussel densities. After mussels were removed from the tanks, eDNA decay was log−linear (Figure 2). For the low density treatments, eDNA was not detected after 7 days in either replicate experiment (Figure 2). eDNA in the moderate density treatment was detected up to 31 days in the first replicate, but the concentration was <1% of the starting concentration 22 days after the mussels were removed. In the second moderate density treatment, eDNA was not detected after 15 days. eDNA in the high density treatment was not detected after 12 days (Figure 2). The decay rate constants range from 9.7×10^{-3} to 5.3×10^{-2} per hour (Table 2). First-order decay rate constants statistically differed between experimental treatments (ANCOVA $F = 8.8$, df = 5, $p = 1.4 \times 10^{-6}$; Table 2). Differences between decay rate constants do not appear to be dependent on mussel density. No statistical difference was observed between the moderate and high density experiments in 2016, nor between the low and moderate density experiments in 2017 (Table 2).

Similar to the experimental treatments, eDNA in the environmental treatment log−linearly declined (Figure 3). The initial eDNA concentration in the environmental tank was similar to the low, moderate, and high density treatments, and the concentration of eDNA in the environmental tank was quantifiable on day 3, but below the level of quantification after day 4. The decay rate constant for the environmental treatment, $k = 0.029 \pm 0.0093$, was the same order of magnitude as the

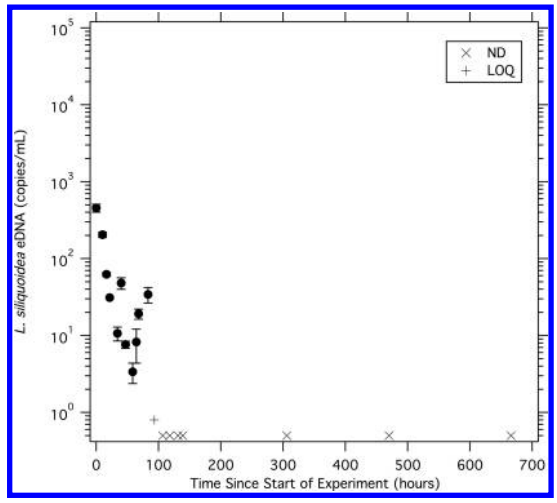

Figure 3. eDNA concentrations in the environmental treatment experiment. The *y* axis is the concentration of DNA (copies per ml) determined with synthesized standards. The *x* axis is the time since the start of the experiment in hours. Error bars on each panel represent ±1 standard error of triplicate qPCR measurements. "+" symbols represent samples below the level of quantification (LOQ) while "x" represents nondetect (ND) samples.

decay rate constants for the other treatments. It was not statistically different than the low density (replicate 2) and moderate density (replicate 2) experiments but was significantly different than the low (replicate 1), moderate (replicate 1), and high density experiments (Table 2).

The first-order eDNA decay rate constants here are also among the first reported for freshwater mussels and are consistent with other studies in freshwater systems. Reported decay constants ranged from 0.06 to 0.116/hour for bluegill (*Lepomis macrochirus*),[45] 0.015 to 0.1/hour for common carp (*Cyprinus carpio*),[58] 0.002 to 0.014/hour for American bullfrog (*Lithobates catesbeianus*),[59] and 0.068 to 0.079/hour for the Idaho giant salamander (*Dicamptodon aterrimus*).[54]

The eDNA signal in our different density treatments decayed below or close to our limit of detection within 7−31 days. eDNA concentration has been reported to decay below detection limits in 4.2 days for carp,[60] 8−18 days for salamanders,[54] 21−44 days for mudsnails,[30] up to 54 days for bullfrogs,[59] and >28 days for silver carp.[61] Because eDNA persistence is dependent upon the starting concentration and the detection limit of the assay,[61] future studies should report decay rate constants, rather than days of detection, to facilitate comparisons across studies and for use in eDNA modeling in environmental systems.

The differences in *k* values across the density treatments in our study suggest that the mechanisms driving eDNA decay do not depend on the density of mussels that contributed that eDNA. eDNA decay rates can be influenced by abiotic (e.g., sunlight and temperature) and biotic (e.g., microbial processes and extracellular enzyme) factors.[40,58−60] Recent studies, however, show mixed results on the impact of sunlight on eDNA decay.[54,59,61,62] The experiments reported here were exposed to indirect sunlight, consistent across treatments, and water temperatures maintained at room temperature (22 ± 1 °C) for the duration of all experiments. Therefore, we hypothesize that the biological activity of filter feeding while the mussels were in the tanks contributed to certain biologically mediated processes ongoing in the tank after the mussels were

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

removed. Although mussels were scrubbed free of visible algae and fasted during the experiment, the filtering process excretes cells, ammonium, feces, and pseudofeces into the environment.[63,64] The filter excreta contributes to an increase in both microbial biomass and microbial growth rates.[65] The increased microbial activity and likely enzymatic activity could lead to biologically mediated DNA degradation. Future research investigating the effect of the microbial community on DNA decay in natural waters in warranted. In addition, similar studies to ours should characterize water quality parameters such as nutrient levels, temperature and pH which may also effect DNA decay.

Modeling eDNA in Tonawanda Creek. On the basis of previous field data (base flow depth = 0.7 m; *L. siliquoidea* density = 0.1 mussels/m^2 − Sansom et al., unpublished data), a time of 12 h, and the mean shedding rate from the mesocosm experiments, we estimated the concentration of *L. siliquoidea* at our study site in Tonawanda Creek, C_{bed}, is 1.4 copies/mL. We set t = 12 h since our shedding and decay experiments indicated that the eDNA concentrations were at steady state after 12 h. Using this concentration (C_{bed} = 1.4 copies/mL) as the input to our model (eq 3), along with a base flow velocity of 0.09 m/s (Sansom et al., unpublished data), the decay constant from the environmental experiment (k = 0.029) and the limit of quantification based on the volume of water that is filtered, we can predict the distance downstream that *L. siliquoidea* eDNA can be detected. Assuming a limit of quantification of 0.047 copies/mL based on 500 mL of creek water filtered, we estimate that *L. siliquoidea* can be detected up to 36.7 km downstream.

To verify our model, we collected water samples from Tonawanda Creek downstream of a mussel bed with *L. siliquoidea*. Because of the inhibition in the samples determined by serial dilutions,[66] BSA was added to all qPCRs and a different standard curve was used to quantify samples. The assay efficiency with BSA was 86.9% with a slope = −3.68 and an intercept = 34.1 (r^2 = 0.98; linear dynamic range = 0 to 5 log copies). *L. siliquoidea* eDNA was detected at each sample location up to 1000 m downstream of the mussel bed. With the exception of one location (100 m downstream), the predicted eDNA concentration from the model fit was within ±1 SE of the amplified environmental samples (Figure S2).

The results of our model prediction and environmental samples underscores two important points. First, inhibition is often a challenge with environmental samples and will negatively affect the use of eDNA as a monitoring tool.[40,67] No amplification was observed for the field samples undiluted, however, amplification was observed for the 1:10 and 1:100 dilutions. Second, detection of eDNA downstream is largely influenced by the volume of water that can be filtered given the turbidity of the water and must be optimized for a particular water body.[68,69] For example, we determined the detection limit based on our standard curves for a range of filter volumes. Given this range (25−500 mL), the downstream detection for *L. siliquoidea* in Tonawanda Creek ranges from 4.3 km to 36.7 km, respectively (Figure S3). Future research on the effects of inhibition and filtration volumes on eDNA detection limits in different water matrices is warranted.

While eDNA has proven to be a useful tool for lentic systems, many challenges remain for lotic environments. Thomsen et al.[20] report that the eDNA detection rate for fish decreased nearly 50% in a lotic system compared to a 100% detection rate in ponds. Further, Stoeckle et al.[34] detected eDNA of a freshwater mussel (*Margaritifera margitifera*) 25 m downstream of mussel populations, but did not detect eDNA at further distances of 500 and 1,000 m. Downstream detection of eDNA is limited by decay, but dilution and settling of DNA fragments (particularly when attached to sediments) can also contribute to reduced detection. Therefore, more research is needed to understand how settling and dilution of eDNA influence downstream transport.

Our model provides a complementary monitoring tool to detect presence/absence and predict the density of *L. siliquoidea* in natural waters. The range of downstream transport predicted by our model is comparable to a study in a similar sized stream that detected eDNA of a mussel (Unio tumidus) up to 9 km downstream of the source population.[31] The results of the model, however, need to be interpreted in the context of the river system. Our model assumes no additional mussel beds are present within the predicted downstream detection distance. Because mussel populations have a patchy distribution throughout a river, it is important to consider additional mussel beds that may influence downstream detection. The model presented here can also be applied as a management tool to understand the interaction between downstream transport and the concentration of eDNA collected at a known distance downstream of a mussel bed (Figure 4). Once shedding and decay rates are better

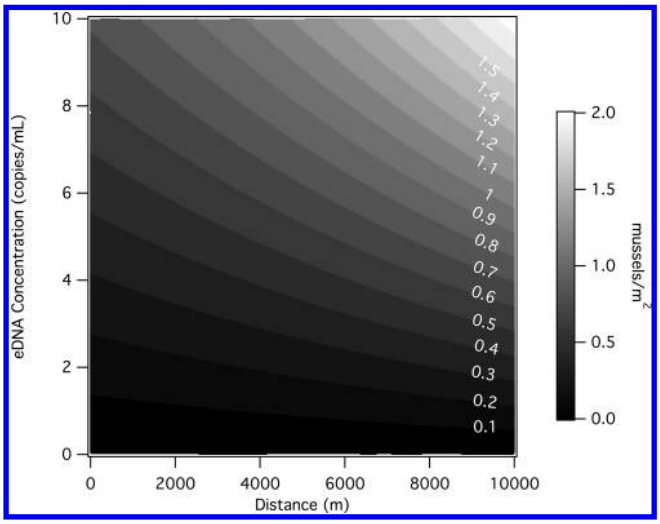

Figure 4. *Lampsilis siliquoidea* density (mussels/m^2) as a function of distance from mussel bed (m) and concentration of eDNA (copies/mL) collected at that distance.

understood for additional freshwater mussel species, application of this model can assist managers to determine the downstream transport of eDNA from a mussel bed, or to predict or verify mussel density by sampling water at a known distance downstream of a mussel bed. Because no study to date has made direct comparisons of mussel density between traditional mussel surveys and eDNA concentration, we suggest that eDNA be used to identify presence/absence or as a complementary tool for traditional mussel density estimates.

Although our study is limited to a single species and stream, the model we presented can be applied to other benthic invertebrates in freshwater systems once the shedding and decay rates are determined, and general site characteristics are made (i.e., mean flow depth, mean flow velocity, and distance from source population). However, improvements can be made

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

by taking into consideration additional processes that may influence eDNA persistence. First, for benthic organisms such as mussels, DNA that is shed may be located in the sediment. More information is needed on how much eDNA is in the sediment, and whether sediment resuspension contributes to total eDNA concentration in the water column. Second, our model was a simplified, one-dimensional mass-balance equation that only considered advection and shedding and decay rates. Additional processes such as dilution and settling of eDNA have been suggested to impact downstream transport,[40] and could improve the model estimates. Finally, sampling considerations, such as downstream distance,[31,34] amount of water collected and filtered,[68] and time or season of sampling[28,31] will influence eDNA detection limits and must be considered.

Broader Implications. Accurate species distribution and diversity counts are critical for proper conservation and management of freshwater mussels. eDNA provides a promising technique to complement current survey methods. Here, we developed a qPCR assay that was specific within the *Lampsilis* genus, determined eDNA shedding and decay rates, and predicted the downstream transport of eDNA for *L. siliquoidea*. On the basis of our results, eDNA has the potential to be a complementary tool to enhance the efforts to monitor and protect freshwater mussel biodiversity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05199.

Expanded methods section describing the genus-specific qPCR assay, propagated error, and mass-balance model derivation, supplemental results showing the qPCR efficiency, mass-balance model agreement with environmental samples, and the effect of sample volume on predicted eDNA concentration in the environmental samples, and the experimental design (i.e., sample collection dates and times) for the mesocosm experiments (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Vaughn, C. C.; Hakenkamp, C. C. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biol.* **2001**, *46* (11), 1431−1446.

(2) Strayer, D. L. *Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance*; University of California Press: Berkeley, CA, 2008.

(3) Allen, D. C.; Vaughn, C. C.; Kelly, J. F.; Cooper, J. T.; Engel, M. H. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology* **2012**, *93* (10), 2165−2174.

(4) Atkinson, C. L.; Vaughn, C. C.; Forshay, K. J.; Cooper, J. T. Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology* **2013**, *94* (6), 1359−1369.

(5) Howard, J. K.; Cuffey, K. M. The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biol.* **2006**, *51* (3), 460−474.

(6) Spooner, D. E.; Vaughn, C. C.; Galbraith, H. S. Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. *Oecologia* **2012**, *168* (2), 533−48.

(7) Atkinson, C. L.; Sansom, B. J.; Vaughn, C. C.; Forshay, K. J. Consumer aggregations drive nutrient dynamics and ecosystem metabolism in nutrient limited systems. *Ecosystems* **2017**, DOI: 10.1007/s10021-017-0166-4.

(8) Williams, J. D.; Warren, M. L.; Cummings, K. S.; Harris, J. L.; Neves, R. J. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **1993**, *18* (9), 6−22.

(9) Strayer, D. L.; Downing, J. A.; Haag, W. R.; King, T. L.; Layzer, J. B.; Newton, T. J.; Nichols, S. J. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* **2004**, *54* (5), 429−439.

(10) Obermeyer, B. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. *Am. Midl. Nat.* **1998**, *139* (2), 331−339.

(11) Smith, D. R.; Villella, R. F.; Lemarie, D. P.; von Oettingen, S. In *How Much Excavation Is Needed to Monitor Freshwater Mussels*; Freshwater Mollusk Conservation Society, Ohio Biological Survey, 2000.

(12) Vaughn, C. C.; Taylor, C. M.; Eberhand, K. J. In A comparison of the effectiveness of timed serches vs. quadrat sampling in mussel surveys, Conservation and Management of Freshwater Mussels II: Initiative for the Future. *Proceedings of A UMRCC Symposium, St. Louis, MO, 1997*; Cummings, K. S., Buchanan, A. C., Mayer, C. A., Naimo, T. J., Eds.; Upper Mississippi River Conservation Committee: Rock Island, IL, 1997.

(13) Miller, A. C.; Payne, B. S. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. *Am. Midl. Nat.* **1993**, *130* (1), 133−145.

(14) Cawley, E. T., Sampling adequacy in population studies of freshwater mussels. In *Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium*; Cummings, K. S., Buchanan, A. C., Koch, L. M., Eds.; St. Louis, MO, 1993; pp 168−172.

(15) Strayer, D. L.; Claypool, S.; Sprague, S. J., Assessing unionid populations with quadrats and timed searches. In *Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium.*, Cummings, K. S., Buchanan, A. C., Mayer, C. A., Naimo, T. J., Eds.; St. Louis, MO, 1997.

(16) Hornbach, D.; Deneka, T. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. *J. North Am. Benthol. Soc.* **1996**, *15* (4), 587−596.

(17) Thomsen, P. F.; Willerslev, E. Environmental DNA − an emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* **2015**, *183*, 4−18.

(18) Jerde, C.; Mahon, A.; Chadderton, W.; Lodge, D. "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* **2011**, *4* (2), 150−157.

(19) Thomsen, P. F.; Kielgast, J.; Iversen, L. L.; Møller, P. R.; Rasmussen, M.; Willerslev, E. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One* **2012**, *7* (8), No. e41732.

(20) Thomsen, P. F.; Kielgast, J.; Iversen, L. L.; Wiuf, C.; Rasmussen, M.; Gilbert, M. T.; Orlando, L.; Willerslev, E. Monitoring endangered

DOI: 10.1021/acs.est.7b05199
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

freshwater biodiversity using environmental DNA. *Mol. Ecol.* **2012**, *21* (11), 2565−73.
(21) Takahara, T.; Minamoto, T.; Yamanaka, H.; Doi, H.; Kawabata, Z. Estimation of fish biomass using environmental DNA. *PLoS One* **2012**, *7* (4), No. e35868.
(22) Kelly, R. P.; Port, J. A.; Yamahara, K. M.; Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS One* **2014**, *9* (1), No. e86175.
(23) Doi, H.; Uchii, K.; Takahara, T.; Matsuhashi, S.; Yamanaka, H.; Minamoto, T. Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. *PLoS One* **2015**, *10*, e0122763.
(24) Port, J. A.; O'Donnell, J. L.; Romero-Maraccini, O. C.; Leary, P. R.; Litvin, S. Y.; Nickols, K. J.; Yamahara, K. M.; Kelly, R. P. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Mol. Ecol.* **2016**, *25* (2), 527−541.
(25) Sassoubre, L.; Yamahara, K.; Gardner, L.; Block, B.; Boehm, A. Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. *Environ. Sci. Technol.* **2016**, *50* (19), 10456−10464.
(26) Yamamoto, S.; Minami, K.; Fukaya, K.; Takahashi, K.; Sawada, H.; Murakami, H.; Tsuji, S.; Hashizume, H.; Kubonaga, S.; Horiuchi, T.; Hongo, M.; Nishida, J.; Okugawa, Y.; Fujiwara, A.; Fukuda, M.; Hidaka, S.; Suzuki, K.; Miya, M.; Araki, H.; Yamanaka, H.; Maruyama, A.; Miyashita, K.; Masuda, R.; Minamoto, T.; Kondoh, M. Environmental DNA as a 'snapshot' of fish distribution: a case study of Japanese Jack Mackerel in Maizuru Bay, Sea of Japan. *PLoS One* **2016**, *11* (4), No. e0149786.
(27) Ficetola, G. F.; Miaud, C.; Pompanon, F.; Taberlet, P. Species detection using environmental DNA from water samples. *Biol. Lett.* **2008**, *4* (4), 423−425.
(28) Goldberg, C. S.; Pilliod, D. S.; Arkle, R. S.; Waits, L. P. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One* **2011**, *6* (7), No. e22746.
(29) Katano, I.; Harada, K.; Doi, H.; Souma, R.; Minamoto, T. Environmental DNA method for estimating salamander distribution in headwater streams, and a comparison of water sampling methods. *PLoS One* **2017**, *12* (5), No. e0176541.
(30) Goldberg, C.; Sepulveda, A.; Ray, A.; Baumgardt, J.; Waits, L. Environmental DNA as a new method for early detection of New Zealand mudsnails (Potamopyrgus antipodarum). *Freshwater Science* **2013**, *32* (3), 792−800.
(31) Deiner, K.; Altermatt, F. Transport Distance of Invertebrate Environmental DNA in a Natural River. *PLoS One* **2014**, *9*, e88786.
(32) Bass, D.; Stentiford, G. D.; Littlewood, D. T. J.; Hartikainen, H. Diverse applications of environmental DNA methods in parasitology. *Trends Parasitol.* **2015**, *31*, 499−513.
(33) Bastos Gomes, G.; Hutson, K. S.; Domingos, J. A.; Chung, C.; Hayward, S.; Miller, T. L.; Jerry, D. R. Use of environmental DNA (eDNA) and water quality data to predict protozoan parasites outbreaks in fish farms. *Aquaculture* **2017**, *479*, 467−473.
(34) Stoeckle, B. C.; Kuehn, R.; Geist, J. Environmental DNA as a monitoring tool for the endangered freshwater pearl mussel (*Margitifera margaritifera* L.): a substitue for classical monitoring approaches? *Aquatic Conservation: Marine and Freshwater Ecosystems* **2016**, *26* (6), 1120−1129.
(35) Cho, A.; Morris, T.; Wilson, C.; Freeland, J. Development of species-specific primers with potential for amplifying eDNA from imperilled freshwater unionid mussels. *Genome* **2016**, *59* (12), 1141−1149.
(36) Pilliod, D. S.; Goldberg, C. S.; Arkle, R. S.; Waits, L. P. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Can. J. Fish. Aquat. Sci.* **2013**, *70*, 1123−1130.
(37) Ikeda, K.; Doi, H.; Tanaka, K.; Kawai, T.; Negishi, J. N. Using environmental DNA to detect an endangered crayfish *Cambaroides japonicus* in streams. *Conservation Genetics Resources* **2016**, *8* (3), 231−234.
(38) Wilcox, T.; McKelvey, K.; Young, M.; Lowe, W.; Schwartz, M. Environmental DNA particle size distribution from Brook Trout (Salvelinus fontinalis). *Conservation Genetics Resources* **2015**, *7* (3), 639−641.
(39) Deiner, K.; Walser, J.; Machler, E.; Altermatt, F. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biol. Conserv.* **2015**, *183*, 53−63.
(40) Jane, S. F.; Wilcox, T. M.; McKelvey, K. S.; Young, M. K.; Schwartz, M. K.; Lowe, W. H.; Letcher, B. H.; Whiteley, A. R. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Mol. Ecol. Resour.* **2015**, *15* (1), 216−27.
(41) Doi, H.; Inui, R.; Akamatsu, Y.; Kanno, K.; Yamanaka, H.; Takahara, T.; Minamoto, T. Environmental DNA analysis for estimating the abundance and biomass of stream fish. *Freshwater Biol.* **2017**, *62* (1), 30−39.
(42) Henley, W. F.; Grobler, P. J.; Neves, R. J. Non-invasive metho to obtain DNA from freshwater mussels (Bivalvia: Unionidae). *J. Shellfish Res.* **2006**, *25*, 975−977.
(43) Palmer, A. N. S.; Styan, C. A.; Shearman, D. C. A. Foot mucus is a good source for non-destructive geneti sampling in. *Conserv. Genet.* **2008**, *9*, 229−231.
(44) Geist, J.; Wunderlich, H.; Kuehn, R. Use of mollusc shells for DNA-based molecular analyses. *J. Molluscan. Stud.* **2008**, *74*, 337−343.
(45) Maruyama, A.; Nakamura, K.; Yamanaka, H.; Kondoh, M.; Minamoto, T. The release rate of environmental DNA from juvenile and adult fish. *PLoS One* **2014**, *9* (12), No. e114639.
(46) Dejean, T.; Valentini, A.; Duparc, A.; Pellier-Cuit, S.; Pompanon, F.; Taberlet, P.; Miaud, C. Persistence of environmental DNA in freshwater ecosystems. *PLoS One* **2011**, *6* (8), No. e23398.
(47) Ye, J.; Coulouris, G.; Zaretskaya, I.; Cutcutache, I.; Rozen, S.; Madden, T. L. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinf.* **2012**, *13*, 134.
(48) Sivaganesan, M.; Haugland, R. A.; Chern, E. C.; Shanks, O. C. Improved strategies and optimization of calibration models for real-time PCR absolute quantification. *Water Res.* **2010**, *44* (16), 4726−4735.
(49) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013.
(50) McDonald, J. H. *Handbook of Biological Statistics*, 3rd ed.; Sparky House Publishing: Baltimore, MD, 2014.
(51) Marangelo, P. J.; Strayer, D. L. The freshwater mussels of the Tonawanda Creek basin in western New York. *Walkerana* **2000**, *11*, 97−106.
(52) Schriewer, A.; Wehlmann, A.; Wuertz, S. Improving qPCR efficicency in environmental samples by selective removal of humic acids with DAX-8. *J. Microbiol. Methods* **2011**, *85* (1), 16−21.
(53) Bustin, S. A.; Benes, V.; Garson, J. A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M. W.; Shipley, G. L.; Vandesompele, J.; Wittwer, C. T. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **2009**, *55*, 611.
(54) Pilliod, D. S.; Goldberg, C. S.; Arkle, R. S.; Waits, L. P. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.* **2014**, *14* (1), 109−16.
(55) Strayer, D.; Malcom, H. Shell decay rates of native and alien freshwater bivalves and implications for habitat engineering. *Freshwater Biol.* **2007**, *52* (8), 1611−1617.
(56) Gutierrez, J. L.; Jones, C. G.; Strayer, D. L.; Iribarne, O. O. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* **2003**, *101* (1), 79−90.
(57) Strayer, D. L.; Hunter, D. C.; Smith, L. C.; Borg, C. K. Distribution, abundance, and roles of freshwater clams (Bivalvia: Unionidae) in the freshwater tidal Hudson River. *Freshwater Biol.* **1994**, *31*, 239−248.
(58) Eichmiller, J. J.; Best, S. E.; Sorensen, P. W. Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Environ. Sci. Technol.* **2016**, *50* (4), 1859−1867.

DOI: 10.1021/acs.est.7b05199
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(59) Strickler, K.; Fremier, A.; Goldberg, C. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biol. Conserv.* **2015**, *183*, 85−92.
(60) Barnes, M. A.; Turner, C. R.; Jerde, C. L.; Renshaw, M. A.; Chadderton, W. L.; Lodge, D. M. Environmental conditions influence eDNA persistence in aquatic systems. *Environ. Sci. Technol.* **2014**, *48* (3), 1819−27.
(61) Merkes, C. M.; McCalla, S. G.; Jensen, N. R.; Gaikowski, M. P.; Amberg, J. J. Persistence of DNA in Carcasses, Slime and Avian Feces May Affect Interpretation of Environmental DNA Data. *PLoS One* **2014**, *9* (11), No. e113346.
(62) Andruszkiewicz, E. A.; Sassoubre, L. M.; Boehm, A. B. Persistence of marine fish environmental DNA and the influence of sunlight. *PLoS One* **2017**, *12* (9), No. e0185043.
(63) Burton, R. F., Ionic regulation and water balance. In *The Mollusca: Physiology, Part 2*, Saleuddin, A. S. M., Wilbur, K. M., Eds.; Academic Press: New York, 1983.
(64) Atkinson, C. L.; Kelly, J. F.; Vaughn, C. C. Tracing consumer-derived nitrogen in riverine food webs. *Ecosystems* **2014**, *17* (3), 485−496.
(65) McCall, P. L.; Matisoff, G.; Tevesz, M. J. S. The effects of a unionid bivalve on the physical, chemical, and microbial properties of cohesive sediments from Lake Erie. *Am. J. Sci.* **1986**, *286*, 127−159.
(66) Cao, Y.; Griffith, J. F.; Dorevitch, S.; Weisberg, S. B. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring Enterococcus in environmental waters. *J. Appl. Microbiol.* **2012**, *113* (1), 66−75.
(67) Gibson, K. E.; Schwab, K. J.; Spencer, S. K.; Borchardt, M. A. Measuring and mitigating inhibition during quantitative real time PCR analysis of viral nucleic acid extracts from large-volume environmental water samples. *Water Res.* **2012**, *46* (13), 4281−4291.
(68) Machler, E.; Deiner, K.; Spahn, F.; Altermatt, F. Fishing in the water: effect of sampled water volume on environmental DNA-based dection of macroinvertebrates. *Environ. Sci. Technol.* **2016**, *50* (1), 305−312.
(69) Rees, H. C.; Maddison, B. C.; Middleditch, D. J.; Patmore, J. R. M.; Gough, K. C. The detection of aquatic animal specieis using environmental DNA - a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* **2014**, *51*, 1450−1459.

DOI: 10.1021/acs.est.7b05199
Environ. Sci. Technol. XXXX, XXX, XXX−XXX