Environmental DNA as a cost-efficient alternative method to fish monitoring in high-energy environments

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I. Introduction

BECAUSE offshore energy converters (e.g., wave and tidal devices, offshore wind turbines, floating solar) have the potential to affect marine habitats around them, regulatory bodies often require pre- and post-installation monitoring to track potential changes. Common means of surveying and monitoring marine habitats and species for environmental impact assessments for marine renewable energy (MRE) projects include active and passive gear types and approaches. Traditional active sampling methods include bottom and pelagic trawls, nets and grabs, whereas passive sampling can include non-invasive underwater visual surveys or acoustic sonars. While the latter rarely provides truly reliable identifications to the species level, the former comes with the inherent drawback of killing most of the catch. Combined with the high-energy environments usually targeted for MRE deployments, sampling can be particularly challenging (e.g., time, cost, reliability of observations).

Environmental DNA (eDNA) methods could alleviate these challenges by not only providing a more reliable methodology to detect organisms, but also an approach that offers significant cost savings over traditional sampling techniques [1]. Every animal sheds cells in its environment, which can be sampled and DNA extracted from, in order to identify local organisms. This non-invasive method, developed and improved over the last decade, is called eDNA metabarcoding (similar to using a wide net to catch everything) or eDNA profiling (similar to targeted hook-and-line fishing). In the aquatic environment, this method has been applied for detecting and monitoring rare [2] and/or invasive species [3] and has been shown to outperform several other sampling methods in many circumstances [1]. Although eDNA shedding and decay rates vary among organisms [4], eDNA communities seem stable over time and tidal cycles within a sampling location [5].

While the science and technology associated with eDNA has been applied in numerous aquatic environments, the novelty of the approach requires optimization of protocols to ensure adequate matching of environmental conditions to the detection of target species. In addition, the vast majority of eDNA protocols produce presence detections, and quantification of a species abundance is still in its infancy [6]. With well-established, easy-to-use protocols, eDNA could become a cost-effective method for rapidly monitoring marine species (e.g., threatened or endangered, invasive) in habitats of interest. However, an eDNA approach may also result in extremely high numbers of sequences from a great diversity of species, and monitoring for a specific species at MRE sites may be overwhelmed by extremely large datasets.

In this study, we assessed the efficiency of eDNA compared to conventional methods following two axes: 1) comparison of the costs of conducting fish surveys with eDNA, beach seine, and scuba divers; and 2) biodiversity survey in a tidal channel using eDNA and underwater images. We used an eDNA metabarcoding approach to characterize the eDNA background signature due to the presence of marine species on and around a bottom-mounted artificial structure as well as a floating dock in a tidal channel, in lieu of an actual MRE converter.

II. Methods

The project followed two related axes: 1- a review of necessary eDNA supplies and cost comparison of surveys conducted with eDNA and conventional methods (desktop study); and 2- an analysis of eDNA samples collected in a tidal channel and compared to species identified on underwater images taken alongside the water samples (field study).

A. Desktop study

A literature review and cost analysis were completed to identify the diversity and cost of supplies specific to eDNA field collection and to evaluate different laboratory methodologies for DNA extraction. Peer-reviewed articles detailing eDNA surveys in marine and riverine ecosystems were selected from generalist scientific journals (e.g., PLoS ONE, Scientific Reports) and additional articles were derived from online knowledge

base searches. In addition, all articles from the dedicated journal *Environmental DNA* were also evaluated. A total of 202 articles were reviewed. Information relevant to articles' metadata and supplies used for eDNA sampling, filtering, and extraction was collected from each document. Equipment and supply costs were obtained from internet searches for each supply type and brand. Supplies were divided into categories (e.g., collection method, filtration method, filter pore size, extraction kit) and graphs were used to represent the diversity and proportions of supply types and brands per category, as well as their price ranges. This literature review highlighted the most commonly used supplies, which were then used to design a hypothetical eDNA survey in order to compare its cost to the costs of conducting comparable beach seine and scuba diver video transect surveys in Sequim Bay. These surveys assumed four sites where data were collected over the course of a single day during daylight hours. The eDNA survey consisted of two staff (one boat operator and one water sampler), the scuba survey included three staff (one boat operator and two scuba divers), and the beach seine survey included four staff (one boat operator and three field biologists to deploy the seine and process the catch). Parameters taken into consideration included cost of supplies, and labor costs to obtain necessary permits, conduct the field research, and process and analyze field-collected data (i.e., preliminary data processing steps to convert raw, field-collected data ahead of robust data analyses, including by potential subcontractors). Labor costs incurred to projects (i.e., including overhead and other related costs) were calculated based on assumed average hourly rates of $20/hour for students and $130/hour for senior researchers.

B. *Field study*

Triplicates of 1-L water samples were collected in the tidal channel at the entrance of Sequim Bay, WA (USA), at the seafloor and at the sea surface. Seafloor water samples were collected by scuba divers in the immediate vicinity of an artificial structure heavily colonized by invertebrates and macroalgae at ≈ 10 m deep. Surface water samples were collected by hand near the underside of a less-heavily colonized floating dock. Sample collections occurred in March, May and August 2021 and coincided with slack tide. Underwater photos were opportunistically collected with the seafloor and surface water samples to compare the biodiversity identified on the images to the species list obtained from eDNA sequencing. Organisms on the images were identified to the lowest taxonomic level possible by a trained benthic ecologist. In addition, triplicates of mid-depth water samples were collected in the summer of 2022 near the floating dock every two hours during the full cycle (ebb and flow) of a spring and neap tide to analyze the tides' influence on the variability in fish eDNA and quantify patterns in assemblages relative to variations in the tidal cycles.

Water samples were filtered using a vacuum pump and single-use filter funnels equipped with a 0.45 μm cellulose nitrate filter. Filters were preserved in 95% ethanol and stored in the -20°C freezer until processing. For each collection period, DNA of one surface and one seafloor samples was extracted from the filters using an extraction kit and quantified with a microvolume UV spectrophotometer. Samples (filters and DNA extracts) were sent to a subcontractor for DNA extraction (filters) and sequencing (all samples) of the genetic marker 12S. Upon receipt of the data from the subcontractor, sequences were aligned, identified against sequences available in existing online nucleotide databases (i.e., blast), and analyzed using phylogenetic tools. Results were compared between the sampling periods and locations using univariate (t-tests on Shannon diversity and Pielou evenness indices) and multivariate statistical analyses (i.e., non-metric multidimensional scaling [nMDS], permutational multivariate analysis of variance [PERMANOVA]) to identify consistencies and discrepancies in species present at the sampling site.

III. RESULTS

A. *Desktop study*

The thorough literature review of more than 200 journal articles revealed an eDNA toolkit more diverse than expected and highlighted the absence of consistency in field and lab methods and supplies used for eDNA collection and processing. The literature review identified 56 different collection methods, ranging from water samplers (e.g., Kemmerer, NIOZ, Niskin) and sampling bottles (e.g., Corning, Nalgene) to syringes and capsule filters. Bottles were the most common eDNA collection method, and among them Nalgene was the most common brand of bottles. These bottles are available in a diversity of volumes, and 1 L was by far the preferred volume of sampling, which costs around $8 per unit.

The filtration step of the eDNA method was also associated with a range of approaches and supply types. Examples included various techniques to pump the water sample through a filter, numerous types of filters to process the water, and a range of pore sizes for these filters. A large majority of the reviewed studies used peristaltic or vacuum pumps to draw the water samples through the filters. The most common types (material) of filter were glass fiber and cellulose nitrate, in very similar proportions. The most common filter pore size used was 0.45 μm, available for both glass fiber and cellulose nitrate filters. Filter costs ranged from $0.57 to $4.46, with an average of $1.51 per unit.

The total cost of conducting a hypothetical eDNA survey in Sequim Bay was $4190, which was lower than the scuba diver and the beach seine surveys ($11,580 and $14,953 respectively) (Figure 1). Permitting costs and extra labor needed for field data collection resulted in increased costs for the beach seine survey, and the greatest expenses for the scuba diver survey were associated with data

collection and post-processing of field data. Data processing (i.e., sequencing by a subcontractor) was the highest cost for the hypothetical eDNA survey.

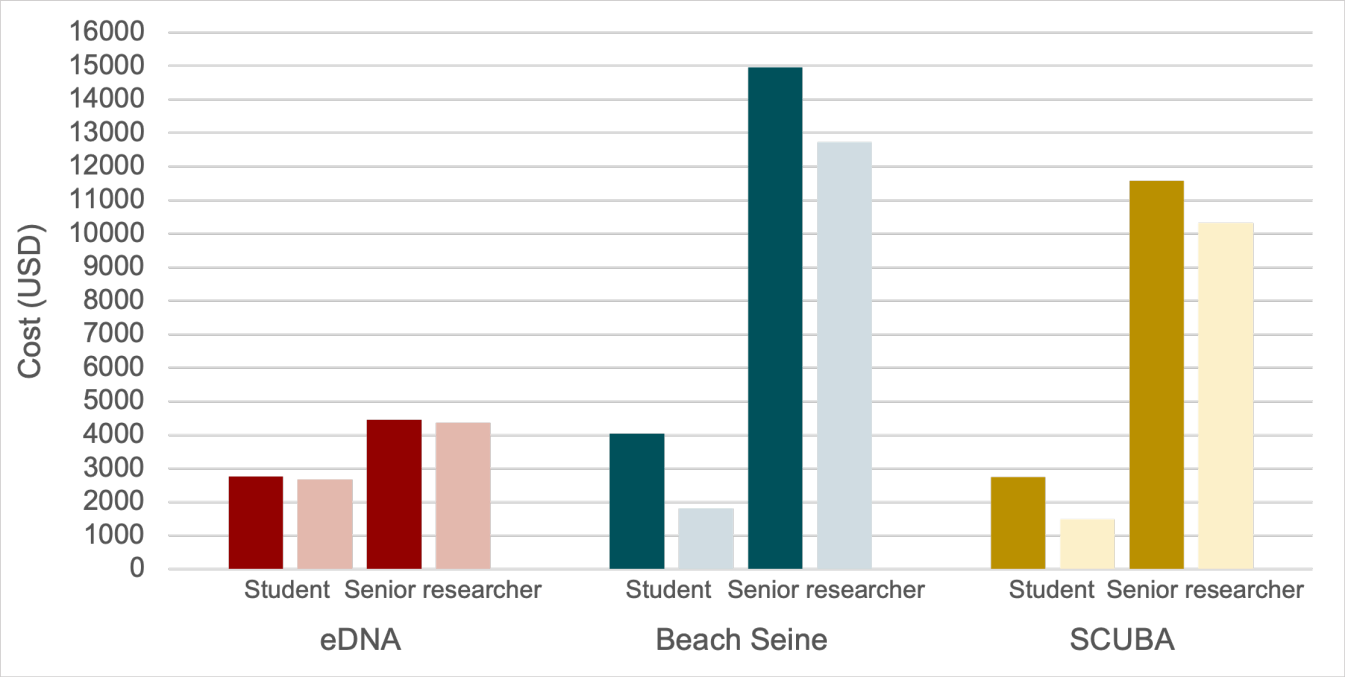

Fig. 1. Cost comparison of eDNA, beach seine, and scuba surveys undertaken by teams of students or senior researchers with all new supplies (indicated by the darker colors), as well as follow-up surveys that would reuse part of the supplies (lighter colors).

B. Field study

A diversity of 12S fish sequences was obtained, belonging to 38 species, and 15 others identified to higher taxonomic levels, belonging to 24 families. Pelagic fish species identified in the eDNA samples included salmonids (chum salmon, coho salmon, cutthroat trout), Pacific herring, shiner perch and surf smelt. Benthic fish species included bay goby, buffalo sculpin, flounder, kelp greenling, saddleback gunnel and sturgeon poacher. About 280 species of fish have been identified in the Salish Sea, belonging to 72 families. Habitat diversity in Sequim Bay and its channel are relatively poor, which explains the limited representativeness of its fish diversity as compared to the whole Salish Sea.

Despite the diversity of fish identified from eDNA, scuba divers reported only observing buffalo and scalyhead sculpins during sample collection at the seafloor site. Most of the benthic fish species identified from eDNA are small fish that hide in cracks, which may explain why most of these species were not observed. Additionally, divers were not conducting systematic transect surveys because observations were made to opportunistically coincide with collecting water samples. It is possible that fish whose eDNA material was collected were not in the immediate vicinity of the sampling site. Small pelagic fish were visible from the floating dock during collection of the surface samples, but identification was impossible without actually capturing fish.

Multivariate statistical analyses (nMDS and PERMANOVA tests) showed no significant differences between depths and months of sampling (Figure 3), nor between spring and neap tides or ebb and flow (Figure 4).

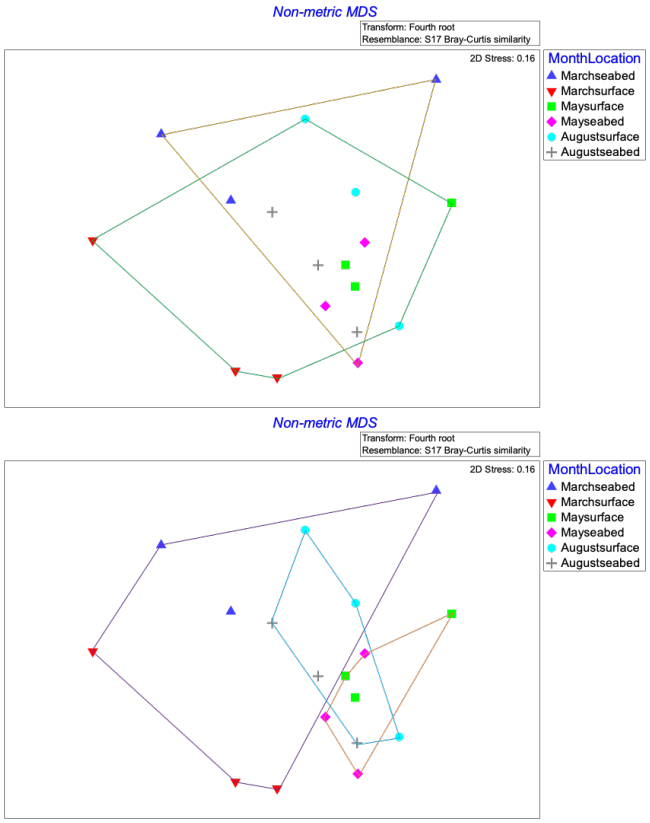

Fig. 3. Non-metric multidimensional scaling plots with 2021 samples identified by location and month status, highlighting all surface vs. seabed samples on the top, and all March, May and August samples on the bottom.

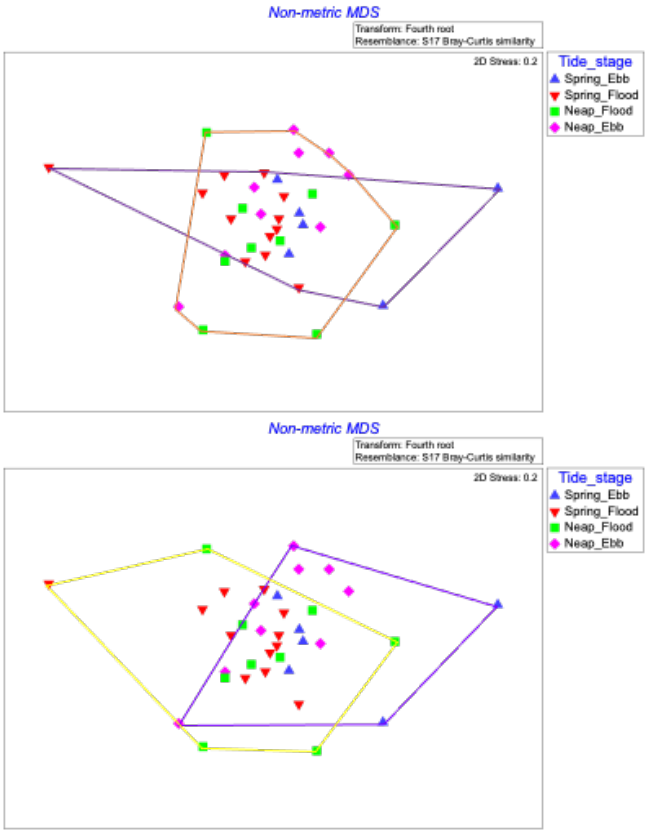

Fig. 4. Non-metric multidimensional scaling plots with 2022 samples identified by tide and tidal stage status, highlighting all spring tide vs. neap tide samples on the top, and all ebb flow vs. flood flow on the bottom.

In addition, the Shannon diversity was reasonably high and in line with other marine environments (e.g., New Jersey [7], Indonesia [8], or Florida [9]), and not significantly different between sampling month or location (2021), or between tide or cycle (2022). Community evenness was also not significantly different in our data – Pielou evenness values between 0.5 and 0.75 are reasonable given the observed diversity in the environment.

IV. Conclusion

Our study highlighted the range of eDNA supplies and methods reported in the scientific literature and provided support for our method development approach. In addition, we confirmed eDNA methods provide a more cost-effective alternative to conventional capture methods of surveying marine biodiversity that are often challenging to implement in high-energy environments. From these preliminary results, eDNA methods provided insights into the local biodiversity of fish in the tidal channel of Sequim Bay. Similar to findings reported in the literature, these methods support the identification of more species than observed by other methods such as scuba, and spatiotemporal variability was not significant at the scale of a tidal channel, across tidal cycles, and throughout months. Our results show that molecular techniques such as eDNA offer a cost-efficient, comprehensive, and reliable alternative to conventional methods, adapted to fish monitoring in high-energy environments.

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