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Using environmental DNA for mega- and microorganisms

Advancements in sequencing technologies have uncovered a wealth of information about the diversity of natural systems– particularly that of the uncultured microbial world (Hug et al. 2016). More recently, there has been an increased interest in using these techniques from microbial community ecology to rapidly assess the presence of larger organisms for conservation and monitoring efforts. Sampling for environmental DNA (eDNA) provides an opportunity for ecologists to understand the trophic structuring and diversity of a habitat without invasive sampling or taxonomic expertise in the field, though the computational effort is much larger for large sequencing datasets. Despite methodological constraints that largely involve uncovering the “ecology of eDNA”, there have been successful studies that are able to uncover community patterns in terrestrial, aquatic, and marine ecosystems for micro and macroorganisms (Deiner et al. 2017). The two papers chosen for discussion, Sunagawa et al. (2015) and Boussarie et al. (2018), show how seawater samples can be utilized for microbial and megafauna groups (respectively). While there have been large sequencing efforts aboard research vessels for microorganisms, the same effort has not yet been done for animals – the stark contrast between the conclusions the authors are able to draw in each of these papers reflect this difference (see Table 1 for summary).

The *Tara* Oceans expedition represents the largest sequencing effort ever done in ocean science, producing >40 million mostly novel sequences from microbes and samples with >35,000 species in them. Of the five papers published in a special *Tara* Oceans edition of *Science*, Sunagawa et al. (2015) revealed that temperature was the main driver of vertical stratification and found an “ocean microbial core” of functions that are ubiquitous in the ocean (regardless of taxonomic composition). Because the researchers were collecting whole organisms in their seawater samples rather than DNA fragments shed from target organisms, they were able to do deep Illumina shotgun sequencing of 243 samples, which is an incredibly expensive undertaking. This sequencing effort produced 5x more data than the human gut microbial reference gene catalog, the only comparable dataset to the one generated in this study. Their success in analyzing this huge dataset can be attributed to the strength of publicly available ocean metagenomes and reference genomes that they were able to build upon, ultimately leading to the creation of the Ocean Microbial Reference Gene Catalog (OM-RGC).

Boussarie et al. (2016) on the other hand, was more concerned with whether or not eDNA was a reasonable sampling method for uncovering the “dark diversity” of shark species in New Caledonia. Ultimately, they found that eDNA had a lower field sampling effort than traditional survey methods (underwater visual censuses and baited remote underwater video stations) and detected species that were missed in those traditional survey methods. To do this, they used a set of DNA barcoding primers specific to elasmobranchs (rather than a universal metabarcoding primer, which would have yielded ~300 bp rather than 127 bp amplicons). This is likely the reason why they were unable to identify their molecular operational taxonomic units (mOTUs) to the species level – the primer they chose was too conserved among the target shark species of interest. In addition, there is likely not a large database of shark DNA for the COI region they targeted, particularly in this region of the world. They ended up finding 16 species in total using all three methods, suggesting that eDNA is a complementary approach to visual surveys. With their Venn diagram (Fig. 2), it is challenging to justify why an organism would be detected visually at a site, but not leave behind DNA for sample collection (like in the other sampling locations where there is overlap between eDNA and visual detection). They do not talk about this detection difference (- eDNA/+visual) at all in their discussion.

These two papers highlight the different utilities that eDNA sampling has, depending on the taxonomic context. For microorganisms, sampling whole organisms means that genomes can be annotated for their functions, but taxonomic identity is less certain at the species level due to high amounts of lateral gene transfer among microbial “species”. Generally, microbes are only classified to the phylum level, whereas resolving shark taxa to the genus or even family level in Boussarie et al. (2016) would not have been adequate resolution for the purposes of their research. This difference in taxonomic resolution is also reflected in the amount and origin of functional resolution needed to infer overall ecosystem functions/health– while microorganisms can be assigned specific functions based on genomic databases, macro and megafauna are assigned ecological functions based on their diet and previous field observations. The role of functional redundancy is more favored in a microbial context (in other words, specific species/OTUs are not as important), while largely ignored when talking about larger organisms like sharks. In part, this is because marine megafauna are charismatic species, so the local extirpation of one species prompts a large conservation response. In fact, Boussarie et al. (2018) completely disregards why shark diversity is ecologically relevant in their paper, only justifying the study because it is inherently important to detect shark species. Regardless of this paper, the discrepancy in functional assignment between micro and macrofauna could be lessened if macrofauna had a robust and standardized functional database, the ecological equivalent to GO-terms. These different viewpoints for the valuation and protection of specific species brings into question how ecological function is valued between large and small organisms – will we be able to accept functional redundancy in macrofauna communities as readily as we do in microbial communities, even if that means a loss in species diversity?

**Table 1:** Comparison of major methods and take-aways from both papers.

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| --- | --- | --- |
|  | **Sunagawa et al. (2015)** | **Bousarie et al. (2018)** |
| **Target organisms** | Microbes (viruses, prokaryotes, picoeukaryotes) | Sharks |
| **Location** | All over the world (except Indonesia!) | New Caledonia (southwestern Pacific) |
| **Environmental data** | Yes | No |
| **Other surveying methods?** | No | Yes – UVC and BRUV |
| **Origin of eDNA** | Whole organisms (mainly) | Shed DNA (feces, skin, etc) |
| **Sequencing effort** | Shotgun sequencing (+ used 16S region to assign OTUs @97% sequence similarity) | Amplicon sequencing only (barcoding primers + BOLD database @ 99% sequence similarity) |
| **Taxonomic resolution** | Phylum | Species |
| **Functional resolution** | Assigned/annotated using a generated set of 433 ocean microbial reference genomes | Interestingly, no mention of sharks’ function in an ecosystem – just that they need to be detected for conservation |
| **Functional redundancy** | “Core” set of oceanic functions irrespective of location and taxonomy | N/A – see above |
| **Spatial/depth heterogeneity?** | Communities differed between depths, dependent on temperature | N/A – though impacted areas (based on proximity to human pops and not fishing pressure) had less diversity than “wild” areas |

Works Cited

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