Environmental DNA for fish monitoring around tidal energy devices

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Abstract— **Marine renewable energy (MRE) development faces many challenges. Uncertainty from the regulatory community regarding environmental effects can often result in project delays and costly monitoring campaigns. Additionally, effectively studying organisms in dynamic and variable conditions is difficult under normal circumstances; high-energy environments like tidal streams and channels add to this difficulty. Traditional tools and approaches for monitoring the potential effects of MRE technologies are often insufficient or challenging to implement around tidal turbines due to the strong currents, limited visibility, and short slack windows between ebbs and floods. The analysis of environmental DNA (eDNA) is a relatively new method that uses molecular techniques to detect and identify species from genetic material shed in their environment. This material is collected through water samples and does not require the physical capture or handling of any organisms. A recent study in a tidal channel in Washington State (U.S.) showed that eDNA is a cost-effective, comprehensive, and reliable alternative to conventional methods for evaluating fish presence and habitat use in the context of tidal energy development. A follow-up study is now looking at establishing the efficacy of eDNA in the same high-energy environment by quantifying the effects of hydrodynamic and seasonal variations on molecular response variables, validating eDNA results using underwater images, and evaluating the relevance of findings to the regulatory context for MRE monitoring programs. This paper will present an overview of the relevance of the eDNA approach to the U.S. regulatory context for fish monitoring around tidal energy projects, as well as preliminary results of the field data collection campaign.**

Keywords—**Environmental DNA, environmental effects, environmental monitoring, regulatory context.**

I. INTRODUCTION

MARINE renewable energy (MRE) converters have the potential to affect marine habitats around them and the various associated species, especially those already threatened by global changes and other human activities at sea [1], [2]. The scientific community has come together to identify the main environmental effects associated with MRE, which are the risk of collision for marine animals with turbine blades, the emissions of underwater noise and electromagnetic fields (EMFs), changes in marine habitats and oceanographic conditions, the risk of entanglement for animals with mooring lines and cables, and the potential displacement of marine animals from their usual habitats [3]. However, uncertainties about these effects on marine species, habitats, and ecosystem processes have caused project delays and costly monitoring campaigns to assess potential risks during the regulatory processes. Consequently, the use of new, cost-effective monitoring methodologies to assess these risks is timely and needed.

Common methods of surveying and monitoring marine species, especially fish, and their habitats for environmental impact assessments for MRE projects include active and passive gear types and approaches [4], [5]. 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 passive methods rarely provide truly reliable identifications to the species level, the active methods come with the inherent drawback of killing most of the catch. High-energy environments like

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tidal streams add challenges to collecting meaningful data with traditional sampling tools, such as entrained air and low visibility limiting the use of sonars or cameras, or strong currents and short slack windows preventing scuba divers from sampling adequately.

The environmental DNA (eDNA) approach could alleviate these challenges by providing a comparably reliable methodology to detect organisms, as well as offering non-negligible cost savings over traditional sampling techniques [6], [7]. Moreover, regulators need to know about fish presence, distribution, and habitat use at MRE sites to evaluate how proposed projects may impact fish populations, especially those from species of conservation, economic, or cultural value [8]. Molecular tools like eDNA can help gather this information. For instance, a recent study in a tidal channel suitable for MRE development (Sequim Bay tidal channel, in Washington [U.S.]) evaluated temporal and environmental factors that may affect fish eDNA data throughout seasons and tidal cycles and demonstrated that eDNA is a cost-effective, comprehensive, and reliable alternative to conventional methods for evaluating fish habitat use associated with tidal energy projects [9].

In this context, eDNA can also help gather information on fish assemblages, which are affected by a combination of environmental and seasonal conditions. Some fish are local resident while others use nearshore coastal habitats intermittently at different times of the year [10]. Likewise, changes in hydrodynamic and environmental conditions will also affect variations in fish assemblages. Monitoring tools must be sensitive enough to detect and estimate these sources of variation. For instance, previous work has started to show that tides and tidal cycles have little influence on eDNA data, which tend to be endogenous to the sites sampled [11], [12]. With optimized sampling designs, eDNA monitoring methods will become a common robust and affordable tool for assessments at MRE sites to identify whether changes in presence and habitat use occur, which is particularly valuable for evaluating impacts to threatened species.

The present study builds upon the findings from the previous project in Sequim Bay tidal channel [9] to evaluate the variation of fish eDNA data in relation to environmental factors and help establish a robust eDNA procedure for surveying fish at MRE sites. The goal of the study is to develop a cost effective, easy to implement molecular sampling approach for generating reliable data on fish presence at MRE sites. The research objectives include 1) establishing the efficacy of in-situ sampling in a high-energy environment by quantifying the effects of hydrodynamic and seasonal variation on eDNA; 2) validating eDNA results using an underwater camera; and 3) evaluating the relevance of findings to the regulatory context for MRE monitoring programs. As such, the study was designed to evaluate the variation in fish eDNA data over 12 consecutive months to determine the efficacy of eDNA and inform design of future monitoring programs by optimizing the selection of environmental parameters, sample size, and timing of data collection. In addition, validation will help provide evidence of reliability of the eDNA method and support adoption into future fish monitoring campaigns. Visual data, obtained from underwater cameras, are used to confirm which fish were present at the time of water sample collection. It is likely that eDNA is more sensitive than visual observations and may result in higher diversity [13], [14]. In addition, the study inventories the information requested about fish at consented MRE projects in the U.S. to identify circumstances where eDNA could provide comparable information and how the molecular approach could be implemented in fish monitoring plans. The present paper summarises preliminary results of a project that will be accomplished over two years, here focusing mainly on objectives 2 and 3 after a year into the project.

II. METHODS

A. *Relevance to regulatory context*

A review of the nine U.S. tidal and riverine energy projects listed on the Tethys website [i] and associated documents was undertaken to assess the relevance of eDNA to the U.S. regulatory context for fish monitoring around MRE projects. Regulatory entities for these projects were the U.S. Federal Energy Regulatory Commission (FERC), the U.S. Army Corps of Engineers (USACE), the U.S. Environmental Protection Agency (EPA), and several state and local agencies. This review enabled the identification of the regulatory concerns pertaining to fish species, any required monitoring measures and the approaches taken by the project proponents, any challenges in implementing these measures, and instances where eDNA may have been an alternative method. Table I summarizes the metrics evaluated for this review.

TABLE I

DESCRIPTION OF THE CATEGORIES USED IN THE REVIEW OF U.S. TIDAL AND RIVERINE ENERGY PROJECTS.

Category	Description
Project name	Project name as listed on Tethys
Location	Geographic location of the project
Type of energy	Type of turbine/device used
Status	In operation, decommissioned, operation ended, cancelled, planned
Environmental requirements	Types of information required in licensing processes
Objectives of monitoring	Main goals of fish monitoring
Methods used	Main methods used in fish monitoring to meet requirements
Main results	Main results of fish monitoring
Risk of impact	Conclusions from fish monitoring against regulatory requirements
Environmental assessment	Deliberation from licensing agencies
Missing information	Information relative to proposed methods that was not acquired
Role for eDNA	How eDNA may have been used to meet regulatory requirements

B. *Fish eDNA monitoring*

For this part, the project relies on 12 consecutive months of collection of eDNA samples, underwater images, and ancillary environmental data in Sequim Bay channel, Washington (U.S.).

1) *Data collection and processing*

Using a horizontal water sampler, samples were collected monthly on one day of a spring tide, when tidal exchange is strongest (Table II). Water was collected in triplicates at 3-m deep from a floating dock within the tidal channel, on the hour before slack tide, at slack tide, and the hour after slack tide. Water samples were filtered on site, then the filters were sent to a subcontractor to undertake the molecular (12S metabarcoding) and bioinformatic analyses.

Concurrent to water sample collection, a 360-degree video camera (see [15]) was lowered to the seafloor at 5-m depth to record timelapse images at the sampling location to validate eDNA results by providing visual images for fish identification and count data collected over the same time period. The camera started recording images two hours prior to the first water sampling and ran until all samples were collected. Images were reviewed by two trained biologists to count and identify to the lowest taxonomic level possible all fish in field of view.

Ancillary environmental data were gathered for the days and times of sampling from sensors (i.e., tide gauge, acoustic Doppler current profiler [ADCP], conductivity-temperature-density [CTD], dissolved oxygen [DO], photosynthetic active radiation [PAR], weather station) deployed under the floating dock in the channel[ii]. These ancillary data (e.g., current speed, water temperature, pH, etc.) will be analyzed alongside eDNA data as environmental explanatory variables.

TABLE II
SAMPLING DATE AND TIME OF SLACK TIDE FOR EACH MONTH'S FIELD COLLECTION EVENT AND REMAINING SAMPLING PERIODS OVER THE TWELVE MONTHS OF THE PROJECT.

2024 sampling		**2025 sampling**	
July	7/22, slack low tide 11:12 PDT	January	1/30, slack low tide 10:31 PDT
August	8/20, slack low tide 10:50 PDT	February	2/26, slack high tide 13:18 PDT
September	9/22, slack high tide 11:37 PDT	March	04/01, slack low tide 12:49 PDT
October	10/21, slack low tide 13:34 PDT	April	04/27 to 05/01
November	11/15, slack high tide 13:40 PDT	May	05/25 to 05/30
December	12/16, slack low tide 10:21 PDT	June	06/23 to 06/27

2) *Statistical data analyses*

eDNA 12S metabarcoding data have been received from the subcontractor for the samples collected from July to October 2024. These data consisted of ESVs (exact sequence variants) and numbers of reads detected in each sample. In a preliminary assessment of the results, species richness was calculated in R, using the number of sequence reads per species as pseudo-abundance, for each sampling time each month, and categorized as "ebb", "slack", or "flood". A Kruskal-Wallis test was computed in R to determine whether the species richness differed between tidal stages and across months.

Thus far, underwater images collected from August to November have been processed; however, the camera failed to record any image in October. Species identification quality control was conducted for the August dataset to test any differences in the fish identifications and counts by the two trained biologists. For each of the three cameras making up the 360-degree camera (see [15]), 100 images were randomly selected and differences in fish identifications and counts were noted. Chi-square tests were computed in R to test for the significance of the observed differences. In addition, Shannon-Wiener diversity and Pielou evenness indices were calculated for the months of August, September, and November, and compared with pairwise Hutcheson t-tests [16].

III. RESULTS

A. *Relevance to regulatory context*

The review identified five U.S. legislations related to fish that apply to MRE development [17]: Section 7 of the Endangered Species Act, regulated by the National Marine Fisheries Service (NMFS); Section 10 of the Federal Power Act, by NMFS; Section 18 of the Federal Power Act, by the U.S. Fish and Wildlife Service (FWS); the Fish and Wildlife Conservation Act, by FWS; and the Magnuson-Stevens Fishery Conservation and Management Act (Essential Fish Habitat assessment), by NMFS.

While most applications were required to evaluate fish species diversity and presence at the project site, other requirements were objective- and site-specific. For instance, some projects were asked to collect baseline data while others were only required to collect post-installation data. Some of the other data collection requests for these nine projects covered the risk of collision of fish with MRE systems; impacts to habitats of known endangered or threatened species; the emissions of noise, electromagnetic fields, and/or pollutants; the movement and behaviour of fish near turbines; and potential population effects.

The U.S. tidal and riverine energy projects reviewed for this study used a combination of field methods and desktop approaches to collect data and compile information to meet the regulatory requirements. Field methods included underwater video imagery, acoustic monitoring, and trawl surveys. The desktop approaches included literature reviews of best available scientific information, and numerical modelling for fish collision.

Among the challenges that stemmed from the fish monitoring at the projects reviewed were the difficulty in distinguishing small fishes from debris in high-energy turbid environments; differentiating between fish and blade movement in close proximity to the turbines; quantifying fish entrainment, behavior, or habitat use; and estimating population effects from the data collected. In addition, the sheer amount of video footages recorded at some sites would have required the implementation of automated data processing and annotating algorithms to fully analyze the collected data. Lastly, it was noted that some data collection methods would need refinement to provide suitable datasets for collision risk models.

With this information in mind, if eDNA had been a common and validated method at the time these nine projects took place, it could have provided reliable and manageable data on the presence in the project area of fish species, and their habitat use, over tide cycles, months, seasons, and years. eDNA would have also enabled the quantification of population size for some species of focus, especially endangered or threatened fish species, without harming individuals in trawl hauls.

B. *Fish eDNA monitoring*

After sorting out all potential contaminations (i.e., any freshwater fish species or marine species unknown to the U.S. West Coast), the sample processing resulted in 212 unique ESVs, 172 of them identified to a fish species with over 97.5% match. These resulted in 62 different fish species belonging to 24 families. Cottidae (sculpins) was the family with the highest number of species (15), followed by Pleuronectidae (righteye flounders) with five species. While species richness varied from 5 to 12, most of the values were between 7 and 9 (Fig. 1). No statistically significant differences were observed between tidal stages or months.

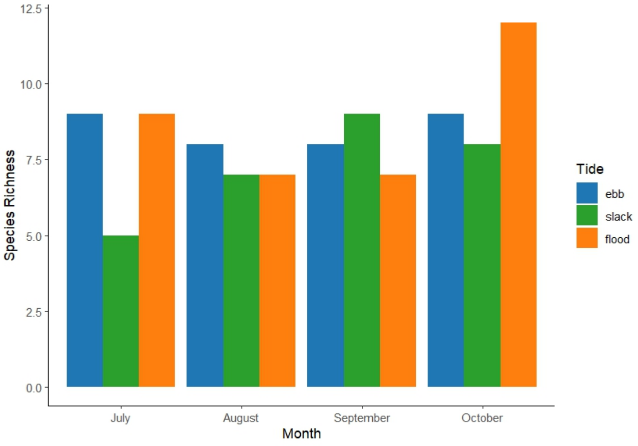

Fig. 1. Species richness obtained from the 12S environmental DNA metabarcoding data for samples collected between July and October 2024 and identified by tidal stage based on the time of sampling.

A total of seven different fish species were identified in the three monthly imagery surveys from the waters around the floating dock, from the seafloor to the sea surface, as well as several unidentified sculpins. The most common species, present on a majority of the images, was the shiner perch (*Cymatogaster aggregata*), in groups of a few individuals to several dozens of fishes. The quality control comparisons for the August dataset between the two trained biologists resulted in significatively different numbers of fish. Specifically, one person consistently counted more fishes than the other in larger schools of shiner perches and sand lances (*Ammodytes personatus*). The statistical comparisons of diversity indices returned significant differences between months. The Shannon values ranged from 0.02 (August) to 0.62 (September) and the Pielou values ranged from 0.005 (August) to 0.22 (September), with November indices in between. Shannon index in August was significantly lower than September. None of the other pairwise comparisons of the diversity indices were statistically different (Tables III and IV). Looking at species composition, August and September were dominated by the shiner perch while November was dominated by Pacific sand lance, potentially indicative of a seasonal shift in fish assemblages.

TABLE III
VALUES OF THE SHANNON DIVERSITY INDEX FOR THE MONTHS OF AUGUST, SEPTEMBER AND NOVEMBER (DIAGONAL) AND P-VALUE OF THE HUTCHESON T-TESTS, WITH STATISTICALLY SIGNIFICANCE OF PAIRWISE TESTS INDICATED BY * (LOWER HALF).

	August	**September**	**November**
August	0.0198	-	-
September	*0.0017**	0.6245	-
November	*0.1914*	*0.4280*	0.3807

TABLE IV
VALUES OF THE PIELOU EVENNESS INDEX FOR THE MONTHS OF AUGUST, SEPTEMBER AND NOVEMBER (DIAGONAL) AND P-VALUE OF THE HUTCHESON T-TESTS, WITH STATISTICALLY SIGNIFICANCE OF PAIRWISE TESTS INDICATED BY * (LOWER HALF).

	August	**September**	**November**
August	0.0048	-	-
September	*0.2045*	0.2219	-
November	*0.5935*	*0.8094*	0.1481

IV. DISCUSSION

Numerous freshwater and marine fish species around the world have faced threats of extinction for decades, resulting from overexploitation, habitat degradation, global warming, or other causes [18], [19]. Human industrial activities in coastal areas can be particularly harmful to fish populations, warranting special protection measures for species of conservation, commercial, cultural, or recreational interest. Even though MRE is a reliable source of sustainable energy, tidal and riverine turbines may pose an additional threat to vulnerable fish species and scientific research is conducted to better understand the potential effects [8].

What drive regulatory requirements and monitoring efforts for tidal and riverine energy projects in the U.S. are the potential presence of endangered or threatened species in the vicinity of a project site and the possibility of harm from installation, operation, and/or decommissioning activities [17]. Environmental effects from MRE turbines that are most likely to impact fish at various life stages are the risk of collision between fish and rotating turbine blades, the exposure to underwater noise from turbine generators (both the pressure wave and particle motion components of noise) or to EMFs from power export cables (for electro- or magneto-receptive species), changes in fish habitats (feeding, spawning, or nursery), and the displacement of fish to (attraction) or from (exclusion) a device area [3], [8], [20].

In order to evaluate these various effects of MRE turbines on fish, regulators need information such as the overall species diversity and distribution at a project's site, the presence of endangered and threatened species, the spatiotemporal trends of these species and their life stages, these species' ability to avoid and/or evade moving objects, and their sensitivity or receptivity to underwater noise and EMFs. While some of this information can be gathered from scientific literature, or transferred from research studies at comparable sites [21], new data on fish diversity and spatiotemporal distribution may need to be collected during the lifetime of an MRE project. A baseline assessment, whether from comprehensive historical data or from a detailed site survey, will provide an initial inventory of fish species and their life stages present at a project site throughout the year. Follow-up surveys would then allow an assessment of any changes in fish diversity and/or spatiotemporal trends after turbine installation, during operation, and after decommissioning, especially as species distributions shifts with climate change and other anthropogenic pressures [22], [23].

Numerous studies over the past decade have proven that eDNA is an efficient method for detecting fish in marine and freshwater environments (e.g., [7], [12], [13], [14]), paving the way for a study specifically targeting high-energy environments and their inherent monitoring challenges. Our present study demonstrates that eDNA is able to capture much higher numbers of fish species in a tidal channel than underwater imagery methods (62 species vs. 7 species, respectively). eDNA also delivers species diversity trends that seem more balanced over the few months studied here than the underwater imagery data, which would need to be reassessed upon completion of the 12 months of field data collection. These preliminary results still demonstrate that eDNA can be a valuable method for collecting baseline and follow-up data on the diversity and distribution of fish at MRE sites, increasing the chances of detecting species of interest (endangered or threatened species, or species sensitive to underwater noise or EMFs) over underwater imagery surveys. However, eDNA surveys are currently limited in quantifying fish abundance, unlike imagery- and catch-based methods, although recent developments are inching toward eDNA quantification [24], [25]. In addition, it is difficult with the eDNA approach to distinguish between endogenous eDNA, synonym to a species locally present at the moment of sampling, and exogenous eDNA, coming from individuals present on a broader spatiotemporal scale and brought to the sampling site by current or particle resuspension [26]. In the MRE context, this limits the ability to tell whether an animal was near a device or in its greater vicinity, even when a water sample is collected next to an MRE device.

Underwater imagery methods (both optical and acoustic) remain necessary as part of an environmental monitoring plan for MRE projects. While they may not record every species present in a project area, they provide eyes underwater and especially enable an assessment of fish behaviour around devices, especially in close proximity [4], [27]. Evaluating fish avoidance and evasion behaviour around rotating turbines is particularly crucial to understand their risk of collision at project sites [8]. eDNA would complement these underwater imagery methods by providing a more accurate estimate of fish biodiversity and spatiotemporal trends, and enable cost-efficient replication of the sampling [28].

V. CONCLUSION

While the results presented here are preliminary, after nine months of field data collection and four of those months analysed, it appears evident that eDNA will be a valuable monitoring method for collecting field data on fish relevant to regulatory requirements for tidal and riverine energy projects in the U.S. With 12 months of eDNA data collection, the project will eventually be able to also evaluate seasonal trends in fish diversity and use of Sequim Bay tidal channel.

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