

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



Taylor M. Wilcox^{a,b,*}, Kevin S. McKelvey^a, Michael K. Young^a, Adam J. Sepulveda^c, Bradley B. Shepard^{d,1}, Stephen F. Jane^e, Andrew R. Whiteley^e, Winsor H. Lowe^b, Michael K. Schwartz^a

^a U.S. Department of Agriculture, Forest Service, National Genomics Center for Wildlife and Fish Conservation, Rocky Mountain Research Station, Missoula, MT 59801, USA

^b Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

^c US Geological Survey Northern Rocky Mountain Science Center, Bozeman, MT 59715, USA

^d B.B. Shepard and Associates, Livingston, MT 59047, USA

^e Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, USA

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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., 2014c). 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

* Corresponding author at: U.S. Department of Agriculture, Forest Service, National Genomics Center for Wildlife and Fish Conservation, Rocky Mountain Research Station, Missoula, MT 59801, USA.

E-mail address: taylor.wilcox@umontana.edu (T.M. Wilcox).

¹ B.B. Shepard was with the Wildlife Conservation Society, Bozeman, MT 59715, USA during most of this study.

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).

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

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- μ m-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 μ L 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- μ L 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 C_{y0} 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

very small animals. However, in some sites these small, unquantified animals may also have contributed to eDNA production and their relative eDNA contribution likely varied among sites. We used multiple electrofishing passes to derive a removal estimate at 15 sites (assuming no fish movement out of the reach during sampling; Otis et al., 1978), and a single electrofishing pass at 34 sites (includes sites in Buck Creek and Deep Creek). We calibrated estimates from our single-pass sites with estimates of capture efficiency from removal estimates in the same stream or a nearby stream of similar size (Appendix A). We measured total length and weight of all brook trout captured, except for eight sites where we used length–weight regressions from the same stream ($n = 7$ in Deep Creek) or from a nearby stream ($n = 1$) to predict weight from fish length (Appendix A). We also measured stream discharge at the downstream end of the site at the time of eDNA sample collection (midsection method; Hauer and Lamberti, 2007), except for three sites where estimates from nearby sites (<1 km upstream or downstream) were used. Discharges on Buck and Deep Creeks were averaged from three transects.

2.3. Analysis for observational study

The eDNA concentrations in the downstream samples at these sites were a function of eDNA produced by fish within the reach (known abundance) plus eDNA produced by fish upstream of the reach. Environmental DNA produced by fish within the reach is represented by Eq. (1), where we assumed that there is no eDNA degradation from fish within the site. The abundance of fish upstream of the study reach is unknown, but their total eDNA input is known (*upstream input*), and we expect this eDNA to decline to a concentration of $1 - k$ (where k is the settling velocity per 100 m) over a 100 m reach (Eq. (2)). Forty of the 49 sites were 100 ± 10 m (mean = 108 m) and only one site was less than 90 m, so we assumed a 100 m site length for analysis. Sites longer than 100 m underestimate persistence per 100 m, making this approximation conservative. Further, a more complex model incorporating eDNA degradation within the site and variable reach lengths resulted in a somewhat larger estimate of eDNA production per fish, but no difference in downstream persistence of eDNA per 100 m (data not shown).

$$eDNA\ conc. = \frac{(fish \times production) \times (1 - k)^0}{discharge} + \frac{(upstream\ input) \times (1 - k)^1}{discharge} \quad (2)$$

Discharge is known at each site, so we can rearrange this equation into a simple regression model to solve for production rate per fish and persistence of eDNA per 100 m where β_0 is the intercept, $\beta_{production}$ the production rate of mtDNA per brook trout (mitochondrial DNA copies/s), and $\beta_{(1-k)}$ is the proportion of eDNA that persists per 100 m of stream length.

$$eDNA\ output = \beta_0 + \beta_{production} fish + \beta_{(1-k)} upstream\ input \quad (3)$$

We then tested the ability of these parameter estimates and our model (Eq. (1)) to predict eDNA concentrations in the longitudinally-sampled Deep and Buck Creeks. We also performed complementary stochastic simulations, which are described in Appendix C.

2.4. Mesocosm experiments

To generate an independent estimate of eDNA production per fish (*production* from Eq. (1)), we conducted mesocosm experiments in nine tanks ($0.35\text{ m} \times 0.5\text{ m} \times 0.22\text{ m}$) with water fed to the top of the tank from an ephemeral stream, as described in Wilcox et al. (2015). No substrate was added to the bottom of the tanks to prevent any influence due to differences in tank composition. We captured brook trout

from West Fork Lolo Creek, MT using backpack electrofishing (MFWP sampling permit SCP-33-14). We then added a single brook trout (mean mass = 32 g, range = 13–69 g; mean total length = 147 mm, range = 112–187 mm) to each of eight mesocosm tanks, leaving one tank as a negative control. Fish were not fed for the duration of the 48 h study. Water flow was maintained through each tank at a minimum of 0.3 L/min. One-liter eDNA samples were taken from all nine tanks 24 and 48 h after fish addition. Each day that samples were collected, we also collected an equipment control using 0.5 L of distilled water. After 48 h, each fish was weighed, measured, and released back into West Fork Lolo Creek. Tanks were then sterilized with a 50% bleach solution, rinsed with water from the fishless tributary, and the experiments were repeated for a total of 16 individuals.

2.5. Caged fish experiments

We also used data from a caged fish experiment (Jane et al., 2015) to estimate brook trout eDNA production and downstream persistence (*production* and $1 - k$ from Eq. (1)). Jane et al. (2015) introduced caged brook trout to two otherwise fishless streams (Amethyst and Avery) in Massachusetts, USA, and then measured eDNA concentrations at regular intervals downstream. Samples were collected by drawing 6 L of stream water through a $1.5\text{-}\mu\text{m}$ -pore glass filter, as in the observational and mesocosm studies. Extraction and analysis methods (and thus likely eDNA yield) differed from those in this study, but were expected to result in similar longitudinal loss rates. To compare eDNA production rates observed in Jane et al. (2015) with our observational and mesocosm studies, we used the eDNA concentrations from 27.5 m downstream from the caged fish (first downstream sample taken) to estimate mtDNA production/fish/second.

We estimated the longitudinal loss rate (k ; per meter) as the slope in the regression of log mtDNA copy number + 1 against distance downstream for each sampling session separately. This single variable regression approach to estimate k represents a new analysis from that presented in Jane et al. (2015) that explored multiple predictors of downstream eDNA concentration. From these estimates of k , we calculated DNA transport distance (S_p ; per session) as $1/(1 - k)$, which describes the mean distance that a particle is transported downstream prior to deposition (Paul and Hall, 2002). We compared these estimates of S_p with those from the observational study (also calculated as $1/(1 - k)$), and with the range of S_p values reported in five fine particulate organic matter (FPOM) and FPOM-surrogate stream transport studies (reviewed in Paul and Hall, 2002). As in the observational study, we did not have a conservative tracer to correct for dilution from groundwater inputs, so these analyses probably overestimate the longitudinal loss rate (k) and underestimate transport distance (S_p), making our estimates of downstream eDNA persistence conservative.

2.6. Non-detection probability

We used simple models to compare the non-detection probability (failure to detect) of eDNA and electrofishing when sampling for rare brook trout in streams. The number of fish (*fish*) in a given 100 m stream reach is a random variable and a function of animal density (which follows a Poisson distribution when animals are randomly-distributed). Electrofishing has some rate of failure to detect a single fish within a 100 m reach (f) so that the probability of a non-detection (F) in a reach is a function of fish density (*density*; Eq. (4)). The expected value of F can be expressed as below (ignoring any value of *fish* greater than 10 because $F < 0.001$ when *fish* > 10 as long as $f < 0.5$).

$$E(F|density) \approx \sum_{k=0}^{10} f^k \times \Pr(\text{fish} = k | \text{density}) \quad (4)$$

For eDNA, there is an additional sampling step. The expected concentration of eDNA in a stream is explained by Eq. (1). The mean number of mtDNA copies from this site that end up in PCR reactions (λ) depends on the sampling protocol (*protocol*; Eq. (5)). For example, in our observational study, we sampled 5 L of the stream, and performed PCR on 6% of this sample (50% of the filter extracted and then 12% of the elution was in PCR reactions), so $\lambda = 30\%$ of mtDNA copies/L at the site.

$$\lambda = \frac{(\text{fish} \times \text{production}) \times (1 - k)^{\text{distance}}}{\text{discharge}} \times \text{protocol} \quad (5)$$

Because of stochasticity in the capture of individual mtDNA copies, the actual number of copies in PCR reactions (N) is a random variable with a mean of λ . Again, there is a per mtDNA copy failure rate (f) so that the expected probability of non-detection (F) can be described similarly to that for electrofishing.

$$E(F|\lambda) \approx \sum_{k=0}^{10} f^k \times \Pr(N = k|\lambda) \quad (6)$$

In electrofishing, detection is only possible if there are fish in the sampled reach. In eDNA, there is a probability of detection (i.e., $\lambda > 0$) even when fish are only present in reaches upstream of the sampled reach. Based on previous sampling, we can conservatively suggest that $\lambda > 0$ when *fish* > 0 at least up to 300 m upstream (e.g., Jane et al., 2015 report 100% detection rates of fish 240 m upstream). For a given fish density, we have the probability of detecting fish in the first 100 m reach upstream (distance_1), the second 100 m reach (distance_2), or the third (distance_3). A non-detection means that fish were not detected from any of these three reaches, so the expected non-detection rate is:

$$\text{Total } E(F) = E(F|\lambda|\text{distance}_1) \times E(F|\lambda|\text{distance}_2) \times E(F|\lambda|\text{distance}_3) \quad (7)$$

We used these models and empirical estimates of per target failure rate for eDNA amplification ($f = 0.2$; estimated from Wilcox et al., 2013) and electrofishing 100 m reaches ($f = 0.3$; approximate mean from the observational study in this paper), *production*, and eDNA downstream persistence ($1 - k$; using mean estimates and 95% CI bounds around each from the observational study) to predict probability of non-detection for both sampling methods over a range of fish densities (0.01–5 individuals per 100 m) in 65 L/s discharge streams (mean for streams in our observational study).

3. Results

3.1. Observational study

Low levels of brook trout mtDNA were observed in two of the equipment controls and one upstream sample was lost. After removing these three sites, there were 46 sites for further analysis. No amplification was observed in any of the other field equipment controls, extraction controls, or qPCR setup controls. We detected brook trout mtDNA at all of the sites where brook trout were detected using electrofishing (estimated detection probability from depletion estimates 0.38–1.00). We also detected brook trout at six sites using eDNA where electrofishing did not capture any brook trout at the site; however, all of these sites were in streams where brook trout were detected using electrofishing in another site on the same stream ($n = 5$), or had been detected upstream in previous years ($n = 1$; B.B. Shepard, unpublished data). As expected, brook trout were not detected by electrofishing or eDNA at two sites believed to be above the species' upstream distribution. Because both eDNA and electrofishing have non-zero false negative rates, we cannot determine with certainty if we failed to detect brook trout DNA at these two sites because fish were in fact absent, or because we simply failed to detect fish using both methods.

Brook trout abundance alone was correlated with eDNA concentration ($P < 0.001$, $r^2 = 0.592$; Fig. 1), and our model predicting downstream eDNA from brook trout abundance (estimated by electrofishing) and upstream eDNA input was highly significant ($P < 0.001$, $r^2 = 0.754$). The model intercept was not significant ($P = 0.451$). Coefficients for eDNA production per fish ($\beta_{\text{production}}$) and downstream persistence ($\beta_{(1-k)}$) were highly significant (mean = 495 mtDNA copies/s and 0.63/100 m for $\beta_{\text{production}}$ and $\beta_{(1-k)}$, respectively; $P < 0.001$). Using fish biomass in the place of fish number did not improve model fit ($r^2 = 0.737$; range = 0–3911 g fish), so we used fish number for further analysis. Because this model used a normal error distribution, we compared our mean coefficient estimates and the 95% CI around them with the median and 0.05–0.95 quantile range observed in the mesocosm and caged fish experiments.

3.2. Mesocosm experiments

During the first mesocosm session, four of the eight experimental tanks did not have any water flow when checked at 48 h. Because eDNA probably accumulated in tanks during this time, samples were not taken from these tanks at 48 h. During the second session, one fish was taken from its tank (presumably by a predator), so this tank was dropped from the analysis. This left data from 15 individuals after 24 h and 11 individuals after 48 h.

The distribution of eDNA production rates was strongly right-skewed (24-h median = 990 copies/s, 0.05–0.95 quantile range = 115–3084; Fig. 2). Environmental DNA production rates did not significantly differ over time (Wilcoxon rank-sum test comparing 24- and 48-h samples: median = 990 and 977 copies/s, respectively; $n = 11$, $W = 68$, $P = 0.474$), or with individual weight (Spearman's rank correlations: $\rho = 0.063$ and 0.118 , $P = 0.825$ and 0.729 for 24 and 48 h, respectively), or with flow rate ($\rho = 0.104$ and 0.200 , $P = 0.714$ and 0.558 for 24 and 48 h, respectively). Therefore, we used eDNA production rates 24 h after fish addition for comparison with results from the observational study and caged fish experiments.

3.3. Caged fish experiments

Environmental DNA production rates in the caged fish experiments were also right-skewed, with four observations $> 22 \times$ the median (median = 430 copies/s, 0.05–0.95 quantile range = 60–12,104; Fig. 2). One of the longitudinal loss curves from Amethyst was slightly positive

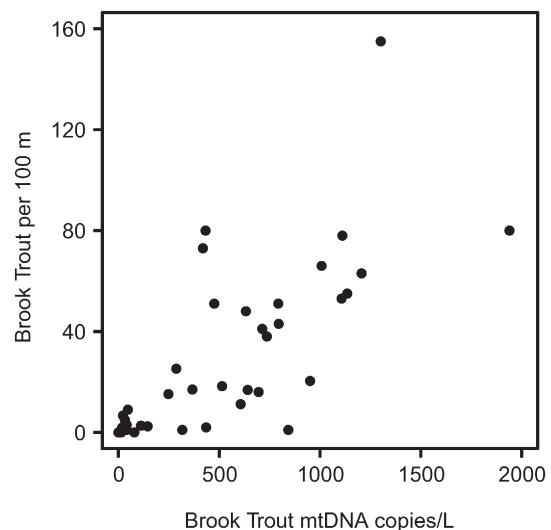


Fig. 1. Brook trout eDNA concentration (mitochondrial DNA copies/L) versus estimated number of brook trout ≥ 75 mm TL per 100 m of stream as estimated by backpack electrofishing in the observational study. Environmental DNA concentration and brook trout number were positively correlated ($P < 0.001$, $r^2 = 0.592$, $n = 46$ stream reaches).

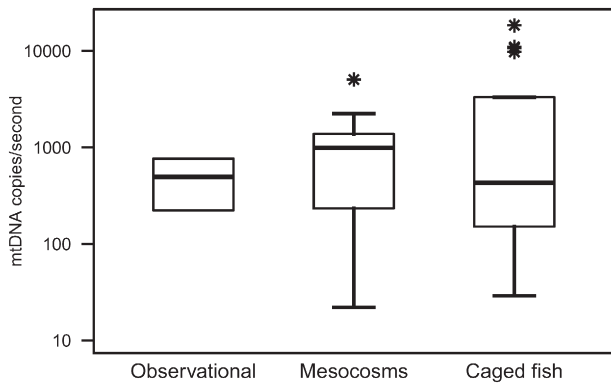


Fig 2. Estimated eDNA production rates per fish from the observational study (mean = 495 copies/s), mesocosm (median = 990 copies/s), and caged fish (Jane et al., 2015; median = 430 copies/s) experiments. For the observational study, the middle line and box represent the model coefficient estimate and 95% CI. For the caged fish and mesocosm experiments the boxplots represent the minimum, median, maximum, and interquartile ranges of the observed data.

(eDNA concentration increased with distance downstream), yielding a nonsensical estimate of transport distance (i.e., $1/k$ when k is positive results in a negative number), so we dropped this session from the analysis. This result could be due to stochasticity in the capture of DNA-containing particles in the environment or to stochasticity in the quantification of rare mtDNA copies. Amethyst had higher median transport distance (median = 145 m, 0.05–0.95 quantile range = 72–1459) and stream discharge (median = 9.7 L/s, 0.05–0.95 quantile range = 1.0–96.0) than Avery (transport distance: median = 74 m, 0.05–0.95 quantile range = 41–222; stream discharge: median = 5.9 L/s, 0.05–0.95 quantile range = 1.4–9.5; Figs. 3 and 4).

3.4. Comparison of production and transport parameter estimates

Mean eDNA production from the observational study (mean = 495 copies/s, 95% CI = 222–768) was similar to the median value from the caged fish study (430 copies/s), but was about half the median value from the mesocosm experiments (990 copies/s).

We converted our estimate of downstream persistence of eDNA per 100 m from the observational study into eDNA transport distance (S_p ; the mean distance that a particle is transported prior to deposition) for comparison with the caged fish study. This estimate of transport distance (mean = 270 m, 95% CI = 167–769) was greater than the median for either stream in the caged fish study. Of the two streams sampled in the caged fish study, the median value for Amethyst (145 m) was greater than for Avery (74 m). These values fall within the large variation in transport distances reported in other studies of FPOM in streams (Fig. 3).

We used parameter estimates from the observational study to predict eDNA concentrations in Buck Creek and Deep Creek from the distribution of brook trout determined by electrofishing. Our eDNA model resulted in predicted eDNA concentrations that were on average lower than, but not significantly different from, the observed concentrations in Buck Creek and Deep Creek (mean predicted – observed = –85 and –88 mtDNA copies/L, $t = -1.51$ and -1.48 , $df = 7$ and 9 , $P = 0.174$ and 0.174 , respectively). Brook trout were common in Deep Creek (estimated 38–155 fish ≥ 75 mm total length per 100-m reach) and rare in Buck Creek, with five of the eight most upstream reaches (500 m) containing no brook trout. Our model resulted in a larger underestimate of eDNA concentration in the three Buck Creek reaches with brook trout (mean = –169 copies/L), but this difference was also not significant ($t = -1.71$, $df = 3$, $P = 0.186$).

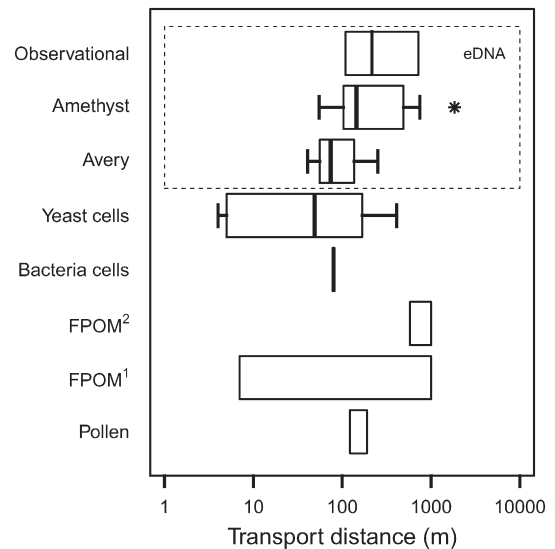


Fig 3. Transport distance (m) of eDNA in the observational study (*Observational*) and two caged fish experiments (the streams *Amethyst* and *Avery*) and of fine particulate organic matter (FPOM) and FPOM-surrogates from five studies reviewed in Paul and Hall (2002). Transport distance (S_p) is a metric of persistence that describes the mean distance that a particle travels downstream before deposition. Data from this study (eDNA) are enclosed in a black dotted box. The FPOM and FPOM-surrogate studies include brewer's yeast (*Yeast cells*; Paul and Hall, 2002), *Escherichia coli* cells (*Bacteria cells*; Hall et al., 1996), radio-labeled fine particulate organic matter (FPOM; Minshall et al., 2000, Cushing et al., 1993), and corn (*Zea mays*) pollen (*Pollen*; Miller and Georgian, 1992). The boxes for *Amethyst*, *Avery*, and *Yeast cells* show the distribution of individual estimates ($n = 8, 9$, and 6 , respectively), the other FPOM studies show only minimum and maximum transport distances, and the *Observational* box shows the model coefficient estimate and 95% CI.

3.5. Non-detection probability

Using parameter estimates from the observational study, which represents our best estimates of these parameters in natural systems, our models predicted that eDNA probability of non-detection is substantially lower than that for electrofishing 100 m stream reaches at low fish densities (e.g., estimated non-detection probabilities at densities of one fish per 100 m are 0.130 and 0.497 for eDNA and electrofishing, respectively; Fig. 5). This was consistent with results from stochastic simulations using a similar model of eDNA production, downstream persistence, and sampling in streams (Appendix C) and may result in lower eDNA sampling costs to achieve a minimum detection probability relative to electrofishing (e.g., at least 10% cost savings for a 95% detection probability when there is less than one fish per kilometer;).

4. Discussion

4.1. Environmental DNA production

There was high variation in production rates in the mesocosm and caged fish experiments, and these distributions were strongly right-skewed by rare observations of very high production rates. This is common among animal eDNA studies (e.g., Maruyama et al., 2014) and could be in part due to capture of rare clumps of cells or pieces of tissue in a single sample (Pilliod et al., 2013; Wilcox et al., 2015). We sampled relatively large volumes of water (5 L for the observational study and 6 L for the caged fish study), which should minimize the effect of rare clumps of DNA-bearing particles. However, clumping could be an important factor, particularly in the mesocosm experiments where small sample volumes were taken (1 L).

Even allowing for variation in particle capture, our data suggest there is high variation in DNA production rates among individual fish

that is unrelated to body size. For example, the two fish in our mesocosm experiments with the highest DNA production rates at both 24 and 48 h (> twice the median for each time period) were smaller than four other fish and less than half the size of the largest fish. This is also consistent with work in other species. For example, Pilliod et al. (2014) observed four-fold variation in eDNA production rates among Idaho giant salamanders (*Dicamptodon aterrimus*) held in an aquarium ($n = 5$ individuals, mass = 44–55 g).

The source of variation in eDNA production rates among individuals is unknown, but could be related to aspects of animal physiology, such as stress (Pilliod et al., 2014), breeding readiness (Spear et al., 2015), diet (Klymus et al., 2015), or metabolic rate (Maruyama et al., 2014). Stress due to handling could help explain the high variation that we observed in both experimental studies, and our observation of higher median eDNA production rates in our mesocosm experiments. High eDNA concentrations within a few days of fish addition to tanks have been observed in other studies (Maruyama et al., 2014; Takahara et al., 2012). Differences in flow among studies could also affect eDNA production rate estimates, though Klymus et al. (2015) observed no influence of flow on eDNA production rates in captive silver carp (*Hypophthalmichthys molitrix*).

4.2. Environmental DNA transport

Our estimates of eDNA transport distance were similar to those of fine particulate organic matter (and its surrogates) in streams (Paul and Hall, 2002; Fig. 3). We previously confirmed that most eDNA particles from brook trout eDNA are approximately 1–10 μm across, which may be grouped in loose aggregations (Wilcox et al., 2015), as was also found for Common Carp (*Cyprinus carpio*; Turner et al., 2014a). These eDNA particle sizes are similar to some other types of organic matter, including bacterial cells (mean diameter = 2 μm ; Hall et al., 1996) and brewer's yeast (*Saccharomyces cerevisiae*; mean size = 5.8 μm ; Paul and Hall, 2002), and similar to mitochondria (~0.5–10 μm). However, it is likely that eDNA represents a heterogeneous mixture of different size particles and aggregations (Turner et al., 2014a) which behave differently in the environment. Further, eDNA may adsorb to sediments, creating further aggregates with differing transport dynamics and persistence times (e.g., as has been observed for fecal indicator bacteria; Jamieson et al., 2005). These may have contributed to the variation in eDNA concentrations that we observed in our experimental and observational studies.

Across experiments, the median transport distances in the caged-fish streams and estimated transport distance from our observational study were positively related to median stream discharge, similar to the observed relationship for brewer's yeast cells (Paul and Hall, 2002). This observation corroborates the relationship between eDNA transport and stream discharge detected in Jane et al. (2015) using a different analytical approach. However, transport distances of FPOM are influenced by a large number of factors including stream velocity and depth (Minshall et al., 2000). It is likely, therefore, that transport distances of eDNA are influenced not only by total stream discharge, but also by stream morphology, including gradient and channel confinement. Further, increased shear stress on the bottom of the stream, which is also influenced by substrate roughness, may result in greater settling distances for FPOM (Minshall et al., 2000). Future studies might attempt to partition the effects of stream velocity, water depth, channel roughness, and transient storage dynamics that influence eDNA transport.

We did not have a conservative tracer to correct for changes in eDNA concentration due to dilution by inflows from groundwater (Hauer and Lamberti, 2007). As a result, we may have underestimated the true transport distance in our eDNA experiments. Additionally, we cannot separate the effects of eDNA particle settling and DNA degradation. We believe that the primary source of the decline in eDNA concentrations was settling, particularly because our streams were cold

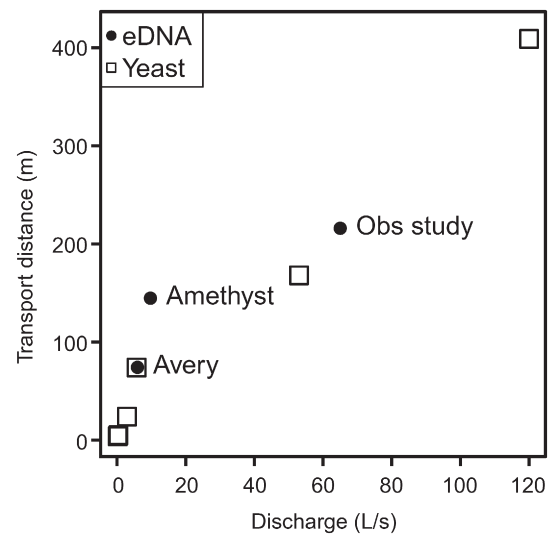


Fig 4. Median and model-estimated particle transport distance (estimated mean number of meters that a particle travels downstream before deposition; eDNA, black circles; brewer's yeast, open squares) versus median stream discharge (L/s). Environmental DNA values are from our observational study (*Obs study*; model estimate) and median transport distances and stream discharges from caged-fish experiments in the streams Avery and Amethyst ($n = 9$ and 8, respectively). Transport distance of brewer's yeast cells are from those observed in six streams in the Hubbard brook watershed from Paul and Hall (2002).

(mean = 13 °C). Whereas previous research on eDNA degradation suggests that this takes hours to days (Barnes et al., 2014; Pilliod et al., 2014; Strickler et al., 2015), we observed approximately 50% declines in eDNA per 100 m, which is only a few minutes of travel time. This is also consistent with research finding that eDNA is much more concentrated in sediments than in the water column of rivers (Turner et al., 2014c).

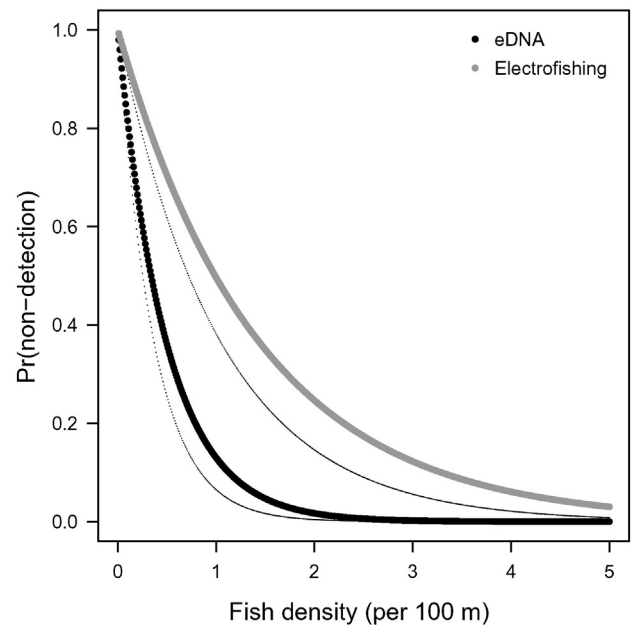


Fig 5. Predicted non-detection rates for electrofishing (gray) and eDNA (black) for randomly-distributed 0.01–5 fish per 100 m reach using protocols described in this study. Thick black points show calculations using eDNA production and downstream persistence ($1 - k$ per 100 m) mean estimates from the observational study. Thin black points show calculations using the lower and upper bounds of the 95% CI around these estimates. These results are consistent with stochastic simulations (Appendix C) and suggest lower costs for eDNA relative to electrofishing when animals are rare (Appendix D).

We applied a discharge of 65 L/s in our models estimating detection probability, which was similar to the mean from our observational study. We sampled streams at base flow, when electrofishing is likely to be most efficient and eDNA transport distances are likely to be at their lowest (Jane et al., 2015). Increasing discharge would dilute the eDNA, but may also increase transport distance. The complex relationship between flow and eDNA concentration will require further empirical investigation to predict eDNA detection probability under different hydrological conditions. As shown in this study, drawing on literature from related fields such as sediment transport and nutrient cycling in streams is a potentially fruitful source for study design and analytical methods.

4.3. False negative probability

Our simple detection models support the conclusion that eDNA is substantially more sensitive than traditional methods for detecting rare aquatic species, even when assuming high electrofishing efficiency (0.7) relative to many studies of stream salmonids (e.g., mean = 0.28 in Peterson et al., 2004 for bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Oncorhynchus clarki lewisi*); mean = 0.58 in Letcher et al., 2014 for brook trout). Our findings suggest that eDNA provides high detection probabilities with a single sample at reasonable animal densities for natural populations (estimated non-detection rate <0.01 when ≥ 3 fish per 100 m and 100% detection rate of known populations in the field during our observational study). With multiple samples, eDNA is also practical for very low animal densities (estimated non-detection rate <0.01 with 5–10 samples when there are 0.25–1 fish per 100 m).

Our modeling approach allowed us to predict detection probabilities for very low animal densities, including those which would be too low to accurately quantify using traditional methods. This has been a problem in other systems as well; eDNA may detect organisms at lower densities than they can be detected using traditional methods, making a direct comparison of their sensitivity difficult (Mahon et al., 2013). As a further example, in our study we cannot know with certainty if the two sites where we did not detect brook trout with either electrofishing or eDNA sampling that no animals were present, or if we simply failed to detect rare animals with both methods. This underlines the utility of models (mechanistic models, as in this study, or occupancy modeling, as in Rees et al., 2014) and controlled experiments for assessing eDNA detection probability.

Our models also draw attention to the importance of eDNA sample collection and analysis protocols for detection, because the expected non-detection rate is inversely proportional to the number of mtDNA copies analyzed. For example, our model predicts that with the sampling protocols used in our observational study, the probability of failing to detect five fish 200 m upstream is 0.027, which is comparable to the 100% detect rate of five caged fish 240 m upstream in Jane et al. (2015). However, if we only took 1 L samples our estimated non-detection rate would be 0.484. Conversely, further optimizing our eDNA protocol to double extraction yields would decrease our expected non-detection rate to <0.001. Sample volume may further be important because if eDNA is clumped into aggregations capture of these aggregations may be important for sensitivity (Turner et al., 2014a; Section 4.1). In this case our model assumption that mtDNA copies are randomly distributed at a given stream site could over-estimate sensitivity (Furlan et al., 2015). Finally, different analysis platforms or protocols may change the non-detection rate per mtDNA copy. For example, qPCR protocols can be substantially more sensitive than traditional end-point PCR (Turner et al., 2014b; Wilcox et al., 2013), but digital PCR may be even more sensitive in the presence of PCR inhibitors (Doi et al., 2015).

Because of its high sensitivity and rapid field collection, eDNA sampling is likely to be more cost-effective than electrofishing when animals are rare (Appendices C and D). Moreover, eDNA reduces the costs associated with uncertainty about the presence of species of

conservation concern, which can dictate land or water management by government agencies. Further, unlike electrofishing, eDNA sampling does not require intensive technical training or scientific sampling permits, which may be difficult to obtain, making eDNA potentially appropriate for citizen science projects (Biggs et al., 2015). However, it is also important to also recognize that the high sensitivity of eDNA sampling is a double-edged sword: Our ability to detect a single copy of target DNA in a sample makes this approach very sensitive for the detection of rare organisms, but also very sensitive to contamination. In our observational study we detected low level contamination in two out of our 31 field controls. We subsequently adopted a more rigorous protocol for equipment sterilization and field collection that minimizes collector contact with samples and employs single-use, sterile materials (Carim et al., 2015). This protocol has subsequently been used to collect hundreds of samples for analysis at the National Genomics Center for Wildlife and Fish Conservation (Missoula, Montana, USA) without any signs of field contamination. Further, because sample collection is rapid and inexpensive, it may be prudent to use repeated sampling to confirm the presence of rare species of special conservation concern, particularly in systems where alternative vectors of eDNA may be a concern (e.g., piscivorous birds; Merkes et al., 2014).

Beyond confirmation of positive detections, spatially and temporally repeated eDNA sampling may be incorporated into an occupancy modeling framework to account for imperfect detection of rare species (Mackenzie et al., 2002; Rees et al., 2014). Rapid sample collection makes eDNA a particularly powerful tool for broad-scale sampling of species of special conservation concern (McKelvey et al. in press). Combining eDNA with occupancy modeling could allow for robust determination of species habitat associations, estimation of colonization and extinction probabilities (Mackenzie et al., 2003), and tests of species interactions (Richmond et al., 2010; Wagner et al., 2013) over scales which have been prohibitively labor and cost-intensive using traditional sampling approaches.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.biocon.2015.12.023>.

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