Guidance for environmental DNA sampling design and effort

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The use of environmental DNA (eDNA) was born out of a recognized need for early detection of aquatic invasive species (Lodge et al. 2006, Ficetola et al. 2008). Initial eDNA efforts attempted to sample in locations with presumably the highest probability of detection if the target species was present, such as downstream from where a species would likely aggregate and where DNA might accumulate in the water column (Jerde et al. 2011). The implementation of high-throughput sequencing (HTS) allowed for evaluations of species richness (counts of unique species found within the aquatic systems) (Thomsen et al. 2012, Olds et al. 2016), and has motivated different sampling efforts focused on broad coverage of either the area being surveyed or stratified sampling within habitat types (e.g,. near shore, cobble bottom, sand bottom, etc.) (Evans et al. 2017).

In searching for guidance on sampling design, it is critical to realize that the same effort and inferential related pitfalls (Gu and Swihart 2004) apply to eDNA applications (Darling and Mahon 2011) as they do to traditional capture-based sampling approaches. While the protocols and procedures from eDNA extraction to screening are rapidly evolving (Goldberg et al. 2016), the sample collection process has the same questions when designing an eDNA survey:

How much water should we collect per sample?

Practicality has driven many of the decisions regarding water volume. In the search for Asian carp, two-liter water samples were used with a glass microfiber filter having an average pore size of 1.5 microns (Jerde et al. 2011). In the search for white sharks in Southern California, 500ml samples using a 0.22 micron filter capsule worked well (Lafferty et al. 2018). Ficetola et al. (2008) used a centrifuge method that relied on 15ml of water per sample. The methods are varied, but all worked. The best recommendation is to collect water from the proposed sample site and determine how much can be pushed through filters of various pore sizes or how much water can be centrifuged, and see what volume and/or pore size works best for a given situation or study. Depending on the particulate matter in the water (e.g., soil, algae, eDNA), there is trade-off between the volume of water that can be pushed through and the pore size of the filter that should be considered (Figure 1, Turner et al.

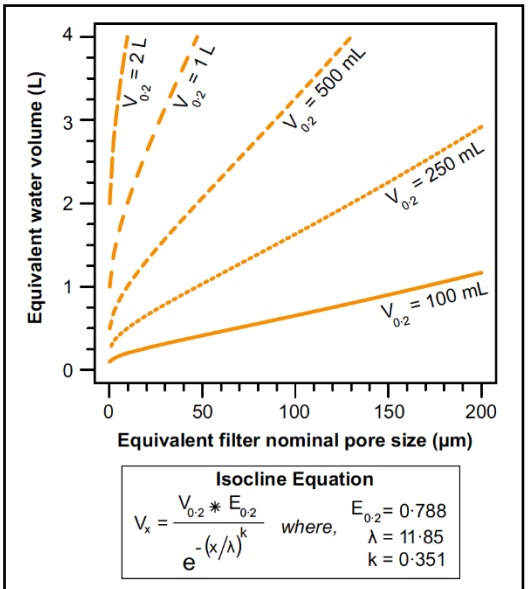

Figure 1: Isoclines showing combinations of filter pore size (x) and water volume (Vx) where the particle size distribution (PSD) of Carp eDNA predicts identical amounts of Carp eDNA captured. Isoclines are shown for five hypothetical examples of maximum throughput water volume for a 0_2 lm filter pore size (V0_2). The equation for calculating isoclines is shown using the 0_2 lm capture efficiency (E0_2) we estimated for Carp eDNA by fitting a Weibull CCDF to our cumulative size fractionation data; the scale (k) and shape (k) parameters from the Weibull CCDF model of Carp eDNA PSD are also used in this equation (see Results and Appendix S1 of Turner et al. (2016) for details). Figure used under Creative Commons Attribution-Non-Commercial License.

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2014). It is generally thought that increased total water volume filtered and screened increases your chances of detecting rare species.

When should we collect samples?

We know eDNA can persist longer (10-58 days) in colder water, with low UV radiation, and alkaline conditions (Strickler et al. 2015), but in natural systems persistence of eDNA may be much shorter, on the range of less than two days (Li et al. 2019). We also know that some organisms change their behavior seasonally and in response to some of the same factors that slow DNA degradation. For example, Asian carps exhibit upstream movements and spawning during flooding events (Kocovsky et al. 2012), which can also be correlated with cooler water temperatures and lower UV radiation into the water column during spring runoff (Erickson et al. 2016). Add to this the pulse of eDNA from broadcast spawning of eggs and milt, and the detection probability using eDNA may be much higher during spawning periods (Bylemans et al. 2017) just below any barriers to upstream dispersal (i.e., dams). This was part of the motivation in the sampling effort for Asian carp in the Great Lakes (Jerde et al. 2013).

There is growing evidence for the positive relationship between the concentration of DNA recovered during a sampling effort and density or activity of the target species across time and/or space (Doi et al. 2015, Dougherty et al. 2016, Lacoursière-Roussel et al. 2016, Bista et al. 2017). Consequently, for some species, it may be useful to have temporal eDNA monitoring to track population density trends, albeit with consideration of the biotic and abiotic influences on the probably of detection. As with traditional sampling, such as knowing when the fish are biting, there may be conditions when deploying eDNA is optimal and can be guided by our understanding of the system and the species.

Where should we collect samples?

For the two applications, (early) detection of rare species and estimation of species richness, there are two different motivations for spatial sampling design. For rare species, sampling in preferable habitat is advocated. The assumption is that when a species is rare, most of the individual samples will contain no target DNA. The species will likely be able to occupy its most preferred habitat without intraspecific competition. Therefore, if we have some information about the distribution of preferred habitat in the lake or river, then we can more intensively sample these habitats, thus increasing our probability of detection. This sampling approach can be effective for early detection applications (Jerde et al. 2011), but has pitfalls when using the same data to making inferences about population trends (Staples et al. 2004).

The problem of where to sample is particularly acute across large geographies, such as the entirety of the Great Lakes watershed. In this case, sampling locations may need to be further narrowed down. For example, in the search for Asian carp, Jerde et al. (2013) limited sampling to just below upstream barriers to dispersal, and focused on the rivers near likely entry points into the system, namely rivers near the Chicago Area Waterway Systems (CAWS) and rivers of the Sandusky Bay. This approach of triaging sampling effort instead of broad coverage surveillance comes with risk. If we errantly misunderstand Asian carp habitat preferences or where the potential sources of Asian carp introduction are, focusing on some areas at the expense of broader sampling may risk inadvertently missing early detection of an incipient invasion.

Environmental DNA applications that seek to gauge trends or estimate species richness should seek coverage of the location (Evans et al. 2017). While much of the work has been conducted in closed

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systems (ponds and small lakes) or small streams (Olds et al. 2016), work by Yamamoto et al. (2016) in an open ocean bay showed the estimated eDNA concentration reflected the biomass of fish (jack mackerel) within 10-150 m from the sampling location. Spacing these samples and then using spatial distribution modeling may provide an interpolated model of total fish biomass. Further refinement of microhabitat mapping within systems may provide guidance for stratified sampling to ensure rare species in rare habitats are represented in species richness estimates.

How many samples should we collect?

The theory and application of sampling rare species has been a long-standing subject of discussion (Thompson 2013). Ultimately the target species density at which we want to have some defined confidence in detecting is a management decision that can motivate a specified number of samples to allocate (Kovalak et al. 1986), budgetary constraints notwithstanding. The sampling effort necessary to detect rare species in aquatic habitats is discussed robustly in a number of papers (Olds et al. 2016, McKelvey et al. 2016, Evans et al. 2017) and will vary depending on the goal of the effort, expected species abundance or rarity, and size or area of the habitat to be sampled.

Green and Young (1993) lay out a useful framework and example applied to sampling unionid mollusks. However, the conclusion remains the same for extremely rare, incipient invasive species: the sample size necessary to have reliable detection goes to infinity irrespective of the power to detect (Figure 2, Green and Young 1993). Under this scenario, the recommendation would be to take as many samples as the budget allows, realize that likely all the samples will be non-detections (zeros), and yet accept there are potentially undetected fish present. The more practical recommendation is to reduce the surveillance area to something manageable based on the target organism's habitat preferences and where the organism is likely to be introduced, and to use the sampling effort based on Green and Young (1993) that makes invasive species surveillance tractable.

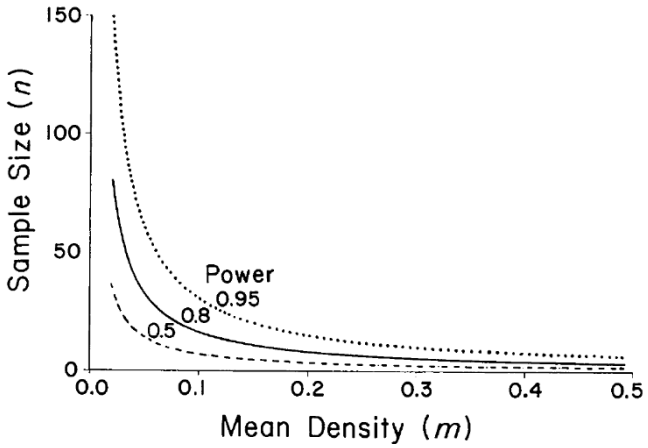

Figure 2: The necessary sample size *n* as a function of the mean density *m*, for various degrees of power 1 - β, when sampling the Poisson distribution. Figure found in Green and Young (1993) and used with permission from the publisher.

Any surveillance sampling for invasive or endangered species should anticipate needing to interpret zero detections in communications with management and policy personnel as well as the public. It should be expected that sampling will result in mostly zeros; without clear communication and interpretation, results such as these may easily be misinterpreted as true species absences rather than effort- and probability-based failure to detect.

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