Natural England Commissioned Report NECR287

Pilot study to validate an environmental DNA sampler for monitoring inshore fish communities

First published 21 May 2020

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NATURAL ENGLAND

Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

DNA based applications have the potential to significantly change how we monitor biodiversity and which species and taxa we monitor. These techniques may provide cheaper alternatives to existing species monitoring, an ability to detect species that we do not currently monitor effectively and the potential to develop new measures of habitat and ecosystem quality.

Natural England has been supporting the development of DNA techniques for a number of years. The use of environmental DNA (eDNA) to determine the presence or absence of great crested newts in ponds is now a standard tool for developers and consultants.

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Samples of water from which eDNA can be extracted and used for monitoring are usually collected manually, which limits the volume of water that can be filtered, however this study trials the novel use of a programmable large volume eDNA filtration system which is deployed on the seabed. The collection of water over a tidal cycle increases the likelihood of the detection of species using eDNA, and this study was intended as a proof-of-concept to determine if the method could be used to monitor inshore fish communities, which are traditionally both expensive and challenging to monitor accurately

This report should be cited as: MYNOTT S., 2019 'Pilot study to validate an environmental DNA sampler for monitoring inshore fish communities'. Natural England Commissioned Report number NECR287

Natural England Project Manager – Maija Marsh

Contractor – Applied Genomics

Keywords – eDNA, metarbarcoding, inshore fish, marine monitoring

Further information
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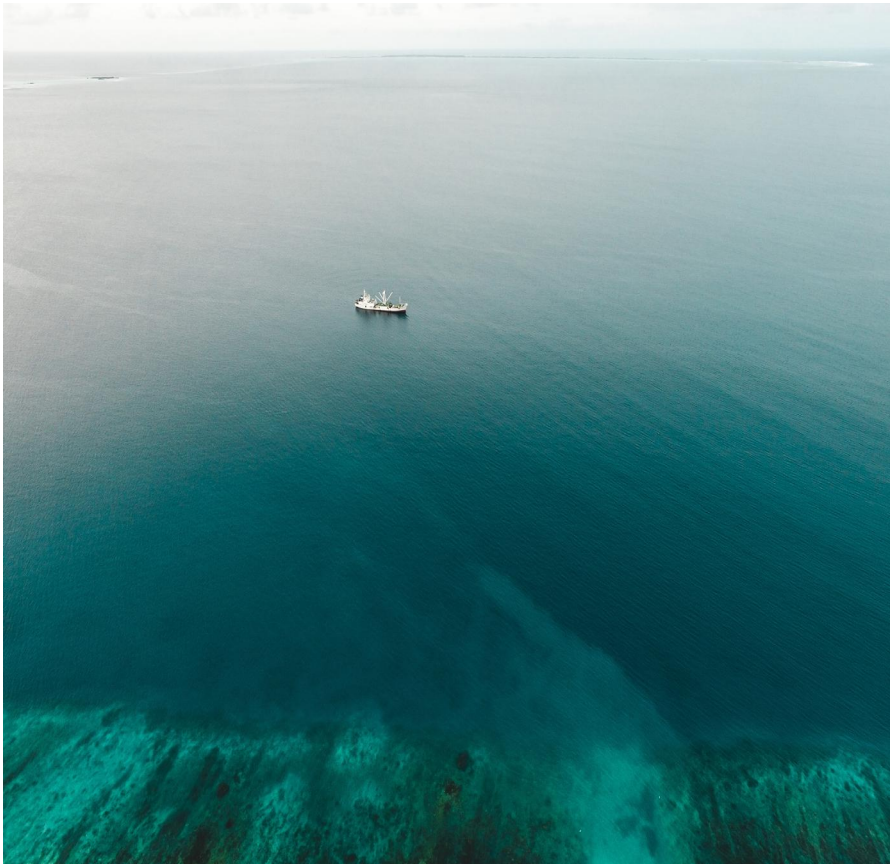
ISBN 978-1-78354-581-0

Applied Genomics



PILOT STUDY TO VALIDATE AN ENVIRONMENTAL DNA SAMPLER FOR MONITORING INSHORE FISH COMMUNITIES

Final Project Report

ABSTRACT

This document reports on the results of a pilot study project to investigate the feasibility of monitoring inshore fish communities using a large-volume marine eDNA sampler. Samples were collected at three locations along the south Devon coast. Recovered samples were processed, DNA was purified and sequenced. The DNA sequence data were bioinformatically processed and analysed to build an understanding of local fish community diversity. It is hoped that this method will provide an independent and unbiased method for continuous monitoring of inshore fish communities.

Sebastian Mynott

Applied Genomics Ltd.

Brixham Environmental Laboratory
Freshwater Quarry
Devon TQ5 8BA
solutions@appliedgenomics.co.uk
01803 611818

Pilot study to validate an environmental DNA sampler for monitoring inshore fish communities

Project Report

Report for:

Dr. Maija Marsh

Natural England

Project number:

PRJ18002

Document control:

STATUS	AUTHOR	DATE
INTERIM REPORT	Sebastian Mynott, Chief Operations Officer	08.MAR.2019
DRAFT FINAL REPORT	Sebastian Mynott, Chief Operations Officer	25.MAR.2019
FINAL REPORT	Sebastian Mynott, Chief Operations Officer	29.MAR.2019
REVISED FINAL REPORT	Sebastian Mynott, Chief Operations Officer	03.APR.2019

Executive Summary

The utility of environmental DNA (eDNA) techniques for monitoring species diversity are gaining acceptance amongst industry and government decision-makers. This interest is driven by the pressing need to better understand the effects of anthropogenic pressures on ecological communities and the capability of eDNA methods to deliver more biodiversity data at reduced effort and cost. Sample collection presents the greatest risk for any environmental survey and particularly so for eDNA surveys. The importance of good survey design should not be underestimated. Despite its known importance in conventional ecology, few eDNA studies have examined sampling effort, the effect of sample volume and sampling duration on the probability of eDNA capture (Grey *et al*. 2018). Simply put, the greater the sampling effort, the greater is the probability of detection. Applied Genomics have addressed this by developing a marine eDNA sampler capable of sampling up to 50 litres of demersal seawater over a programmable time period of several hours to several days.

This document presents a report on a pilot study project to investigate the feasibility of monitoring inshore fish communities using a large-volume marine eDNA sampler. Three replicate sample collections were conducted at each of three sampling sites. Recovered samples were processed, DNA purified, fish-specific metabarcode amplicons for 12S and CytB genes were produced. Sequencing was undertaken on an Illumina MiSeq DNA sequencer. The resulting sequence data were subjected to bioinformatic processing, taxonomic assignment and biostatistical analyses.

Our methods characterised a total of 74 unique fish and marine mammal species to species level, of which 14 were identified as listed protected species. We provided data and visualisation of species haplotype diversity, which is an indication of the diversity of breeding individuals within each sampled population. In addition to assessing the performance of our sampling strategy, we undertook fish community analyses for alpha, beta and gamma diversity, and compared our results to historical data for recorded fisheries landings.

Whilst the study suffered from low statistical power, we were able to demonstrate the considerable potential of this sampling system for the cost-effective, independent and unbiased monitoring of fish communities and of marine biodiversity. The data resulting from the ongoing monitoring of inshore marine habitats using large-volume eDNA samples, such as these, could serve the remit of multiple government agencies, thereby providing an unprecedented level of information about the health of our fisheries and the wider coastal ecosystem at relatively low cost.

Background

Inshore fish communities are poorly understood. There is still a considerable lack of understanding of inshore fish community composition, community health, population status, as well as the scale and footprint of critical pressures and the efficacy of management measures. Filling these evidence gaps is a priority for Natural England, as upon EU Exit, there is a possibility that inshore fishing effort will increase (as a result of increased inshore quotas and vessel displacement), however the UK will still be required to meet its national and international commitments related to fisheries management and marine conservation. When combined with commitments to improve the state of our marine environment identified in the 25 Year Environment Plan and the UK Marine Strategy, there is a clear evidential need to understand the nature and status of our inshore fish populations.

Environmental DNA (eDNA) is genetic material that is released by organisms living in their environment. Sources of genetic material may include, faeces, urine, skin cells, mucous, gametes, etc. The DNA from these sources will degrade over time but persist in the environment for long enough that the presence of organisms in the environment may be detected without their being seen or captured. The marine environment is large, heterogeneous and dynamically variable. Validation studies undertaken by Applied Genomics have shown that large-volume eDNA sample collection is required to maximise probability of detection, reduce inter-sample variability and ensure meaningful results. We have developed proprietary technology for consistent large-volume environmental DNA sample collection, robust laboratory protocols for processing these large samples and a proven bioinformatics pipeline which delivers reliable results.

The sampler was built to operate at depth, sampling the demersal layer currents. Demersal currents are generally slower moving due to laminar boundary layer flows with less influence from surface fluctuations, such as Langmuir circulations (Barstow, 2003) and may have greater directional consistency due to Ekman layer effects (Ekman, 1905). This results in a Eulerian-transect sampling strategy designed to mitigate sampling variability from tidal currents and longshore currents to provide an unbiased method for surveying local area marine life. By its nature, DNA captured from the environment is normally associated with particulate matter, such as soughed skin cells, faeces, etc. which are heavier than water. Whilst these particles may persist in the water column for some time, they will, eventually, precipitate out. We can therefore assume that the demersal layer may contain a slightly higher density of eDNA particulate matter than may be found nearer the surface. However, the rate at which this precipitation may occur in real terms is highly stochastic. Collins *et al*. (2018) experimentally modelled the decay rate of eDNA in the marine environment, estimating an inshore eDNA half-life of 21.2 hours.

This project is a preliminary (proof-of-concept) study to test the use of a marine eDNA sampler for monitoring inshore fish communities at a whole site and habitat specific sub-feature level. It has been designed to complement a number of other ongoing projects that aim to target key knowledge gaps in inshore fish biodiversity identified by Natural England and the HBDSEG (Healthy Biologically Diverse Seas Environment Group) and acknowledged by Defra. This pilot study represents a step in the process of finding a cost-effective method to effectively monitor inshore fish communities, which in turn will underpin our ability to effectively conserve, manage and sustainably exploit these resources and allow the UK to develop a world class marine monitoring system post-EU exit. This

project has been undertaken in SW England and aligns with the pilot areas being used for a related, Defra funded (and Natural England managed) project entitled “Regional pilot of approaches to monitoring inshore fish communities”.

Methods

Field Sampling

The large volume marine eDNA sampler (Figure 1) was deployed in three locations with differing ecological substrata (Figure 2; Site 1: kelp, Site 2: mud, Site 3: rock) and similar hydrological characteristics in the inshore waters around Brixham, Devon. Sites were selected on the basis that they would be phylogeographically comparable but sufficiently separated that they could be treated independently. Each sampling station was similarly exposed to southerly weather and sea states from the English Channel and differences in tide times and ranges between stations were negligible.

Figure 1: Applied Genomics large-volume eDNA sampler.

The eDNA sampler was programmed to undertake repeated periodic sampling over a 25-hour period to cover two full tidal cycles. For each deployment, the eDNA sampler was left on station to collect environmental DNA by filtering seawater through its 1 µm

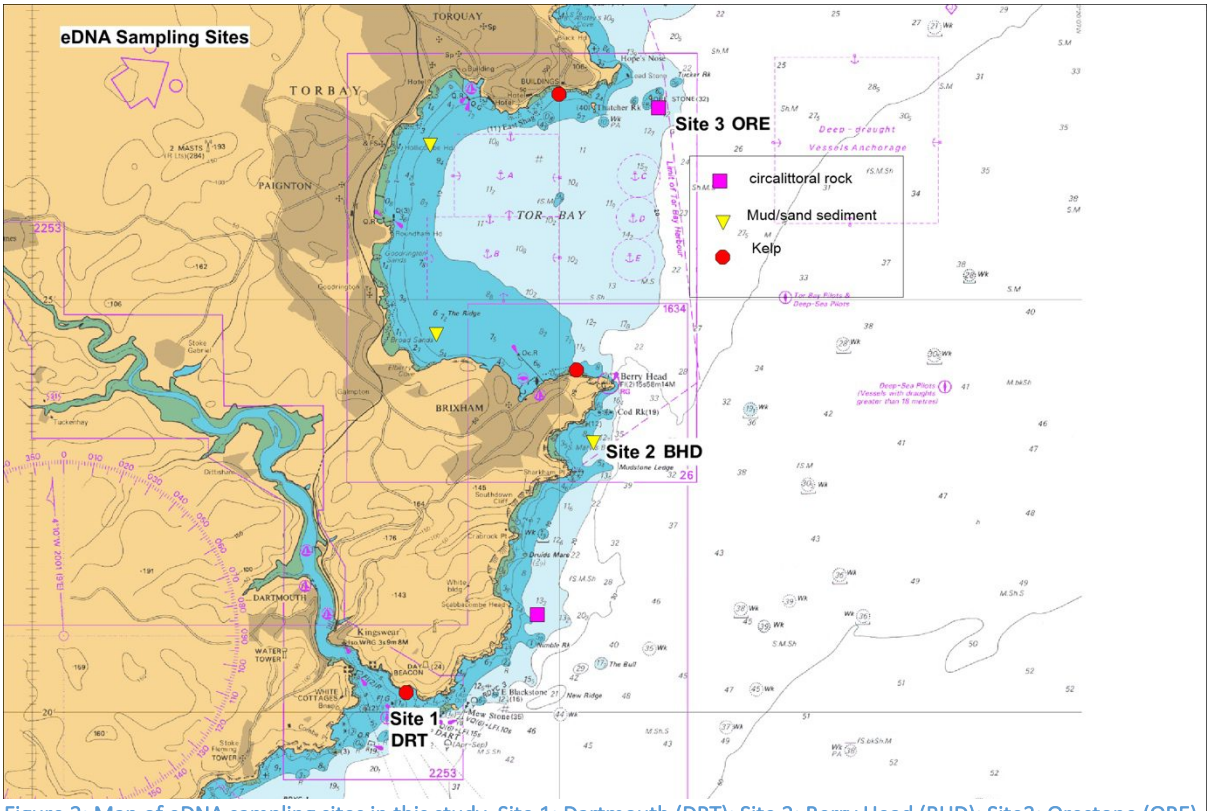


Figure 2: Map of eDNA sampling sites in this study. Site 1: Dartmouth (DRT); Site 2: Berry Head (BHD); Site3: Orestone (ORE).

pore filter membrane from the demersal water layer, 1 metre above the benthos. Sampling depth varied between 8 to 20 metres, depending on location and tidal range.

With the assistance of the Devon and Severn Inshore Fisheries and Conservation Authority (IFCA), three replicates were collected from each sampling station between the 15th November and the 5th February, as frequently as weather permitted (Table 1). Upon recovery of the eDNA sampler, samples were stored in DNA preservative and refrigerated.

Sample Processing & Sequencing

For details, contact Applied Genomics Ltd. directly: solutions@appliedgenomics.co.uk.

Bioinformatics & Taxonomic Assignment

For details, contact Applied Genomics Ltd. directly: solutions@appliedgenomics.co.uk.

Biostatistical Analysis

We undertook fish community analyses for alpha diversity (species richness at each site), beta diversity (the difference in community composition between sites) and gamma diversity (the total species richness among all sampled sites) (Creer *et al.*, 2016; Ritter *et al.*, 2018).

Using available Marine Management Organisation (MMO) historical data for recorded fisheries landings for the months of November to February, 2014 to 2017 by under-10-metre fishing vessels at the Port of Brixham and Dartmouth, the diversity analyses were compared to historical catch data for the local area at the same time of year. The under-10-metre vessel category was selected under the assumption that these vessels would be more likely to be fishing within the local area and near to the coast. These data provide a comparison of our eDNA monitoring approach against fisheries landings data which are necessarily selective and biased but are currently used to inform fisheries management decisions.

The design of this study allows us to explore correlations and potential differences in eDNA capture and fish diversity in the three habitat types, rock, mud or kelp within the context of regional fish community species diversity. The insights delivered are intended to inform the optimisation of future sampling strategies for an independent and unbiased method for continuous monitoring of inshore fish communities.

Results

Field Sampling

Locations and dates of sampler deployment and recovery are given in Table 1, below. The Time Difference shows the delay incurred between sampler deployments due to weather, etc.

Table 1: Locations and dates of sampler deployment and recovery

SAMPLE NAME	SUBSTRATE	LAT	LON	DEPLOYMENT	RECOVERY	TIME DIFFERENCE
ORE1	Rock	50.453100	-3.466083	14 Nov 2018	15 Nov 2018	0
BHD1	Mud	50.387050	-3.488817	11 Dec 2018	12 Dec 2018	27d
DRT1	Kelp	50.337117	-3.548717	3 Jan 2019	4 Jan 2019	23d
ORE2	Rock	50.453100	-3.466083	7 Jan 2019	8 Jan 2019	4d
DRT2	Kelp	50.337117	-3.548717	14 Jan 2019	15 Jan 2019	7d
BHD2	Mud	50.387050	-3.488817	16 Jan 2019	17 Jan 2019	2d
ORE3	Rock	50.453100	-3.466083	22 Jan 2019	23 Jan 2019	6d
BHD3	Mud	50.387050	-3.488817	23 Jan 2019	24 Jan 2019	1d
DRT3	Kelp	50.337117	-3.548717	4 Feb 2019	5 Feb 2019	12d
BLANK	Negative	n/a	n/a	n/a	n/a	n/a

Sample processing & Sequencing

All 9 samples were processed, DNA purified and successful amplification of both CytB and 12S amplicons (Table 2). The CytB amplicons generally had better success with higher concentration of product. The 12S amplicon for sample DRT3 had very low concentration despite efforts to purify the sample.

Table 2: Quantification of sample eDNA and fish-specific amplicons

SAMPLE NAME	SITE	eDNA SAMPLE (NG/UL)	CYT B AMPLICON (NG/UL)	12S AMPLICON (NG/UL)
DRT1	Dartmouth	8.47	59.2	12.9
DRT2	Dartmouth	7.62	48.0	11.1
DRT3	Dartmouth	4.72	74.8	0.714
BHD1	Berry Head	20.0	62.0	23.8
BHD2	Berry Head	15.9	96.0	11.6
BHD3	Berry Head	18.2	60.4	6.22
ORE1	Orestone	9.40	48.4	19.9
ORE2	Orestone	8.68	75.2	8.12
ORE3	Orestone	15.1	72.0	11.4
BLANK	Negative	0.0	0.0	0.0

BIOINFORMATICS & TAXONOMIC ASSIGNMENT

A summary of the bioinformatics analyses are given in the Table 3. Observations are given for each sample, paired with the fish-specific barcode used to generate the amplicons. The variable Input gives the number of raw sequence reads from the MiSeq sequencer; Filtered are the number of sequences remaining after trimming and quality filtering of the sequences; Denoised show the inferred sequence variants calculated from modelled sequencing errors; Merged is the result of merging forward and reverse sequence paired reads; Nonchimeric are the numbers of reads remaining after filtering of chimeric sequence reads, which may occur as artefacts of PCR amplification or sequencing misreads. The % Reads are then presented to provide an indication of the pipeline efficiency. Amplicon sequence variants (ASVs) are the number of unique amplicon sequences from each gene captured in each sample. Many of these sequences are variants of the same species, whilst some cannot be resolved to the species level. Those that can be characterised as species are then tallied and presented as ID'd Species. The % Sample Diversity describes the contribution of each fish-specific barcode amplicon to the number of species characterised in the sample. Where the sums are to greater than 100%, the excess is an indication of the overlapping proportion of species characterised by both genes. For example, in Sample BHD1, the % Sample Diversity of BHD1_CytB is 62.5% and BHD1_12S is 60.4%; therefore, the proportion of species detected by both genes is (62.5 + 60.4 - 100 =) 22.9%. The % Site Diversity shows the contribution of each gene to the total number of species detected by all samples at each site (Table 5).

Table 3: Bioinformatics pipeline throughput for both CytB and 12S barcode genes from sample eDNA.

SAMPLE & GENE	INPUT	FILTERED	DENOISED	MERGED	NONCHIMERIC	% READS	ASVs	ID'd SPECIES	% SAMPLE DIVERSITY	% SITE DIVERSITY
BHD1_CYTB	56318	54358	53802	53275	40817	72.5 %	185	30	62.5%	50.8%
BHD2_CYTB	68206	65487	64728	64028	45924	67.3 %	199	26	74.3%	44.1%
BHD3_CYTB	54257	52003	51403	50839	39408	72.6 %	135	24	64.9%	40.7%
DRT1_CYTB	40946	38951	38302	37705	29050	70.9 %	215	28	57.1%	51.9%
DRT2_CYTB	67609	64752	64022	63325	48795	72.2 %	148	25	69.4%	46.3%
DRT3_CYTB	31702	29541	28759	28012	26651	84.1 %	41	13	50.0%	24.1%
ORE1_CYTB	102577	99483	98730	98018	73796	71.9 %	183	26	63.4%	43.3%
ORE2_CYTB	76950	74289	73788	73319	50381	65.5 %	86	19	54.3%	31.7%
ORE3_CYTB	82531	80256	79465	78709	52746	63.9 %	171	28	56.0%	46.7%
BHD1_12S	29886	27751	27604	24851	24799	83.0 %	55	29	60.4%	49.2%
BHD2_12S	23433	21822	21762	20854	20812	88.8 %	36	19	54.3%	32.2%
BHD3_12S	22764	20903	20802	17336	17331	76.1 %	47	23	62.2%	39.0%
DRT1_12S	50528	45822	45572	39749	39110	77.4 %	100	32	65.3%	59.3%
DRT2_12S	28591	24438	24237	16348	16062	56.2 %	65	23	63.9%	42.6%
DRT3_12S	4092	3669	3536	549	549	13.4 %	27	17	65.4%	31.5%
ORE1_12S	37000	32403	32228	22138	21774	58.8 %	64	25	61.0%	41.7%
ORE2_12S	35473	33032	32866	31965	31593	89.1 %	69	21	60.0%	35.0%
ORE3_12S	46111	40246	40061	26919	26566	57.6 %	80	34	68.0%	56.7%
MEAN								25	61.8%	42.6%
ST.DEV								5	6.0%	9.3%

Mean and standard deviation (St.Dev) are given for the ID'd Species, % Sample Diversity and % Site Diversity as indicators of performance of this approach as a monitoring tool. Though not shown in Table 3, non-target sample contaminants, such as terrestrial species (eg. *Sus scrofa*, *Ovis aries*, etc.), were identified and removed from the data.

Environmental DNA sequences that could not be characterised to species level were removed from the analysis. There were a total of 74 unique fish and marine mammal species characterised (Table 4).

Table 4: Numbers of unique eDNA sequences characterised at each taxonomic level.

GENE	ROOT	DOMAIN	KINGDOM	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	UNIQUES
12S	223	130	129	129	125	116	114	100	90	45
CYTB	934	933	933	933	924	890	882	853	770	46

A full list of the taxa characterised to species used in all subsequent biostatistical analyses, with corresponding haplotype diversity data for each sample is available by contacting Applied Genomics Ltd. directly (solutions@appliedgenomics.co.uk). For clarity, some data visualisations are provided to the genus level. Note that common names have not been used to avoid potential ambiguities.

Effective population size is the number of individuals in a population who contribute offspring to the next generation (Ridley, 2003). Following taxonomic assignment, a number of amplicon sequence variants were assigned to the same species. Where these occurred, the detected variants may be regarded as haplotypic variants for the same genetic locus. Each individual within a population will have one genetic haplotype but several individuals may have the same haplotype (Figure 4). So, whilst not an indication of numbers of individuals, haplotype diversity is an indication of the diversity of breeding individuals within the sampled population.

Figure 3: Indicators of the genetic diversity of breeding fish species for each habitat type

Figure 4 shows the numbers of characterised Genera, separated by Class. Note that two species of *Cetacea*, *Lagenorhynchus albirostris* and *Phocoena phocoena,* were also captured by these 'fish-specific' primers. Whilst clearly non-target species, these *Cetacea* were sighted during a number of sorties and were included in the data because of their importance in the marine ecosystem.

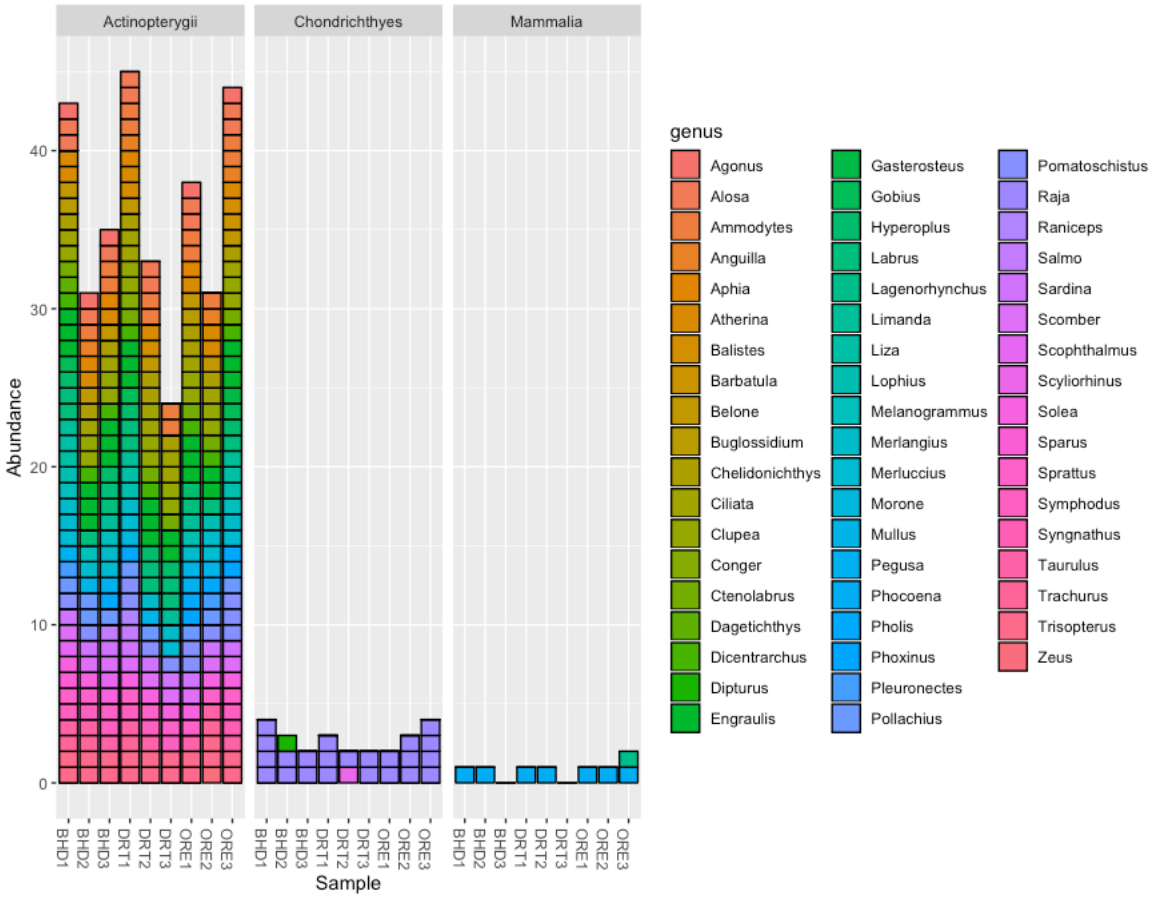


Figure 4: Relative detection efficiency from eDNA

For each sample, the % Site Diversity was calculated as a proportion of the total characterised species at each site and % Total Diversity was calculated as a proportion of all characterised species with mean and standard deviation summary statistics.

Table 5: Sampling performance summary

SAMPLE NAME	IDENTIFIED SPECIES	% SITE DIVERSITY	% TOTAL DIVERSITY
DRT1	49	90.7%	66.2%
DRT2	36	66.7%	48.6%
DRT3	26	48.1%	35.1%
BHD1	48	81.4%	64.9%
BHD2	35	59.3%	47.3%
BHD3	37	62.7%	50.0%
ORE1	41	68.3%	55.4%
ORE2	35	58.3%	47.3%
ORE3	50	83.3%	67.6%
BLANK	0	n/a	n/a
MEAN ± ST.DEV	40 ± 8	68.8% ± 13.8%	53.6% ± 10.9%

To assess sample number adequacy, we calculated the pseudo multivariate dissimilarity-based standard error (MultSE) for all samples in this study (Anderson & Santanta-Garcon, 2015). The point

at which the curve approaches the asymptote can indicate the optimal sample number, beyond which few new species will be encountered.

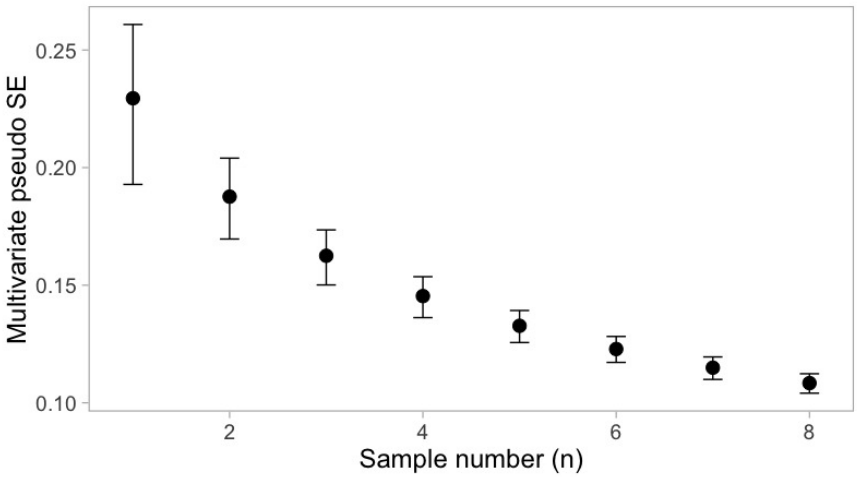


Figure 5: Pseudo multivariate dissimilarity-based standard error for all samples in this study.

Power analysis (Cohen, 2013) indicates that, to achieve a statistical power of 0.8 (1 minus Type II error probability of 0.2), with a significance level of 0.05 (Type I error probability), detecting a "medium" effect size of 0.25 for 3 observation groups, would require n = 52.4 observations per group, or a minimum 159 samples. Alternatively, 38 sites sampled monthly would achieve sufficient statistical power. The statistical power achieved by this study was 0.08.

Biostatistical Analysis

Alpha diversity is the species richness of a small homogeneous community, within a given area (Whittaker, 1972). A community will have higher Alpha diversity when there is a high number of species and their abundances are very similar. Alpha diversity statistics are visualised in Figure 6.

Given that abundance estimates are, strictly speaking, not calculable due to compositional biases in the HTS workflow (Brooks *et al*., 2015), Alpha diversity estimates are calculated using pooled incidence coverage estimates (i.e. detection or non-detection). These calculations use the frequencies of species in a collection of sites. The variants of extrapolated richness with standard-error using Chao's (Chao, 1987) diversity index.

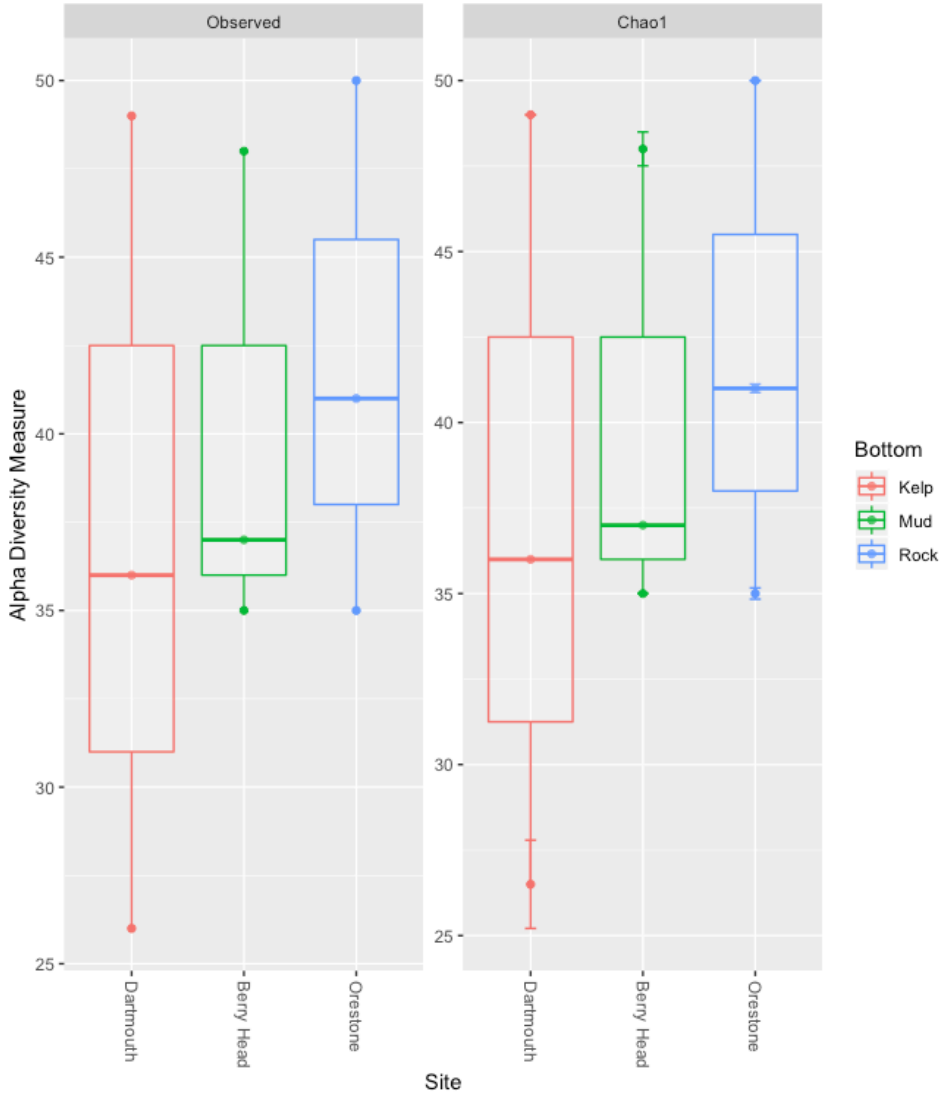


Figure 6: Alpha diversity

Beta diversity, the distinctiveness of biodiversity between sampling locations, was low and there were no statistically significant differences between habitat types (Table 6) or sample collection over time, stratified by habitat type (Table 7).

Table 6: PerMANOVA of species detection by habitat type

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
sample_data(psFishes)$Bottom	2	0.07736767	0.03868384	1.766215	0.3705697	0.064
Residuals	6	0.13141267	0.02190211	NA	0.6294303	NA
Total	8	0.20878034	NA	NA	1.0000000	NA

Table 7: PerMANOVA of species detection over time

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
sample_data(psFishes)$Time	2	0.05109153	0.02554577	0.9720069	0.2447143	0.2777778
Residuals	6	0.15768880	0.02628147	NA	0.7552857	NA
Total	8	0.20878034	NA	NA	1.0000000	NA

Our sampling approach characterised a total of 74 unique fish and marine mammal species to species level. From these, the estimated community diversity from all study samples, the Gamma diversity, was 84 ± 7 species (Chao ± Chao SE; Chao, 1987).

The list of species detected using these fish-specific metabarcoding genes were cross referenced with the National Biodiversity Network (NBN, accessed via JNCC) marine & freshwater non-native species (NNS) list and the United Kingdom Biodiversity Action Plan (UKBAP, 2019) marine protected species list from the priority species accounts spreadsheet. No matches were found for listed NNS; 14 species were returned as UKBAP priority listed species (Table 8). Detection of other anomalous species not included in these lists may indicate novel introductions or may be due to misclassification, over-classification or errors within the reference databases. Further studies should help us to understand if these species are actually present in the environment.

Table 8: Detection of UKBAP species by habitat

species	Kelp	Mud	Rock
Alosa alosa	2	3	2
Anguilla anguilla	1	1	2
Clupea harengus	3	3	3
Dipturus batis	0	1	0
Lagenorhynchus albirostris	0	0	1
Merlangius merlangus	3	3	3
Merluccius merluccius	0	1	2
Phocoena phocoena	2	2	3
Pleuronectes platessa	0	1	1
Raja undulata	0	1	2
Salmo salar	1	1	0
Scomber scombrus	3	3	3
Solea solea	3	3	3
Trachurus trachurus	1	2	1

From MMO data for recorded pelagic and demersal fisheries landings for the months of November to February, 2014 to 2017 by under-10-metre fishing vessels at the Port of Brixham and Dartmouth, a total of 26 species were recorded as landed. Of these, 57.7% of landed species were characterised by our sampling method and 285% more fishes were characterised from 9 eDNA samples than in the MMO landings data (2014 – 2017), where a total of 26 pelagic and demersal fishes were recorded for the same seasons (Figure 7).

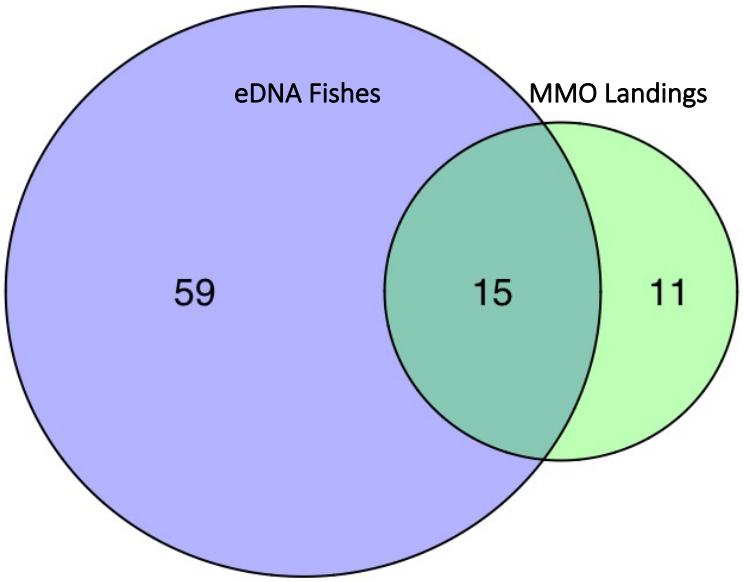


Figure 7: Venn diagram of eDNA characterised fish species to MMO fish landings

By considering the under-10-metre vessel data only, it is assumed that fishes landed by these small vessels at the ports nearest our sampling locations would closely reflect the potential species which may be detected by eDNA.

DISCUSSION

We have demonstrated the effectiveness of our large-volume marine eDNA sampling strategy as a method for the continuous, unbiased and independent monitoring of inshore fish communities. Nearly three times as many fishes were characterised from this eDNA pilot study than appeared in 4 years' landings data over the same season. This translates to a significantly more comprehensive survey of inshore fish communities than what are currently available. Our study showed that by sampling the demersal currents we were able to detect both pelagic and demersal species of fishes. Furthermore, we provided data and visualisation of species haplotype diversity, which is an indication of the diversity of breeding individuals within each sampled population. This level of data is of key importance to fisheries managers wishing to better understand the genetic diversity of local fish communities.

However, eDNA techniques cannot determine population abundance, sizes, life stages or sex ratios, at this time. Beta diversity and statistically significant differences in species detection between habitat types and/or over time were not observed, which are likely due to the very low statistical power of this pilot study. The multivariate standard-error analysis indicate that regular sampling will, with larger sample size over time, build up a complete picture of fish diversity. Whilst our study found there were no significant differences between selected habitat types or between samples collected over time, regular sampling over a larger spatial scale may be adequate to capture community diversity for inshore fisheries.

Given the considerable potential shown by this study for this marine eDNA sampling technique, particularly for continuous monitoring at large spatial scales, we would recommend future studies should include crustacean-specific primers to detect all species of interest to fisheries managers. Additionally, the inclusion of pan-specific primers would provide a more complete picture of marine biodiversity and the opportunity of early detection of potential non-native species invasions. Therefore, with correct placement of the sampler in or near marine protected areas and hotspots at risk of non-native species invasions, the data resulting from these samples could serve the remit of multiple government agencies, thereby providing an unprecedented level of information about the health of our fisheries and the wider coastal ecosystem at relatively low cost, resulting in data-driven insights for deeper due-diligence and more meaningful risk intelligence.

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