GUIDELINES FOR DESIGNING ENVIRONMENTAL DNA SURVEYS FOR TARGET SPECIES

1/13/2017

Caren Goldberg
Katherine Strickler
Alexander Fremier

Washington State University
Pullman, WA 99164

Note: these guidelines were developed on Department of Defense lands in the coterminous U.S. and represent the state of knowledge at this date (January 2017). They are intended for stream and wetland systems and will require adjustment for larger river, lake, or ocean systems.

GUIDELINES FOR DESIGNING EDNA SURVEYS FOR TARGET SPECIES

1.0 Deciding whether to use eDNA surveys for target species

Environmental DNA is a powerful tool for detecting aquatic species, and in many cases it can be more accurate and efficient than traditional field surveys. This may be especially true if the target species occurs in very low densities or is difficult to distinguish from similar species. Environmental DNA surveys may also be advantageous if current survey methods yield low detection probabilities, are destructive to the species or its habitat, or require extensive training or certification for personnel conducting surveys.

However, species detection with eDNA may not be better than conventional field surveys in all monitoring situations. If current survey methods provide high detection rates for a relatively low cost or time investment, eDNA methods are not needed. There may also be species or systems for which detection probabilities for eDNA surveys are low due to environmental conditions that contribute to movement or degradation of eDNA in the aquatic system.

The application of eDNA as a tool for detecting species will be different for every system and depends on the goals of the program, characteristics of the target species, and conditions of the systems to be sampled. The decision tool in Figure 1 can guide managers in deciding whether eDNA surveys may be beneficial as a replacement or supplement to current survey techniques.

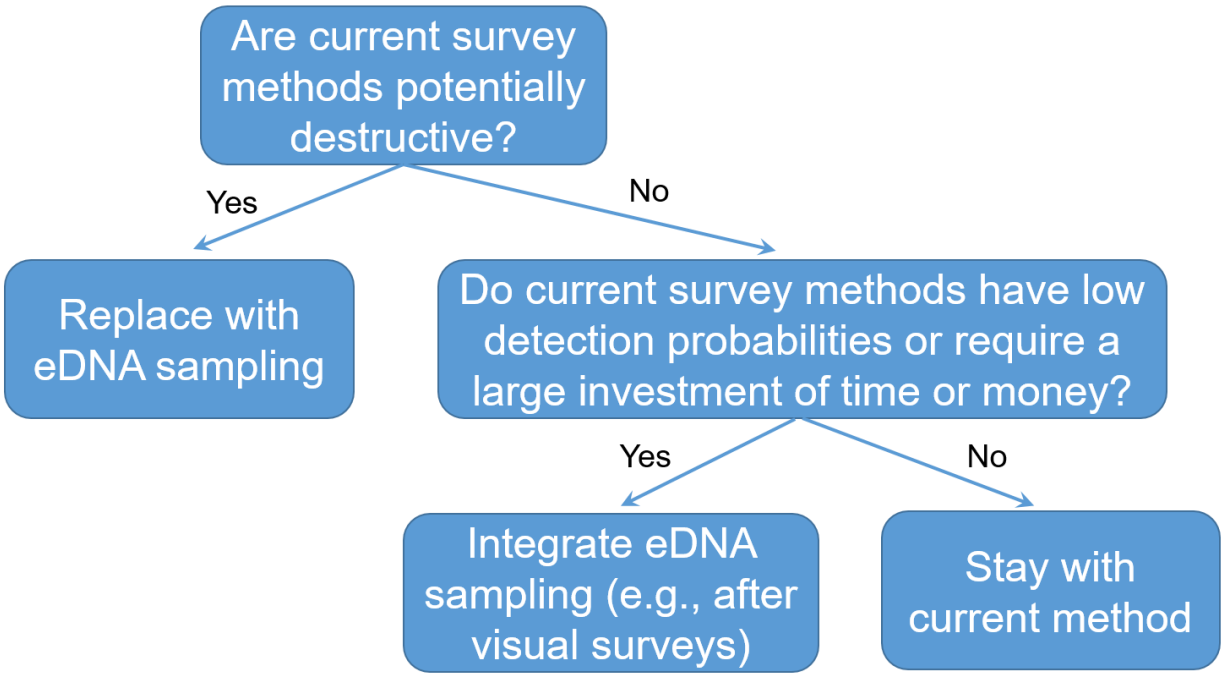

Figure 1. Decision support tool for determining how environmental DNA sampling can complement or replace current survey methods.

2.0 Conducting a pilot survey

Once it has been determined that eDNA surveys can potentially be useful for monitoring of target species, a pilot survey should be developed and applied.

Environmental DNA detection rates depend on a variety of characteristics of the target species, conditions of the aquatic system, and sampling methods. By identifying the factors most likely to influence detection probabilities, managers can adapt sampling strategies to increase the probability of detecting the target species if it in fact occurs at the sampling site. The best way to do this is to conduct a pilot survey in which environmental factors are 1) measured at the same time eDNA water samples are collected, 2) analyzed to determine the most important factors in predicting species detection, and 3) used to modify sampling strategies to improve detection probabilities.

For example, eDNA detection probabilities for Chiricahua leopard frogs in Arizona were found to be strongly influenced by the size of the wetland that was sampled (Goldberg et al. *in prep*). By increasing the number of eDNA sampling locations as wetland size increased, detection probabilities for Chiricahua leopard frogs at large wetlands were improved. Similarly, eDNA detection of Sonora tiger salamanders was limited by the amount of sample water that could be filtered (Goldberg et al. *in prep*). Water samples from Sonora tiger salamander ponds was very silty and the filters tended to clog before the desired water volume was reached. Switching to eDNA filters with a larger pore size greatly improved detection probabilities for this species.

A pilot survey makes adaptive sampling strategies such as these possible. Ideally, the pilot eDNA survey is conducted simultaneously with conventional field surveys so detection probabilities for each method can be directly compared.

2.1 Designing the pilot survey

The pilot survey's timing, sampling intensity, and environmental measurements are determined by the ecology of the target species and its habitat. This section provides guidance about when, where, and how much to sample.

2.1.1 Timing

Time eDNA surveys for the season with the highest species density in the water, as long as that coincides with the life stage of interest. For amphibians, this is likely to be during tadpole development if reproducing populations are the target of the surveys (as opposed to adults that may not be successfully reproducing).

Environmental DNA degrades fairly quickly in water, generally persisting for 1-3 weeks, though eDNA may persist longer in cold water bodies or degrade more quickly in very warm or acidic systems (Strickler et al. 2015, Barnes and Turner 2015). Environmental DNA surveys that are conducted more than a few weeks after the species occupied the site may fail to detect the species' eDNA simply because the eDNA has degraded.

2.1.2 Environmental covariates

Identify characteristics of the aquatic system that are likely to affect eDNA concentrations in the water. These may include factors that may influence eDNA degradation (for example, water temperature, pH, or solar radiation), transport in streams (such as current velocity, discharge, or channel complexity), or diffusion in ponds, lakes, and wetlands (such as water body area, depth, or complexity). Select environmental covariates that are meaningful and can be measured in the field efficiently and accurately. Additionally, record data related to sampling methods, such as volume of water filtered or spatial arrangement of sampling locations, which may also affect eDNA detection.

2.1.3 Number of samples

Collect more than 1 sample at each site so that detection probabilities can be estimated. In streams, 2 samples per site has generally been sufficient for >0.95 detection of amphibians, but in wetlands more samples may be required, and may include pooling samples taken from different areas of the wetland. During the pilot survey, consider collecting 4 samples per site. The occupancy modeling framework used to analyze pilot survey results can help calculate the relative value (in terms of improved detection) of each additional sample.

Additionally, the optimal number of samples may be dependent on sample volume and spatial arrangement of samples.

2.1.4 Sample volume

In streams, eDNA water samples are generally 1 L, while in wetlands 250-500 mL is usually sufficient. The targeted sample volume is a balance between detection and efficiency: larger sample volumes may increase detection, but may be inefficient to collect and filter in the field.

The sample volume is often dependent on how much water can be filtered before the filter clogs, which is related to filter material and pore size. Generally, PES, cellulose nitrate, and mixed cellulose filters all perform equivalently for detecting eDNA of vertebrates (up to 5 µm filter pore size) when the additional amount that can be filtered is taken into account.

2.1.5 Spatial arrangement of samples

Environmental DNA is not uniformly distributed in a water body, so it's important to collect water samples where eDNA concentrations are likely to be high. Concentrations are highest close to the target organism, then decline as eDNA is carried away from the source by factors such as downstream transport in streams or diffusion in ponds and lakes.

Lotic systems: Distribution of eDNA in streams is likely to be heterogeneous. Use your knowledge of the target species' ecology to select sampling locations in the stream habitats likely to be used by the species. Samples can be collected from the stream margin, thalweg, or, in larger streams, from a decontaminated boat. In all cases, collect samples upstream of your position and equipment. When sampling multiple sites on the same stream, always begin sampling at the site that is furthest downstream and sample other sites sequentially as you move upstream.

Lentic systems: Diffusion is often a limiting factor for detection, so within-site sample location can be important for detection. Samples can be collected in association with particular habitat characteristics or evenly spaced. It is easiest to sample from the edge of aquatic sites, but space use by species may indicate that sampling from a (decontaminated) boat will increase detection. Samples should be replicates, so combining sample volumes from multiple spatial locations in equal volumes in each filter is more informative than using a single filter per sampling location at a site. For example, if your goal is to collect four 250 mL samples at a large wetland, you can collect 250 mL from each of four sites around the wetland, pool the samples into a single sample bottle, and filter 250 mL of the pooled sample for each of four individual filters to create four replicate samples. (edit 23 Dec 2019 - note that this would not create independent samples as required for occupancy modeling. For independant samples, collect starting from a random point each time.)

2.2 Analyzing pilot survey results

Detection probabilities can be estimated from replicate samples to determine if the pilot design is efficient enough for application or if changes in survey design need to be applied. Occupancy modeling can be used to determine limiting factors to detection (e.g., area, water quality) if those factors were measured at the same time pilot samples were collected. Concurrent field sampling can be helpful to confirm true occupancy at sampled sites.

3.0 Implementing an adaptive sampling protocol

Pilot survey sampling and analysis provide estimates of the probability of detecting the target species in eDNA samples as well as the environmental factors that affect those probabilities. If field surveys were conducted concurrently with eDNA sampling, error rates (sampling events in which the species was detected with eDNA sampling but not with field surveys, and vice versa) can also be compared.

Detection probabilities for the target species can be considered to be acceptable if they are above an agreed-upon threshold (often 0.75, but may be higher depending on monitoring requirements) or if they are consistently higher than field surveys. In these cases, the original sampling protocol can be used to continue eDNA monitoring with the same timing, sample volume, number of samples, filter pore size, etc.

However, if detection probabilities are low and are strongly influenced by environmental or sampling factors, it may be possible to improve detection probabilities by adapting the sampling strategy to address the influence of those factors. In the Chiricahua leopard frog example above, detection was influenced by the size of the wetland, and detection probabilities were improved by modifying the sampling intensity at larger wetlands.

Environmental factors and sampling approaches should continue to be measured and analyzed as part of a long-term monitoring protocol. It's possible that the relative influence of different factors on detection could change over time if there are changes in environmental conditions, the target species' distribution and abundance, or sampling methods or materials.