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Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms

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ABSTRACT

Environmental DNA (eDNA) degradation is a primary mechanism limiting the detection of rare species using eDNA techniques. To better understand the environmental drivers of eDNA degradation, we conducted a laboratory experiment to quantify degradation rates. We held bullfrog (*Lithobates catesbeianus*) tadpoles in microcosms, then removed the tadpoles and assigned the microcosms to three levels each of temperature, ultraviolet B (UV-B) radiation, and pH in a full factorial design. We collected water samples from each microcosm at six time steps (0 to 58 days). In all microcosms, most degradation occurred in the first three to 10 days of the experiment, but eDNA remained detectable after 58 days in some treatments. Degradation rates were lowest under cold temperatures (5 °C), low UV-B levels, and alkaline conditions. Higher degradation rates were associated with factors that contribute to favorable environments for microbial growth (higher temperatures, neutral pH, moderately high UV-B), indicating that the effects of these factors may be biologically mediated. The results of this experiment indicate that aquatic habitats that are colder, more protected from solar radiation, and more alkaline are likely to hold detectable amounts of eDNA longer than those that are warmer, sunnier, and neutral or acidic. These results can be used to facilitate better characterization of environmental conditions that reduce eDNA persistence, improved design of temporal sampling intervals and inference, and more robust detection of aquatic species with eDNA methods.

1. Introduction

Effective conservation of imperiled species depends on our ability to reliably detect individuals and quantify uncertainties in detection rates. Similarly, control of invasive species is most successful when the species are found while they are still rare. For species that are difficult to find or identify, a recently developed approach using environmental DNA (eDNA) has been found to improve detection rates for aquatic species (Jerde et al., 2011; Dejean et al., 2012; Pilliod et al., 2013). Environmental DNA in aquatic systems is DNA released into water by aquatic and terrestrial organisms, which can be sampled and used as an effective tool for identifying the presence and distribution of target species. As with any method of surveillance, the reliability of eDNA monitoring requires an understanding of factors that improve or detract from accurate detection (Lodge et al., 2012). Although eDNA methods have shown to be reliable, we lack a quantified understanding of the environmental processes that effect eDNA detection (Díaz-Ferguson and Moyer, 2014).

In concept, three processes determine the availability of detectable DNA in environmental samples: (1) eDNA production, (2) transport and removal of eDNA, and (3) eDNA degradation (Fig. 1). For aquatic eDNA, production, the rate at which DNA is released in the water, is a highly variable function influenced by population density and species-specific characteristics, such as metabolic rates and aquatic habitat use. After eDNA is present in water, it is removed from the source by hydrologic processes (diffusion and advection), which vary in time, space, and type of aquatic system (e.g., lentic, lotic, or marine), by other sources of removal such as binding to and settling with sediment, and by *in-situ* degradation.

Degradation of DNA in water is considered a primary agent for reducing detectability over time (Dejean et al., 2012; Barnes et al., 2014; Pilliod et al., 2014), and thus limits the temporal and related spatial inference of eDNA detection results. Understanding the rates and environmental factors controlling degradation is essential to understanding this scope of inference and improving sampling strategies for eDNA monitoring. DNA is broken down in

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water by chemical hydrolysis, primarily through exposure to acid or by enzymatic hydrolysis. Microbial activity in water contributes directly to enzymatic hydrolysis by producing exogenous nucleases that break down DNA into its components (Lindahl, 1993). Although temperature can directly degrade DNA when very high temperatures (>50 °C) cause denaturation, most temperature-related eDNA degradation is likely indirect, as moderately higher temperatures stimulate microbial metabolism and exonuclease activity (Hofreiter et al., 2001; Zhu, 2006; Corinaldesi et al., 2008; Poté et al., 2009; Fu et al., 2012). Exposure to high levels of ultraviolet radiation, particularly ultraviolet B (UV-B) light, can photochemically damage DNA (Ravanat et al., 2001, Häder et al., 2003) to the point where DNA amplification (polymerase chain reaction [PCR]) fails. Naturally-occurring levels of solar radiation can have variable effects on exonuclease activity, and thus eDNA degradation, depending on the type of bacteria present. Ultraviolet radiation can inhibit growth of heterotrophic bacteria or stimulate growth of autotrophic bacteria (Sommaruga, 2001; Häder et al., 2003), consequently decreasing or increasing exonuclease production, respectively. Thus, these factors (pH, solar radiation, and temperature) are likely to interact, either directly or mediated through the biological community, to influence the process of eDNA degradation in aquatic systems.

Recent research has provided experimental evidence that eDNA degrades quickly in water (Dejean et al., 2011; Thomsen et al., 2012a,b; Goldberg et al., 2013; Piaggio et al., 2014). Degradation rates, estimated by measuring eDNA over time following removal of target animals from experimental microcosms or mesocosms, have varied across species and experimental conditions. Dejean et al. (2011) reported that eDNA persisted for 25 days for American bullfrog (*L. catesbeianus*) tadpoles in laboratory microcosms, and Goldberg et al. (2013) found New Zealand mudsnail (*Potamopyrgus antipodarum*) eDNA was detected in laboratory containers for at least 21 days following removal of the organism. In experiments conducted in outdoor containers or ponds, Piaggio et al. (2014) detected Burmese python (*Python bivittatus*) eDNA for at least 2–7 days after removal, Thomsen et al. (2012b) found that eDNA of two larval amphibian species was detectable for 7–14 days (Thomsen et al., 2012b), and Dejean et al. (2011) were able to detect eDNA of Siberian sturgeon (*Acipenser baerii*) for up to 21 days. The lowest eDNA persistence reported to date was for two species of marine fish held in laboratory microcosms, where eDNA was undetectable in about 1–7 days (Thomsen et al., 2012a). None of these studies, however, measured the extent to which environmental conditions influenced eDNA persistence.

Two studies have specifically quantified the effects of particular environmental factors on eDNA degradation. Persistence of Idaho giant salamander eDNA (*Dicamptodon aterrimus*) in outdoor containers was detectable until 8 days under ambient light and temperature conditions, at least 11 days under ambient temperature and reduced light, and at least 18 days in a refrigerated treatment without light (Pilliod et al., 2014). Common carp (*Cyprinus carpio*) eDNA in laboratory mesocosms was estimated to be undetectable at 95% probability after approximately 4 days (Barnes et al., 2014), but was detected in extreme cases as long as 14 days. Degradation rate was negatively correlated with indices of physiochemical factors associated with microorganisms expected to influence degradation (chlorophyll *a*, biochemical oxygen demand (BOD), pH, and total eDNA concentration from any organism in the water sample). Together, these studies illustrate that environmental factors can affect eDNA degradation in different ways. However, none of these studies, nor any others of which we are aware, have explicitly isolated and quantified the primary factors likely to control degradation of eDNA.

In this study, we set out to evaluate eDNA degradation in a controlled setting to better understand the drivers of eDNA degradation and the persistence of eDNA over time. We set up a full factorial study to measure degradation rates of eDNA in laboratory microcosms under different treatments of UV-B, pH, and temperature. Our primary goal was to quantify the effect of these factors, independently and interactively, on the persistence of eDNA. Our second objective was to develop a regression model to help inform eDNA sampling strategies by quantifying variable degradation rates across environments. With this work, we will be better prepared to identify potential areas of high and low degradation and recommend sampling intervals to maximize likelihood of detection under different environmental conditions.

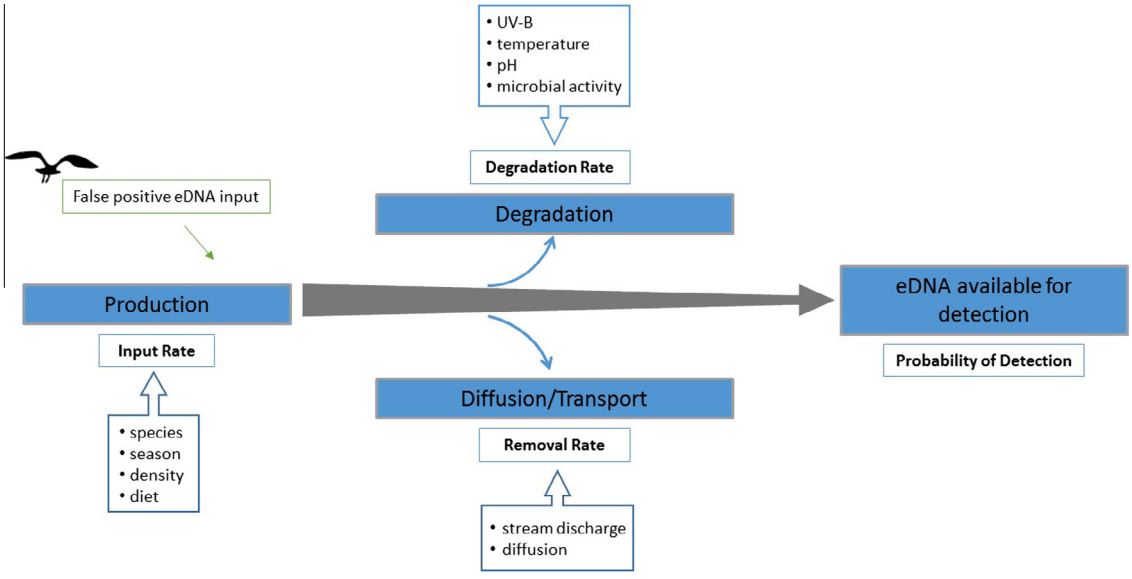

Fig. 1. Conceptual model of factors affecting eDNA detection. Following input of eDNA into an aquatic system, eDNA is removed from the system through degradation and by diffusion and transport processes, reducing the amount available for eDNA detection. Arrow boxes identify some of the biotic and abiotic factors that influence the production, degradation, and diffusion/transport processes. In some situations, it is possible that DNA brought to the sampling site from external sources (including movement by humans, vehicles, or animals) can interfere with estimates of true production by the target species and thus lead to false positive detections.

2. Materials and methods

2.1. Experimental design

We held American bullfrog tadpoles in microcosms for two weeks, then removed the tadpoles and exposed the microcosms to three levels each of temperature, UV-B, and pH in a full factorial design (27 treatments) with three replicates in each treatment. We added nine control microcosms (one to each temperature/pH combination, distributed among UV-B treatments) to monitor potential contamination among treatments, for a total of 90 experimental units. Control microcosms had never been exposed to tadpoles but were otherwise treated the same as experimental microcosms.

We placed large (5–10 cm) tadpoles in polypropylene microcosms at a density of two tadpoles per 3.79-L microcosm. Tadpoles were purchased from Connecticut Valley Biological Supply, where they were sourced from outdoor ponds. Prior to introduction of tadpoles, microcosms were filled with tap water mixed with a mild salt solution (Holtfreter's solution; Cold Spring Harbor Protocol 2009) to prevent infection in experimental animals. Tadpoles were fed twice each day with frozen bloodworms. Uneaten bloodworms were removed after feeding with a clean, disposable pipette. We removed bullfrog tadpoles from microcosms after 14 days (ACUC protocol: University of Idaho 2012-40). Microcosms were then randomly assigned to treatments and placed in growth chambers at the University of Idaho greenhouses. After Day 0 sampling and treatment assignment, we wrapped each microcosm lightly with plastic wrap to prevent contamination.

2.2. Experimental variables

2.2.1. Temperature

We assigned microcosms to one of three temperature levels: 5°, 20°, and 35 °C. Temperature levels were selected to represent the range of temperatures found in streams and ponds in a range of environmental settings. Each treatment temperature level was held in a separate growth chamber automated to maintain constant temperature.

2.2.2. UV-B

We exposed microcosms to three levels of UV-B radiation exposure: 2 kJ/m^2/day, 25 kJ/m^2/day, and 50 kJ/m^2/day. The radiation exposure treatments were selected on the basis of UV-B intensities because, among the wavelengths most likely to be present in terrestrial sunlight, UV-B wavelengths are most harmful to aquatic organisms (Diffey, 2002). Levels of UV-B radiation were selected to represent a range of ambient exposures measured at United States (U.S.) Department of Agriculture UV-B Monitoring Network stations across most of the continental United States (USDA, UV-B-Monitoring and Research Program, Colorado State University, CO, USA; UV-B daily sum data downloaded from http://uvb.nrel.colostate.edu/UVB). We used UVA 340 fluorescent light bulbs (Q-Lab Corporation) to simulate direct solar UV radiation. These bulbs emit light in both the UV-B (315–280 nm) and UV-A (400–315 nm) regions but have no UV output below 295 nm, which is the lower cutoff wavelength for terrestrial sunlight, and do not produce detrimental UV-C radiation (<280 nm) (Q-Lab, 2011). Radiation exposures for each treatment were achieved by varying the height of the lamps over each microcosm.

We monitored UV-B levels in each microcosm every 2–5 days using a hand-held digital Solarmeter radiometer (Model 6.2 UVB, Solar Light Company) to measure UV-B at the water surface, and adjusted lamp height as needed to maintain treatment UV-B levels; water levels declined following removal of water for each eDNA sample. Our radiometers measured irradiance, or the amount of radiation reaching the water surface, measured in W/m^2 (Diffey, 2002). We then controlled radiant exposure, measured in J/m^2/day, in each treatment by adjusting the daily on–off cycles of the UV lamps to maintain the target daily exposure dose. We confirmed that UV-B levels within the microcosms were not affected by plastic wrap covers.

2.2.3. pH

We used levels of pH 4, pH 7, and pH 10 to represent a range of acidic, neutral, and alkaline water found in temperate freshwater systems (Allan and Castillo, 2007). Water in all microcosms was neutral when the experiment began; therefore, microcosms in the pH 7 treatment were not manipulated. Acidic and alkaline treatments were achieved using sterile 1 M HCl or 0.5 M NaOH, respectively. We monitored pH levels to confirm they were stable throughout the experiment.

2.3. Microorganisms

Because we hypothesized that bacteria can play an important role in DNA degradation, we inoculated the microcosms with three bacterial species (*Staphylococcus epidermidis*, *Pseudomonas fluorscens*, *Corynebacterium xerosis*) after tadpole removal. These bacteria are ubiquitous in freshwater systems and have been detected on the skin of American bullfrogs in natural ponds (Culp et al., 2007). We incubated each bacterium overnight and used a spectrophotometer to obtain a turbidimetric measurement of cell density. We then inoculated each container with 10 mL of the bacterial solution after microcosms had been placed in pH, UV-B, and temperature treatments. In addition to the quantified concentrations of bacteria we introduced into microcosms, it is likely that bacteria and other microorganisms were also present on the skin of the experimental tadpoles, and the amount may have varied among microcosms. Although we could not control for experimental changes in the microbial community, we created an index of microbial activity over the course of the experiment by quantifying the total amount of DNA (from any organism) present in each microcosm at Days 0, 21, and 58 and subtracting from it the amount of bullfrog eDNA measured at each time step.

2.4. Sample collection

We collected 250 mL water samples from each microcosm immediately after removal of tadpoles (Day 0) and at 3, 10, 21, 35, and 58 days following removal. Samples were filtered on-site using 0.45 μm cellulose nitrate filters in disposable filter funnels (Whatman product number 1920–7001), and filters were preserved in 95% ethanol. Because extracellular DNA is able to pass through filters with pores larger than 0.2 μm (Matsui et al., 2001, Turner et al., 2014), our filters likely captured DNA that persisted within cells or mitochondria. Filtrate was disposed of following filtration.

2.5. Laboratory analysis

We developed and validated a species-specific quantitative PCR (qPCR) assay for the American bullfrog from previously generated mitochondrial sequence data (Austin et al., 2004) (Table 1).

Table 1
Quantitative PCR assay developed for American bullfrogs (*Lithobates catesbeianus*).

Species	Primer/Probe name	Sequence
American Bullfrog	BullfrogF	TTTTCACTTCATCCTCCCGTTT
	BullfrogR	GGGTTGGATGAGCCAGTTTG
	Bullfrog Probe	NED-TTATCGCAGCAGCAAGT-MGB

Reactions were run using QuantiTect Multiplex PCR Mix (Qiagen, Inc.) with recommended multiplexing concentrations (1X QuantiTect Multiplex PCR mix, 0.2 μM of each primer, and 0.2 μM probe) on an Applied Biosystems 7500 Fast Real-Time PCR System. Reactions were 15 μl in volume and each included 3 μl of sample. Cycling began with 15 min at 95 °C followed by 50 cycles of 94 °C for 60 s and 60 °C for 60 s. We validated this assay *in silico* using PrimerBLAST to confirm that the assay was species specific. We also confirmed that non-target species DNA from a subset of co-occurring species in the western U.S. (Supplementary material, Table A1) did not produce a positive result, defined by any exponential increase in signal from a non-target sample. It is possible that, because these tadpoles were sourced from an outside pond, DNA of eastern U.S. species (e.g., the green frog, *Lithobates clamitans*) was present at the beginning. However, because of time lag between arrival of the tadpoles and the start of the experiment, this DNA is likely to have degraded.

Laboratory analyses were conducted at the University of Idaho's Laboratory for Ecological, Evolutionary, and Conservation Genetics. We extracted filter samples using the Qiashredder/DNeasy method described in Goldberg et al. (2011). All filter sample extractions and qPCR set up was conducted in a lab dedicated to low-quantity DNA samples. Researchers are required to shower and change clothes before entering this room after being in a high-quality DNA or post-PCR laboratory, and no amphibian or reptile tissue samples have been handled in this room. A negative extraction control was included with each set of extractions and an additional negative qPCR control was run with each plate of samples. We used a multi-tube approach for analysis, where multiple reactions were conducted for each sample (Taberlet et al., 1999). We analyzed each sample in triplicate and included an internal positive control (IC; Qiagen) in each well. A positive sample was defined as any sample that showed exponential amplification in all three wells the first time it was tested or in one or more wells from two separate reactions (samples were rerun whenever the original triplicate wells yielded inconsistent results; 6% of samples met this criterion). Quantitative standards consisted of diluted tissue-derived DNA quantified on a Nanodrop spectrophotometer and diluted 10^{-2} through 10^{-6}, run in duplicate. Mean r^2 was 0.99 and mean efficiency was 101.64%.

We quantified the total amount of DNA from all organisms in samples from each microcosm at Days 0, 21, and 58 using a Nanodrop (Thermo Fisher Scientific). We subtracted estimates of bullfrog eDNA from total eDNA to obtain an index of changes in microbial activity over time.

2.6. Statistical analysis

Degradation rate was estimated by the overall change in eDNA detected in each microcosm over the duration of the experiment, as calculated by the slope of the line through all sampling events. Prior to analysis, eDNA values were log-transformed to achieve normality. We used a one-way ANOVA to verify that bullfrog eDNA concentrations at the start of the experiment were not different between treatments. Because starting concentration of eDNA varied among microcosms, we also used linear regression to test whether starting bullfrog eDNA concentration was related to degradation rate. To test for differences in bullfrog eDNA degradation rates among levels of temperature, UV-B, and pH, we used a one-way ANOVA for each factor. We developed a predictive model with main and interaction effects using multiple linear regression. We estimated an overall degradation rate by fitting an exponential decay model to the raw data across all treatments using the exponential decay model $N(t) = N_0 e^{-rt}$, where $N(t)$ is the concentration of bullfrog eDNA measured at time t, N_0 is the bullfrog eDNA concentration at time 0, and r is the degradation or decay rate. We used the estimates for r from the exponential model to calculate the time it would take for eDNA in each treatment to fall below 5% of the mean initial concentration for that treatment. We examined the rate of total eDNA growth over time using linear regression. All statistical analyses were conducted using SAS version 9.3 software (SAS Institute Inc.).

3. Results

Estimated concentration of eDNA in microcosms declined rapidly, with 80–90% of the observed degradation occurring in the first three days after tadpole removal (Fig. 2); however, appreciable amounts of eDNA (>5% of initial concentration) were detected in 53 (of 81) microcosms at Day 3, 35 at Day 10, 21 at Day 21, 13 at Day 35, and 3 at Day 58.

Total eDNA from all sources at Day 0 ranged from 12.55 to 164.53 ng per 250 ml, while starting concentrations of bullfrog eDNA ranged from 0.06 to 5.83 ng DNA per 250 ml water sample. Initial (Day 0) concentrations of bullfrog eDNA did not differ among levels of temperature, pH, and UV-B (Temperature: $F_{(2,80)} = 0.47$; $P = 0.63$; pH: $F_{(2,80)} = 1.65$, $P = 0.20$; UV-B: $F_{(2,80)} = 0.06$, $P = 0.94$). We found no evidence that starting concentration of bullfrog DNA affected degradation rate ($R^2 = 0.02$, $P = 0.13$).

Negative control microcosms (6 of 9) tested positive at 1–3 time steps for minute amounts of tadpole eDNA. This apparent cross-contamination was at very low levels (max = 0.49 pg DNA/250 ml sample) and short-lived (6 microcosms at Day 0, 4 microcosms at Day 3, 1 microcosm at Day 10, and 0 microcosms throughout the remainder of the experiment). Our microcosms were in close proximity to one another (0.1–2 m) within each growth chamber and it is possible that small water droplets were carried between microcosms through aeration, most likely at the outset of the experiment before microcosms were covered with plastic wrap. This issue also occurred in a similar eDNA degradation experiment (Barnes et al., 2014). Because the amounts of eDNA in control microcosms were extremely small, disappeared within the first three time steps, and were detected in all levels of the three treatment factors, it is unlikely that cross-contamination affected the observed patterns of eDNA degradation throughout the experiment.

Across all treatments, temperature exhibited a strong influence on the amount of eDNA detected over time (Fig. 3a; one-way ANOVA $F_{(2,78)} = 9.85$, $P = 0.002$). Degradation rate was significantly lower in the 5 °C treatment compared with 20° and 35 °C

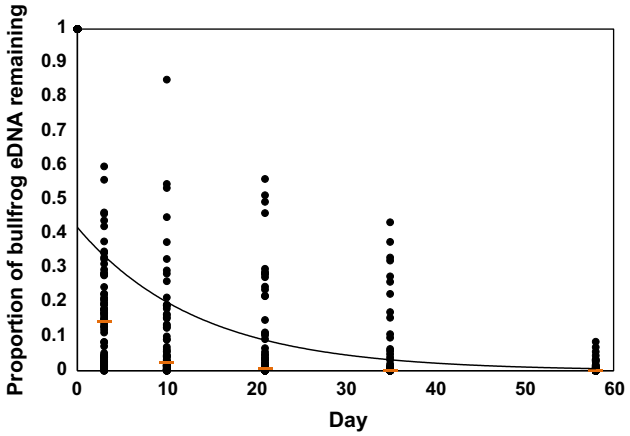

Fig. 2. Proportion of American bullfrog (*Lithobates catesbeianus*) eDNA remaining for 81 microcosms from 0 to 58 days following bullfrog tadpole removal. Horizontal lines represent the median proportion remaining in the sample. Day zero represents the initial amount of detectable bullfrog eDNA in each microcosm.

treatments. Overall degradation rate was higher under pH 4 compared with pH 10 (Fig. 3b; one-way ANOVA $F_{(2,78)} = 3.88$, $P = 0.025$). UV-B did not affect degradation rate when considered across all treatments of pH and temperature (Fig. 3c; one-way ANOVA $F_{(2,78)} = 1.86$, $P = 0.162$).

The full linear regression model with all main effects and interactions (Table 2) was fit to the data ($R^2 = 0.52$, $F = 6.67$, $P < 0.001$). All factors and interactions except pH significantly influenced degradation rate; however, pH had an interactive effect on degradation rate with both temperature and UV-B. The influence of interactions in the model indicate that, while temperature and UV-B affect degradation rate, their effects differ depending on the levels of the other factors. Independently, temperature and UV-B had positive effects on degradation rate, but the direction of the effect reversed when interactions with the other factors were included.

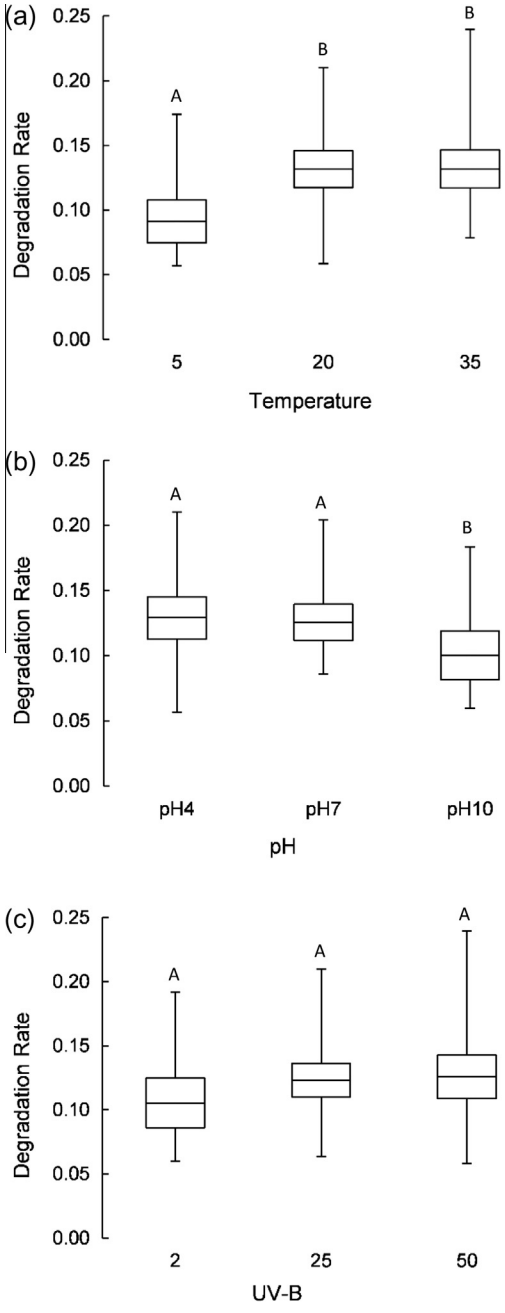

Fig. 3. Effects of (a) temperature, (b) pH, and (c) UV-B radiation on rate of eDNA degradation. Degradation rate represents the rate of change in eDNA detected from Day 0 to Day 58. Horizontal bars in box plots represent mean degradation rates, and upper and lower ends of boxes represent upper and lower 95% confidence intervals. Factor levels with the same letter are not significantly different. Temperature is measured in degrees Celsius (°C). UV-B is measured in kilojoules per square meter per day (kJ/m^2/day).

We found that the exponential decay model represented a high level of variation in the data ($F_{(2,480)} = 377.76$, $P < 0.001$). The degradation rates (r) estimated from the exponential decay model ranged from 0.05 to 0.34 and were lowest for 5 °C for all levels of pH and UV-B (Fig. 4). Estimated time to reach 5% of initial eDNA concentrations was correspondingly highest in low temperature treatments, and ranged from <1 day to 54 days over all treatments. Across all treatments, we estimated a degradation rate of 0.243 ± 0.070, indicating that bullfrog eDNA concentrations decreased by approximately 24% per day.

Total eDNA from non-bullfrog sources ranged from 1.87 to 256.09 ng eDNA/μl (compared to 0 to 5.83 ng bullfrog eDNA/μl) and did not significantly change over the course of the experiment (Fig. 5; $R^2 = 0.004$, $F = 0.10$, $P = 0.75$).

4. Discussion

For eDNA to be a useful tool for monitoring imperiled or invasive species, researchers must be able to focus sampling efforts to periods in which eDNA of the target organism is likely to be present. This requires knowledge about how long eDNA can be detected after the species has left or been removed from the water body of interest. Under controlled conditions, where other forms of eDNA removal (e.g., diffusion, advection, adsorption to particles) were not factors, degradation reduced the mean amount of detectable eDNA by 78% within 3 days and by almost 90% within 10 days of tadpole removal; though eDNA was still detectable at very low concentrations in several microcosms after 58 days.

DNA has been shown to degrade exponentially over long time periods (Willerslev et al., 2004; Allentoft et al., 2012), and we found that bullfrog eDNA degradation followed an exponential pattern of decline, irrespective of experimental treatment. Our estimated degradation rate (0.243) was within the range of similarly estimated rates in other studies (0.322 and 0.701, Thomsen et al., 2012; 0.105, Barnes et al., 2014). The congruence of degradation patterns among studies with a range of environmental covariates (Dejean et al., 2011; Thomsen et al., 2012a,b; Barnes et al., 2014) provides evidence that the initially sharp decrease in eDNA is common across systems and environmental conditions. It is likely that the effects of specific covariates are initially minor but gain influence over time as eDNA becomes more scarce (Fig. 2).

Table 2
Results of linear regression model of the influence of pH, temperature, and UV-B radiation on eDNA degradation rate ($R^2 = 0.52$, $F = 6.67$). pH is treated as a categorical variable with pH 4 as the reference level.

Predictor	Parameter estimate	Standard error	p-Value
Intercept	0.0571	0.0194	0.004
pH 7	0.0370	0.0275	0.190
pH 10	0.0168	0.0275	0.569
Temperature	0.0037	0.0008	<0.001
UV-B	0.0005	0.0006	0.001
pH 7 * UV-B	−0.0005	0.0009	0.038
pH 10 * UV-B	−0.0007	0.0008	0.002
pH 7 * Temperature	−0.0030	0.0012	0.014
pH 10 * Temperature	−0.0032	0.0012	0.008
Temperature * UV-B	−0.00002	0.00003	<0.001
pH 7*Temperature * UV-B	0.00004	0.00004	0.001
pH 10 * Temperature * UV-B	0.00005	0.00004	<0.001

pH4	UV-B (kJ/m²/day) 2	25	50
Temperature (°C) 5	0.05 / 53	0.13 / 22	0.13 / 17
20	0.14 / 14	0.17 / 9	0.14 / 1
35	0.20 / 10	0.15 / 1	0.34 / <1

pH7	UV-B (kJ/m²/day) 2	25	50
Temperature (°C) 5	0.07 / 23	0.12 / 12	0.12 / 12
20	0.14 / 7	0.12 / 1	0.12 / 1
35	0.11 / 8	0.13 / 1	0.15 / 3

pH10	UV-B (kJ/m²/day) 2	25	50
Temperature (°C) 5	0.05 / 53	0.05 / 54	0.05 / 41
20	0.06 / 39	0.12 / 13	0.14 / 13
35	0.09 / 27	0.15 / 11	0.14 / 8

Fig. 4. Estimated within-treatment degradation rate (above diagonal) and estimated number of days for eDNA to fall below 5% of initial quantity (below diagonal) for each full factorial treatment of pH, temperature, and UV-B radiation. Degradation rate and days to 5% were estimated from exponential decay function for bullfrog eDNA.

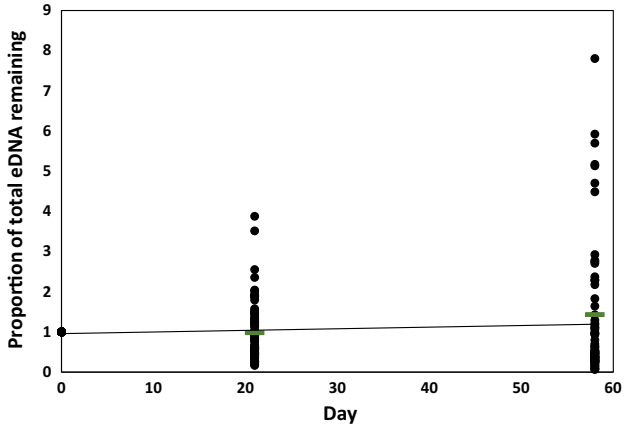

Fig. 5. Proportion of total non-bullfrog eDNA remaining for 81 microcosms at 0, 21, and 58 days following bullfrog tadpole removal. Horizontal lines represent the median proportion remaining in the sample. Day zero represents the initial amount of detectable eDNA in each microcosm.

In our study, temperature had a stronger effect on degradation rate than any other factor, with a greater proportion of bullfrog eDNA remaining at 5 °C than at 25 °C or 35 °C at time steps 10 through 58. Total eDNA concentration in 5 °C microcosms was similar to 25 °C or 35 °C microcosms at the outset of the experiment, but decreased to levels below the other temperatures by Day 58 (Supplementary material, Fig. A1), indicating that microbial growth was lower in the 5 °C microcosms during the later time steps and could have slowed the biotic eDNA degradation process. Regardless of the mechanism, our results provide clear evidence that low temperatures lengthen persistence of eDNA in freshwater and that the impact of temperature is influenced by UV-B and pH.

Solar radiation was also positively associated with increased degradation rate in this study, although this pattern was obscured when considered univariately. Time until <5% of eDNA remained dropped consistently more between UV-B levels 5 kJ/m²/day and 25 kJ/m²/day than between the higher levels of UV-B, suggesting a non-linear effect. Penetration of UV-B in the water column depends on the wavelength of the light and optical qualities of the water (Hargreaves, 2003), and can vary from a few centimeters in humic lakes to 10–20 m in oligotrophic lakes (Kirk, 1994). The water in our microcosms was less than 25 cm deep and likely to be low in humic acids, dissolved organic carbon (DOC), and other substances that may attenuate UV-B radiation (Hargreaves, 2003), suggesting that UV-B penetrated throughout water in the microcosms. In practice, direct effects of UV-B on eDNA degradation are probably limited to shallow or oligotrophic water bodies with low levels of phytoplankton, DOC, and organic matter. This means, since geographic characteristics such as latitude and altitude are primary determinants of UV-B radiation (Godar, 2005), that aquatic environments at higher elevations or closer to the equator are more likely to experience increased effects of UV-B radiation on eDNA degradation rate.

Acidic conditions catalyze hydrolytic processes that degrade DNA (Lindahl, 1993; Alaeddini et al., 2010). Because of this, DNA tends to persist longer in samples with neutral or slightly alkaline pH (Lindahl, 1993) and we expected to find high degradation rates in our acidic microcosms. Overall, our mean degradation rate was higher in pH 4 compared with pH 10 treatments, but in the full model pH was only significant in interactions with other variables and we continued to detect eDNA in some pH 4 microcosms until the end of the experiment. The low pH in our study is at least three units lower than has been reported in other eDNA degradation studies (Zhu, 2006; Barnes et al., 2014), but is similar to the pH of natural forested wetlands in Florida, USA where we have successfully detected eDNA of target amphibians (Goldberg, Strickler, and Fremier, unpublished data). These results show that, by itself, pH as low as 4 does not accelerate eDNA degradation to the point that it limits detection – an encouraging outcome for the use of eDNA in highly acidic aquatic environments. Additionally, systems with higher pH levels, such as alkaline or saline wetlands, may extend the period of time eDNA is detectable. Unexpectedly, neutral conditions in our study were not associated with eDNA persistence, and in fact had generally shorter time to <5% eDNA remaining compared to the other pH levels. We found that total eDNA (which includes eDNA from bullfrog and non-target organisms such as bacteria and algae) increased throughout the study in pH 7 treatments but declined in pH 4 and 10 treatments, suggesting that neutral conditions were more favorable for microbial growth and led to these higher rates of degradation. We note, however, that the bacterial species used in our study were most likely adapted for more neutral environments, which has implications for extrapolating to other environments. In natural settings, bacterial communities adapted to the local pH may result in greater production of exonucleases and correspondingly higher degradation rates than we observed in our acidic and alkaline treatments.

This study was designed to specifically test the influence of degradation on eDNA detection, without consideration of other processes (production and diffusion/advection) that also affect detection rates. Interestingly, however, our experimental setup revealed highly variable rates of DNA production (Fig. 1). Our tadpoles were of similar age and size, housed and fed identically, yet Day 0 DNA levels differed widely across treatments. High levels of individual variability in eDNA production have been documented in other species of amphibians (Pilliod et al., 2014) as well as fish (Klymus et al., 2015), and may be due to differences in individual physiology and behavior. Variability in the production term is a large source of uncertainty in the detection of species using eDNA and warrants further study. Although we found that starting concentration of eDNA did not affect degradation rate, we would expect that the time to reach the threshold of detectability would be shorter when starting concentrations are lower, such as for semi-aquatic or terrestrial species visiting aquatic systems, narrowing the temporal inference for detection of such species. Another unexpected result was the detection of small amounts of eDNA (<0.5 pg/250 mL) in negative control microcosms at the start of the experiment. Detection of this very low level of contamination demonstrates the high level of sensitivity of this detection method.

The results of this experiment indicate that aquatic habitats that are colder, more protected from solar radiation, and more alkaline are likely to hold detectable amounts of eDNA longer than those that are warmer, sunnier, and neutral or acidic. As single factors, warmer temperatures and acidity also degraded eDNA faster while relationships with UV-B were more difficult to discern independently. Factors that contribute to favorable environments for microbial activity (higher temperatures, neutral pH, moderate UV-B) were related to higher degradation rates, lending support to the conclusion that some effects of these factors are mediated in part by biological processes. Although our experiment was conducted under controlled conditions, the levels of temperature, pH, and UV-B we selected are representative of a wide range of aquatic systems. We expect that the general patterns we found in the laboratory for the relative effects of each factor on eDNA persistence will extend to natural environments.

Within the environmental conditions in this study, the variation in the length of time eDNA is predicted to be detectable after the target organism was removed was <1 to 54 days. Because effective eDNA sampling depends on identifying the window of highest probability of detecting the target species, the wide range of predicted persistence times underscores the importance of characterizing degradative conditions to best design sampling intervals and understand the temporal inference of positive and negative detections in each study system. We believe that, with improved understanding of factors influencing eDNA production, transport, degradation, and other mechanisms of removal, eDNA will be an increasingly powerful tool for accurate, robust detection and monitoring of sensitive, rare, or elusive species.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.11.038.

References

Allan, J.D., Castillo, M.M., 2007. Stream Ecology: Structure and Function of Running Waters, second ed. Springer, New York, USA.
Alaeddini, R., Walsh, S.J., Abbas, A., 2010. Forensic implications of genetic analyses from degraded DNA—a review. Forensic Sci. Int. Gen. 4, 148–157.
Allentoft, M.E., Collins, M., Harker, D., Haile, J., Oskam, C.L., Hale, M.L., Campos, P.F., Samaniego, J.A., Gilbert, M.T.P., Willerslev, E., Zhang, G., Scofield, R.P., Holdaway, R.N., Bunce, M., 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proc. R. Soc. B: Biol. Sci. 279, 4724–4733.
Austin, J.D., Lougheed, S.C., Boag, P.T., 2004. Controlling for effects of history and nonequilibrium conditions in gene flow estimates in northern bullfrog (*Rana catesbeiana*) populations. Genetics 168, 1491–1506.
Barnes, M., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L, Lodge, D.M., 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environ. Sci. Technol. 48, 1819–1827.
Corinaldesi, C., Beolchini, F., Dell'Anno, A., 2008. Damage and degradation rates of extracellular DNA in marine sediments: implications for the preservation of gene sequences. Mol. Ecol. 17, 3939–3951.
Culp, C.E., Falkinham, J.O., Belden, L.K., 2007. Identification of the natural bacterial microflora on the skin of eastern newts, bullfrog tadpoles and redback salamanders. Herpetologica 63, 66–71.
Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., Miaud, C., 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS ONE 6, e23398.
Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., Miaud, C., 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. J. Appl. Ecol. 49, 953–959.
Díaz-Ferguson, E.E., Moyer, G.R., 2014. History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. Rev. Biol. Trop. 62, 1273–1284.
Diffey, B.L., 2002. Sources and measurement of ultraviolet radiation. Methods 28, 4–13.
Fu, X.H., Wang, L., Le, Y.Q., Hu, J.J., 2012. Persistence and renaturation efficiency of thermally treated waste recombinant DNA in defined aquatic microcosms. J. Environ. Sci. Health. Part A: Toxic/Hazard. Subst. Environ. Eng. 47, 1975–1983.
Godar, D.E., 2005. UV doses worldwide. Photochem. Photobiol. 81, 736–749.
Goldberg, C.S., Pilliod, D.S., Arkle, R.S., Waits, L.P., 2011. Molecular detection of vertebrates in stream water: a demonstration using rocky mountain tailed frogs and Idaho giant salamanders. PLoS ONE 6, e22746.
Goldberg, C.S., Sepulveda, A., Ray, A., Baumgardt, J., Waits, L.P., 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshw. Sci. 32, 792–800.
Häder, D.-P., Kumar, H.D., Smith, R.C., Worrest, R.C., 2003. Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochem. Photobiol. Sci. 2, 39–50.
Hargreaves, B.R., 2003. Water column optics and penetration of UVR. In: Helbling, E.R., Zagarese, H. (Eds.), UV Effects in Aquatic Organisms and Ecosystems. Royal Society of Chemistry, Cambridge, UK, pp. 59–105.
Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Pääbo, S., 2001. Ancient DNA. Nat. Rev. Genet. 2, 353–359.
Jerde, C.L., Mahon, A.R., Chadderton, W.L., Lodge, D.M., 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv. Lett. 4, 150–157.
Kirk, J.T.O., 1994. Optics of UV-B radiation in natural waters. Arch. Hydrobiol. Beih. Ergebn. Limnol. 43, 1–16.
Klymus, K.E., Richter, C.A., Chapman, D.C., Paukert, C., 2015. Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix. Biol. Cons. 183, 77–84.
Lindahl, T., 1993. Instability and decay of the primary structure of DNA. Nature 362, 709–715.
Lodge, D.M., Turner, C.R., Jerde, C.L., Barnes, M.A., Chadderton, L., Egan, S.P., Feder, J.L., Mahon, A.R., Pfrender, M.E., 2012. Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. Mol. Ecol. 21, 2555–2558.
Matsui, K., Honjo, M., Kawabata, Z.I., 2001. Estimation of the fate of dissolved DNA in thermally stratified lake water from the stability of exogenous plasmid DNA. Aquat. Microb. Ecol. 26, 95–102.
Piaggio, A.J., Engeman, R.M., Hopken, M.W., Humphrey, J.S., Keacher, K.L., Bruce, W.E., Avery, M.L., 2014. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. Mol. Ecol. Resour. 14, 374–380.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P., 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can. J. Fish. Aquat. Sci. 70, 1123–1130.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P., 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Mol. Ecol. Resour. 14, 109–116.

Poté, J., Ackermann, R., Wildi, W., 2009. Plant leaf mass loss and DNA release in freshwater sediments. Ecotoxicol. Environ. Saf. 72, 1378–1383.

Q-Lab, 2011. Sunlight, Weathering, & Light Stability Testing. Q-Lab Technical Bulletin LU-0822. Q-Lab, Westlake, OH.

Ravanat, J.L., Douki, T., Cadet, J., 2001. Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol. B, Biol. 63, 88–102.

Sommaruga, R., 2001. The role of solar UV radiation in the ecology of alpine lakes. J. Photochem. Photobiol. B, Biol. 62, 35–42.

Taberlet, P., Waits, L.P., Luikart, G., 1999. Noninvasive genetic sampling: look before you leap. Trends Ecol. Evol. 14, 323–327.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., Willerslev, E., 2012a. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE 7, e41732.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L., Willerslev, E., 2012b. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. 21, 2565–2573.

Turner, C.R., Barnes, M.A., Xu, C.C., Jones, S.E., Jerde, C.L., Lodge, D.M., 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol. Evol. 5, 676–684.

Willerslev, E., Hansen, A.J., Rønn, R., Brand, T.B., Barnes, I., Wiuf, C., Gilichinsky, D., Mitchell, D., Cooper, A., 2004. Long-term persistence of bacterial DNA. Curr. Biol. 14, R9–R10.

Zhu, B., 2006. Degradation of plasmid and plant DNA in water microcosms monitored by natural transformation and real-time polymerase chain reaction (PCR). Water Res. 40, 3231–3238.