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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.

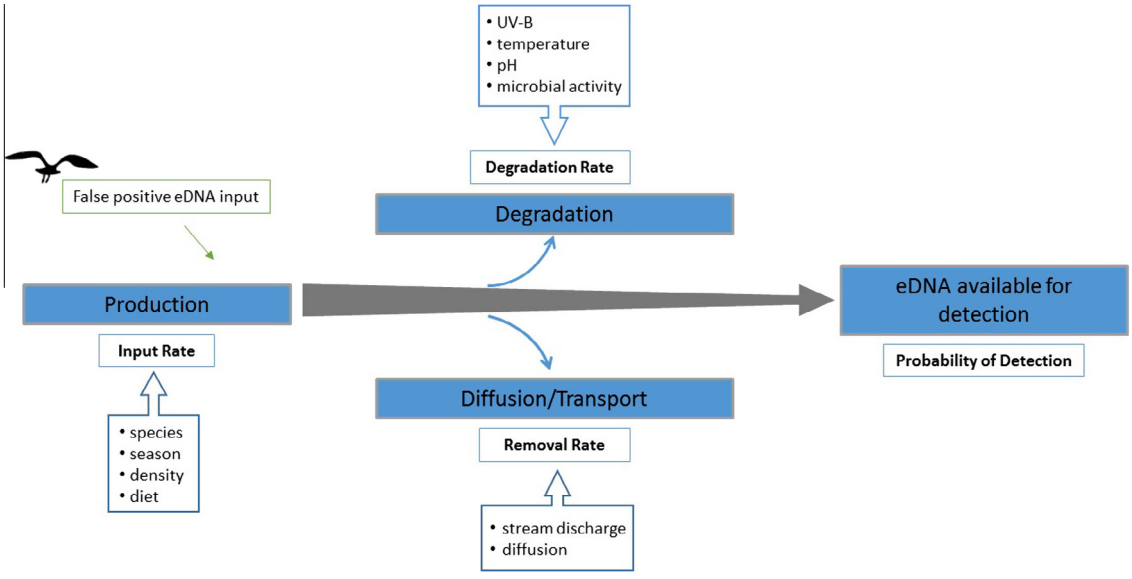

Fig. 1. Conceptual model of factors affecting eDNA detection. Following input of eDNA into an aquatic system, eDNA is removed from the system through degradation and by diffusion and transport processes, reducing the amount available for eDNA detection. Arrow boxes identify some of the biotic and abiotic 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.