Fact Sheet
Recommended Methods for eDNA Water Sampling in Estuarine Systems

In 2018-2019 Six National Estuarine Research Reserves (NERRs) collaborated with the University of New Hampshire to develop and test Environmental DNA (eDNA) sampling methods in estuarine systems. Based on our findings we have put together as set of recommendations for sampling in estuarine systems that balance time and cost of sampling with information gained. These recommendations were developed for the National Estuarine Reserve System, and appropriate for the level of resources and available at most NERRs, but are also relevant to any estuarine sampling program. More information about the overall project, including data results and findings are available at our website www.estuarydna.org.

Collecting eDNA samples in Great Bay NERR

Before you start: Planning and sampling design

Sampling design will depend on project goals, target species, biology and hydrology of the system, accessibility, and budget. Generally, more samples provides greater coverage, but increases cost. Figure 1 shows fish species accumulation curves for three sites sampled multiple times (with triplicate samples) in 2019 in Great Bay. Samples were collected three times over the summer, and we found that even with 35 samples the number of species detected is increasing. Most of the species at the high end of the curves are detected once, in one sample, and may represent DNA transported by birds, boats, or other vectors. The most common species are detected repeatedly, and at different locations.

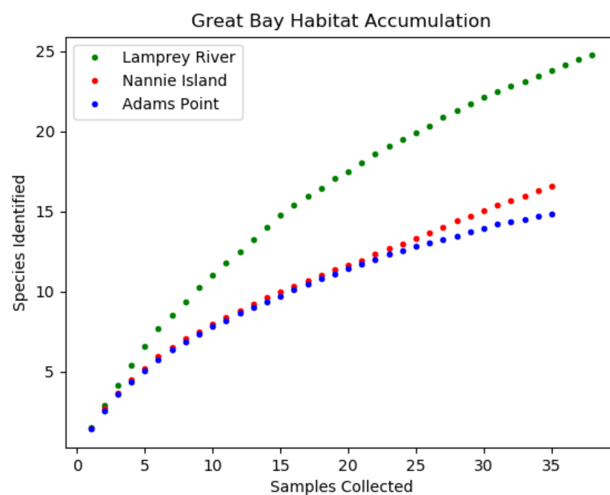

Figure 1. Species accumulation curves for three sites in Great Bay, NH. The Lamprey River site receives freshwater input from the river, and DNA from freshwater species is transported to this location.

Accessibility: Sites which are accessible only by boat will require more coordination and scheduling. Sampling equipment, particularly after samples are collected, may be heavy and difficult to transport without a boat or vehicle.

Timing: At many sites tidal stage will dictate timing of sample collection.

Samples should be filtered as soon as possible after collection, so make sure that personnel and lab space are available to complete filtering within 24 hours of collection.

Leverage other sampling programs: Collection of water samples for eDNA analyses can be accomplished fairly quickly if samples are filtered later in a lab. If samples are collected in coordination with SWMP or other sampling programs the cost and time is reduced. Care must be taken, however, to avoid cross contamination with other activities.

Selecting representative sites: As with any methods, sites should be selected to maximize the opportunity to detect the target species. However, DNA can be transported to a site by several methods including nearby freshwater inputs, fishing or other commercial activities. Transported DNA may provide useful information (by detecting species further up the watershed, for instance), but may also lead to incorrect conclusions regarding species actually present at the site.

In the Field: Sample collection
Preparing sample kits: Sampling supplies should be prepared, and stored in a container that is readily transported and can be kept closed to avoid contamination. Collected samples should be kept on ice until returned to the lab or filtered. A medium size cooler can be packed with sample supplies, then used to store and transport samples. Guidance on preparing sampling kits can be found at www.estuarydna.org/protocols.

Replicate samples: Samples should be collected following a consistent protocol that minimizes the chances of contamination, and can be accomplished in a reasonable time frame. In general, more volume increases the number of species detected. South Slough NERR conducted a volume experiment where replicate 1-liter samples were collected to identify the number of additional species associated with collection of increased volume (up to 12 liters). We found that even at 12 liters, the number of species potentially detected continued to increase (Figure 2a). However, many estuarine sites are very turbid, so filtering large samples becomes very time consuming. Triplicate samples (in our study) yield 1-4 times as many species as single samples (Figure 2b), and provide a reasonable compromise between results, volume, time and effort.

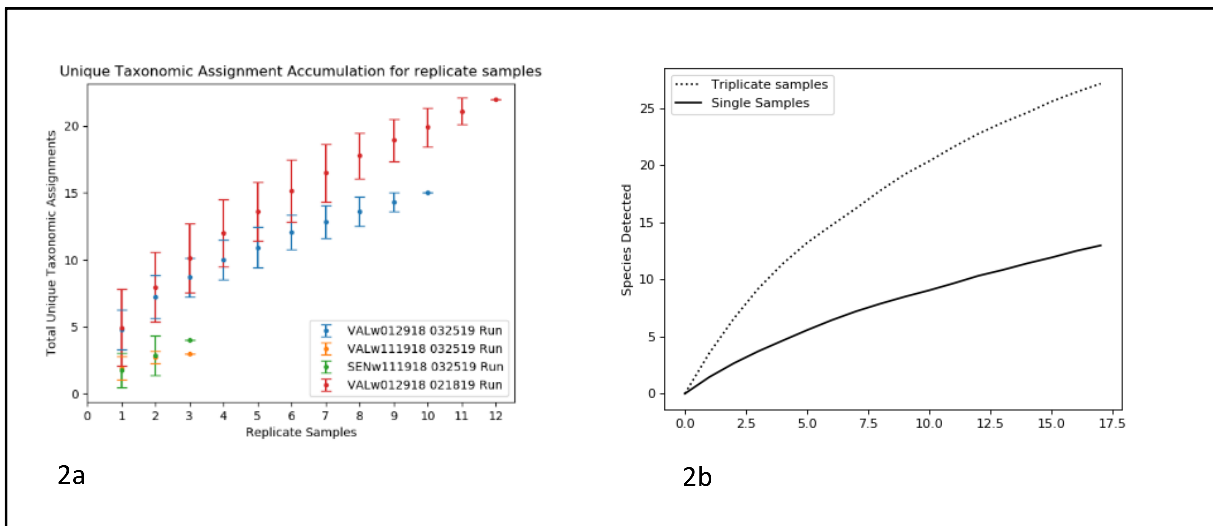

Figure 2a. Number of species detected with increasing volume. Figure 2b. Number of species detected in single vs triplicate 1-liter samples collected at 17 sites.

In the Lab: Sample filtering and processing

Filtering: eDNA sample should be filtered as soon as possible after sample collection. Where possible, field filtering improves preservation of captured DNA and may increase species detection. However, many estuaries are turbid, and sampling windows are often time-constrained, making field filtering impractical. Based on these considerations, and the sampling results discussed above, found that triplicate 1-liter samples, lab filtered through 1.5um glass fiber filters yielded good results without requiring excessive time resources. Alternatively, a pre-filter and smaller core filter size (e.g. 0.45um) may be used, but the use of two filters increases the cost of extraction and processing.

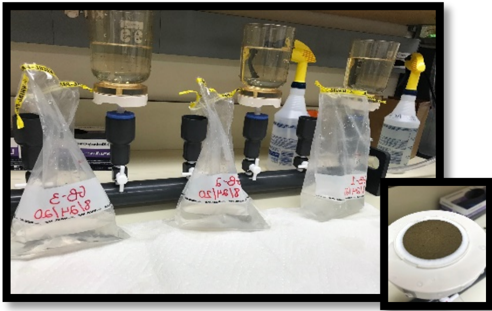

Figure 3. Filtering samples in the lab. Many estuarine samples are turbid, and filters may clog readily. A multihead manifold reduces the time required to process multiple samples.

DNA extraction: DNA extraction is usually performed either using manufactured kits, or with lab specific protocols. The kits are generally more expensive, but we felt that the advantages of a standardized method that is easily replicated in other labs outweighs the cost. Ultimately, we selected QIAGEN PowerSoil Pro for soil extractions, and either the DNeasy Blood and Tissue, or the QIAamp DNA Mini Kit protocols for filter extraction, both manufactured by QIAGEN. All of these methods are commonly used in eDNA studies, based on literature surveys and our discussion with other genomics labs.

Primers and analysis: All samples were amplified using a short metabarcoding primer. We selected published primers that are commonly used to increase cross comparison to other studies. We found that the 12S MiFish primer (Miya et al. 2015) was very effective for identifying fish in most samples, and the region is well represented in databases. However, under some circumstances this primer co-amplifies bacteria in a way that effectively masks any fish species that may be present. A modified touchdown PCR method improves performance (*12S :* dx.doi.org/10.17504/protocols.io.bcppivmn), but ultimately multiple primers may be needed to fully characterize a community in samples with complex matrices or high concentrations of DNA. The Earth Microbiome 18S primer is very effective for characterizing broad biodiversity, and we had no significant laboratory issues.

Making sense of it all: Data analysis and interpretation

All results should be reviewed carefully with local resource managers. Some fish are difficult to distinguish to the species level, and initial database matches should be manually curated to screen species that are not likely to be present in the sampled system. These sequences can be reviewed to determine if they more accurately represent a closely related species, and if not, resource managers should be consulted to identify possible transport pathways for the DNA. It is important to remember that DNA in a sample does not necessarily mean that a living organism is present in the vicinity; DNA can be transported from freshwater sources by tributary streams, by fishing or other commercial activity or even wastewater.