Envisioning the Future of eDNA Sampling and Sample Processing
Virtual Workshop Preliminary Report
23 June 2020, 3:30-5pm EDT

Purpose: To foster discussion about the challenges and obstacles to collecting and concentrating samples for eDNA.

Attendees: The workshop was attended by 30 participants, including members from the scoping working group and invited participants. The research, management, and technology development/transfer sectors were all well-represented.

Synthesis: Following a short introduction of the overall effort and quick presentation of the pre-workshop survey results, the workshop participants were separated into three breakout rooms. Each breakout room was charged with questions around which to discuss current barriers in the different aspects of eDNA sample collection and processing.

Discussion points:

Breakout Room #1

Charge question: What are the main barriers to collecting a clean, representative sample in your environment of interest? Are there certain environments that are harder to collect samples from than others?

- Contamination during field collection is a main concern. This is especially true when samples are collected by field personnel who are unfamiliar with molecular techniques and may not be taking the necessary caution to limit human contamination.
    - In order to minimize contamination researchers typically spend much of their time disinfecting equipment and materials between samples, and when these disinfecting protocols are in place it may not be followed the same way between personnel.
    - Conducting extreme flushes between samples has even shown to be inadequate in limiting sample cross-over contamination (e.g. in ESP with ubiquitous microbes).
    - One solution would be manufacturing enough 3D printed parts to have new materials for every sample collected, though, a downside to this is the problem of plastic waste.
    - Another approach to address contamination is to run many blanks to account for any contamination and cross-over in samples.
- Another concern was to capture true representation of temporal and spatial scales from the sample. Efficient use of field time to get best representation of targeted groups also been discussed. Finding balance between more sampling sites with transport effects (by not filtering on site) or less sites with reduced transport effects (by filtering on site) also highlighted.

- One of the potential solutions discussed was using pre-loaded filter contraptions that could be taken into the field or on a ship so you have one unit per sample and therefore no need for a lot of downtime for decontamination steps. Ideally these contraptions would be balanced with a 'green' approach to production and disposal.

Breakout Room #2

Charge question: What are the main barriers to concentrating a representative sample for your purposes? Are there certain samples that are harder to concentrate than others?

The group outlined several major barriers to concentrating samples for eDNA analyses. These were positioned as a set of conflicting issues that, at present, represent trade-offs in effective, accurate eDNA sample processing.

- The first is the unknown form, or size fraction, eDNA is in when collected. While prescreening can help with apportioning size fractions, there are concerns of wasting eDNA that may be present in unused fractions.
- The second is the use of open vs. closed systems that both have conflicting pros and cons:
    - ease of use in the field (enclosed better)
    - ease of extracting in the lab (enclosed worse)
    - cost (reusable closed are expensive) and plastic use.
- The third barrier is the clogging of filters which can lead to slow speeds and insufficient volumes, especially in environments with high particle loads.
- All participants (with one exception) use filtration to concentrate samples, though it's notable that across the group filter pore sizes range from 0.2µm - 5µm.
    - The other participant utilizes a Subsurface Environmental Sampler (Montana Emergent Technologies - Butte, MT) which uses an internal chamber for media or sample water without enrichment.

Breakout Room #3

Charge question: How well do you trust that samples from other groups are collected and concentrated in a consistent, documented manner?

- The general consensus was that there is little motivation/need for sample sharing in current practice. Participants felt limits on sample sharing stemmed from more than metadata requirements which seems to be well defined at this stage.
- The necessity for holistically prepared key methods with checks and validations pointed out to attain consistency between different researches. The discussion concentrated on filtering and sampling since these stages are more convenient to share the sample than further stages.
- General concerns about consistency fall under categories that map onto other breakout groups' identified challenges, including:

    - Monitoring
    - Standards
    - Contamination.
- For large scale monitoring programs employing eDNA for habitat-community description (e.g. CA State, SCCWRP, NERRs), samplers are trained and field tested on SOPs to ensure consistency in sampling (filter type, sample volumes, metadata).
- Filters can be cut to provide cross calibration of a particular probe sets or sequencing strategies.
- Setting requirements for minimum environmental data also help with consistency in samples collected across habitat, time and depth.
- Application of blanks and standards should be routinely used to improve consistency and validate the accuracy. At minimum field blanks (generally MQ carried from lab) are required for identification of potential contamination. However, it was noted that even field blanks can generate sequence libraries, which makes them difficult to interpret.
    - Ideally, community-specific standards (prokaryote/eukaryote microbes, metazoan) need to be developed to assess efficacy of eDNA processing pipelines.
    - Spike-in standards should be used at extraction. There is interest in developing whole organism standards that could be spiked-in at the sample concentration stage to assess extraction efficiencies and downstream community characterizations.
        - The need was identified for control/standard practices that allow for holistic assessment of quality, agnostic of at which point failures are occurring.
- Contamination control steps should be documented with sample collection to identify potential sources of human contamination (critical for microbial, pathogen tracking), controlling for sample carryover (repeat sampling, transect sampling, diverse habitat sampling on single field session).
    - i.e. What are protocols for flushing sampling apparatus between defined sampling events (i.e. what volumes, time, decontamination reagents and subsequent rinses).
    - As with all studies, contamination control concerns are relative to specific goals of eDNA sampling, i.e. human contamination more important in pathogen studies than shark tracking.
    - Carryover of contamination from pure lab cultures of similar taxa or from processing large biomass samples in same laboratory setting.

Due to technical difficulties, we were unable to initiate the second breakout group.

Whole Group Discussion

- Around this issue of contamination and the need to run blanks or standards as controls, the point was made that that info is often decoupled from the actual data.
    - A potential solution of requiring minimum information to be included by NCBI was suggested, in line with the MIIQE guidelines promulgated for qPCR data (e.g. Goldberg et al. 2016)
- Around issues of filtration and the tradeoffs inherent in finding an effective and economical solution, the suggestion of using peristaltic pumps or serial filtration were also discussed.
- Finally, there a fair bit of discussion around the fact that many of these challenges are context-dependent and there is no "one size fits all" approach to eDNA sampling and processing.
    - Rather, a continuum of methods currently exists, and there are use- and/or target-specific considerations that influence where along that continuum users find themselves.

Workshop Participants

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Workshop Scoping Group

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