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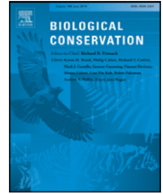

Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*

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ABSTRACT

Environmental DNA sampling (eDNA) has emerged as a powerful tool for detecting aquatic animals. Previous research suggests that eDNA methods are substantially more sensitive than traditional sampling. However, the factors influencing eDNA detection and the resulting sampling costs are still not well understood. Here we use multiple experiments to derive independent estimates of eDNA production rates and downstream persistence from brook trout (*Salvelinus fontinalis*) in streams. We use these estimates to parameterize models comparing the false negative detection rates of eDNA sampling and traditional backpack electrofishing. We find that using the protocols in this study eDNA had reasonable detection probabilities at extremely low animal densities (e.g., probability of detection 0.18 at densities of one fish per stream kilometer) and very high detection probabilities at population-level densities (e.g., probability of detection >0.99 at densities of ≥3 fish per 100 m). This is substantially more sensitive than traditional electrofishing for determining the presence of brook trout and may translate into important cost savings when animals are rare. Our findings are consistent with a growing body of literature showing that eDNA sampling is a powerful tool for the detection of aquatic species, particularly those that are rare and difficult to sample using traditional methods.

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1. Introduction

Environmental DNA (eDNA) sampling has recently emerged as a powerful tool for detecting aquatic animals. These methods detect genetic material in environmental samples (e.g., stream water) to indirectly infer the presence of a species (Jerde et al., 2011). This approach is especially useful for detecting species that are difficult to sample using traditional methods (Taberlet et al., 2012), for non-invasively sampling critically endangered species (Sigsgaard et al., 2015), and for distinguishing cryptic species (Fukumoto et al., 2015). Since it was first used to detect aquatic animals (Ficetola et al., 2008) there has been an explosion of research on eDNA methods, particularly with respect to rare invasive species (e.g., Dejean et al., 2012; Goldberg et al., 2013; Moyer et al., 2014) and threatened native species (Spear et al., 2015; Thomsen et al., 2012).

Previous research suggests that eDNA methods may be substantially more sensitive and cost-effective than traditional sampling for species detection (Biggs et al., 2015; Jerde et al., 2011; Sigsgaard et al., 2015). However, there has been large variation in reported sensitivities, and eDNA production rates are still unknown for most species. Several studies have related eDNA concentration to animal abundance or biomass (Klymus et al., 2015; Pilliod et al., 2013; Takahara et al., 2012), but variation in eDNA production rates among individuals is also very high (Klymus et al., 2015; Pilliod et al., 2014; Strickler et al., 2015).

The eDNA produced by aquatic organisms is distributed in the environment and lost as a function of degradation, dilution, deposition, and re-suspension (Strickler et al., 2015; Turner et al., 2015). Several studies have assessed rates of eDNA degradation, which usually occurs over hours to days (Barnes et al., 2014; Pilliod et al., 2014; Strickler et al., 2015), but the other processes affecting eDNA concentrations in aquatic systems are less understood. For example, the downstream transport of eDNA in lotic systems implies that animals can be detected some distance from their location (e.g., <50 m to up to 12 km; Deiner and Altermatt, 2014; Jane et al., 2015, Pilliod et al., 2014), but because little

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work has been done to quantify the physical transport of eDNA in rivers and streams (e.g., deposition, re-suspension), we do not know the absolute or relative importance of these factors to species detection.

Here we present a simple model of eDNA concentration in lotic systems that is a function of animal abundance (*fish*), how distant those animals are upstream of the sampling site (*distance*), how far downstream the eDNA persists, and the discharge of the stream (*discharge*). If eDNA behaves similarly to other fine particulate organic matter (FPOM), then its longitudinal persistence can be modeled as an exponential decline with a settling velocity k (downstream persistence $= 1 - k$; Paul and Hall, 2002).

$$\textit{eDNA conc.} = \frac{(\textit{fish} \times \text{production}) \times (1-\text{k})^{\textit{distance}}}{\textit{discharge}} \quad (1)$$

We use brook trout (*Salvelinus fontinalis*) as a case study to estimate the rate of eDNA production per individual (*production*) and the downstream persistence of that eDNA ($1 - k$) in streams using data from an observational study (Sections 2.2 and 2.3), mesocosm experiments (Section 2.4), and caged fish experiments (Section 2.5). We then use longitudinal sampling of two streams to internally validate this simple model of eDNA in streams. Finally, we use this model to compare the sensitivity of eDNA and traditional backpack electrofishing to detect rare fish. This is of conservation interest because brook trout are an important invasive species globally (Dunham et al., 2003; Wenger et al., 2011), and of conservation concern within their native range (Hudy et al., 2008). Further, findings from this study will be applicable to other stream organisms of conservation interest, whether as potential invaders or threatened native species, and will increase our general understanding of the relative sensitivity and efficiency of traditional and eDNA-based sampling.

2. Material and methods

2.1. eDNA sampling and analysis

Field samples were collected using a peristaltic pump (GeoTech; Denver, Colorado, USA) to draw stream water through a 47-mm diameter, 1.5-μm-pore glass filter (GE Healthcare; Pittsburg, Pennsylvania, USA) held by either an in-line filter holder (GeoTech) or a disposable filter cup holder (ThermoFisher Scientific; Waltham, Massachusetts, USA). If a filter became clogged with debris, it was replaced with additional filters (≤3 total) until the total sample volume was filtered. Filters were folded with forceps and individually sealed in a plastic bag with approximately 50 mL of silica desiccant. Filters with eDNA stored this way are stable at ambient temperatures for at least two weeks (unpublished data). Samples were protected from direct exposure to sunlight in the field and transferred to a −20 °C freezer within one week of sample collection.

Filter holders, forceps, and any other equipment that came into contact with the eDNA samples were sterilized between each sample by soaking in a bleach solution for >20 min, then thoroughly rinsed with distilled water or tap water that lacked brook trout DNA. We ran water through hosing for the peristaltic pump to remove all traces of bleach. For the observational field study, we used a 20% household bleach (8.25% sodium hypochlorite) solution. After observing low-level contamination in two equipment controls during the observational study, we increased the bleach solution to 50% for subsequent experiments. For the mesocosm experiments we adopted an improved field protocol designed to avoid contamination (Carim et al., 2015). This improved protocol includes single-use kits for each sample. The sample collector only handles the filter holder, filter, and other materials through sterile plastic bags or single-use forceps. We observed no further contamination after revising the field protocol.

One half of each filter was extracted using the Qiagen Blood and Tissue DNeasy Kit with QIAshredder columns (QIAGEN; Valencia, California, USA; protocol adapted from Goldberg et al., 2011). Where a sample required multiple filters each half filter went through lysis separately. The lysates were then combined on the silica spin column for washing and final elution.

The DNA was eluted into 100 μL sterile TE buffer (Integrated DNA Technologies; Coralville, Iowa, USA) for consistency with standard curve dilutions. At least one extraction control was included per batch of 23 samples and field equipment controls. All extracted DNA was stored at −20 or −80 °C until qPCR analysis. The other half of each filter was stored for future analyses.

We used a species-specific qPCR assay (Wilcox et al., 2013) to estimate the brook trout mitochondrial DNA (mtDNA) concentration in all samples. Experiments were run in triplicate 15-μL reactions multiplexed with an internal positive control to test for PCR inhibition. Any samples with PCR inhibition – as evident from a ≥1 C_t shift in the internal positive control – were re-extracted using 1/4 of the original sample filter. This was found to alleviate inhibition in these samples, but did not increase DNA yields in uninhibited samples (unpublished data). Each plate also included triplicate no-template control wells and a five-point standard curve for quantification using the Cy_0 method (Guescini et al., 2008). Details on PCR components, cycling conditions, and standard curve preparation can be found in Wilcox et al. (2013, 2015).

All extractions were done in a room reserved for extracting non-invasive genetic samples where no PCR products or other sources of high concentration DNA are handled. All PCR experiments were set up inside of an enclosure that was irradiated with UV for one 1 h prior to use, along with all consumables and pipettes. Reagents were aliquoted in small quantities prior to experiments such that each reagent tube was opened only once.

2.2. Observational study

We used an observational field study to estimate eDNA production rate per fish and downstream persistence (*production* and $1 - k$ from Eq. (1)). Between July and September 2013, we sampled 49 sites across 16 streams in the Shields River and Blackfoot River watersheds in Montana, USA, using both eDNA and electrofishing. Streams were generally small (mean wetted width and discharge = 3 m and 65 L/s, respectively) and cold (mean approx. 13 °C at time of sampling). Mean stream reach gradients were 2.4% (range = 0.6–6.6%; determined from a 1:24,000 digital elevation map in ArcGIS; a summary of site conditions can be found in Appendix A). At each site, we collected 5-L eDNA samples at the top and bottom of stream reaches (mean length = 108 m, range = 75–330 m). Reach lengths were variable because much of the sampling was conducted in conjunction with electrofishing at traditionally sampled sites. To determine spatial longitudinal variation in eDNA concentration for two streams (Buck and Deep), we sampled every 100 m within 800-m and 1000-m sections (resulting in 8 and 10 contiguous sites, respectively). We used these contiguous data to test predictions of our eDNA model against observed data. Deep Creek was slightly larger than Buck Creek (mean wetted width = 3.4 versus 3.1 m and mean discharge = 104.7 versus 70.4 L/s), but similar in gradient (mean = 2.7 and 3.0% for Deep Creek and Buck Creek, respectively).

Prior to sampling at each site, we collected a field equipment control by filtering 1 L of distilled water through a clean filter and storing as above. For the longitudinal sampling of Buck Creek and Deep Creek, we collected a single equipment control prior to sampling for a total of 31 field equipment controls. Field equipment controls functioned to detect any contamination from the sampling equipment, filter handling, or storage.

After paired downstream–upstream eDNA samples were collected at each site, we sampled the intervening reach using backpack electrofishing (1–24 h following eDNA sampling) to estimate the abundance of brook trout ≥75 mm total length. Detection probabilities for fish <75 mm total length were too low to estimate the abundance of these

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