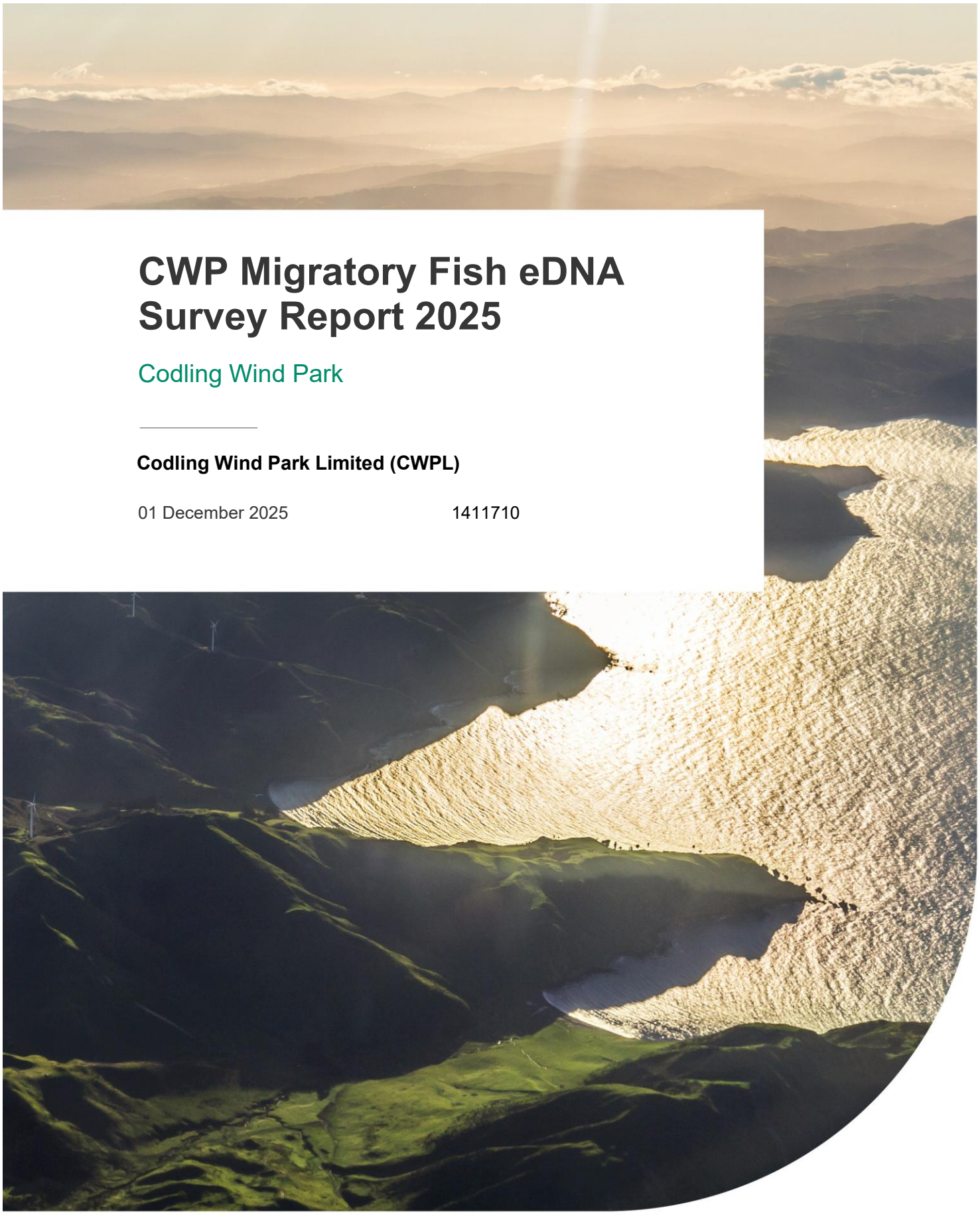
codling wind park

EIAR Addendum

Appendix 9-A CWP Migratory Fish eDNA Survey Report 2025



CWP Migratory Fish eDNA Survey Report 2025

Codling Wind Park

Codling Wind Park Limited (CWPL)

01 December 2025 1411710

OUR VISION

Working to create a world powered by renewable energy

natural power

Document history

Author	Aikaterini Kamposi, Environmental Consultant	10/01/2026
Checked	Michelle Elliott, Principal Environnemental Consultant	13/01/2026
Approved	Stuart McCallum, Technical Director	16/01/2026

Client Details	
Contact	Sean Leake
Client Name	Codling Wind Park Limited (CWPL)
Address	Five South County 2nd Floor South County Business Park Leopardstown Dublin D18 H5H9 Ireland

Issue	Date	Revision Details
A	16/01/2026	First Issue
B	13/04/2026	Final for issue

Local Office:

Units 5 & 10, Stephenson House
Horsley Business Centre
Horsley
Northumberland
NE15 0NY
Tel: +44 (0) 1661 312 100

Reg No: SC177881

Registered Office:

The Natural Power Consultants Limited
The Green House
Forrest Estate, Dalry
Castle Douglas, Kirkcudbrightshire
DG7 3XS

VAT No: GB 243 6926 48

Contents

1. Introduction 3
1.1. Project Background 3
1.2. Document Purpose 3
2. Sampling Design & Methodology 4
2.1. Survey Design 4
2.2. Sampling Methodology 4
3. Data Analysis 7
3.1. Data Processing 7
3.2. Spatio-temporal Maps of Occurrence 7
3.3. Diversity Metrics 7
3.4. Multivariate Analysis 8
3.5. Marine mammals and Seabirds 8
4. Results 9
4.1. Spatio-temporal Maps of Occurrence 9
4.2. Diversity Metrics 23
4.3. Multivariate Analysis 25
4.4. Marine Mammal and Seabird Occurrence 27
5. Discussion 30
6. References 31

Appendices 33
A. Locations of Sampling Stations 33
B. eDNA Laboratory Analysis 34
C. Diversity Metrics Results 36

Table A: Table of Tables

Table Number	Table Title	Page
D	Mean read counts of salmon per station, month and assay	10
E	Mean read counts of herring per station and per month, using the fish assay	12
F	Mean read counts of sandeel per station, month and assay	14
G	All fish species detected by both fish and vertebrate assays, per month	16 - 21
H	Total read counts showing mammal occurrence	27
I	Total read counts showing bird occurrence	27

Table B: Table of Figures

Figure Number	Figure Title	Page
A	eDNA water sampling stations	6
B	Salmon read counts map	11
C	Herring read counts map	13
D	Sandeel read counts map	15
E	Diversity metrics at eDNA sampling stations for June, using the vertebrate assay	22
F	Diversity metrics at eDNA sampling stations for June, using the fish assay	23
G	Diversity metrics at eDNA sampling stations for October, using the vertebrate assay	23
H	Diversity metrics at eDNA sampling stations for October, using the fish assay	24
I	nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the vertebrate assay	24
J	nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the fish assay	25
K	nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the vertebrate assay	26
L	nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the fish assay	26

Table C: Abbreviations used with the text

Acronym	Definition
CWP	Codling Wind Park
CWPL	Codling Wind Park Limited
EIAR	Environmental Impact Assessment Report

Acronym	Definition
eDNA	environmental DNA
GBIF	Global Biodiversity Information Facility
GMT	Greenwich Mean Time
GPS	Global Positioning System
GRIIS	Global Register of Introduced and Invasive Species
IUCN	International Union for Conservation of Nature
MAC	Marine Area Consent
nMDS	Non-Metric Multidimensional Scaling
OTUs	Operational Taxonomic Units
OWF	offshore wind farm
SIMPROF	Similarity Profile Analysis
WoRMS	World Register of Marine Species
zOTUs	zero-radius Operational Taxonomic Units

1. Introduction

1.1. Project Background

Codling Wind Park Limited (CWPL) is proposing to develop the Codling Wind Park (CWP) Project, which is located in the Irish Sea approximately 13 - 22 km off the east coast of Ireland, at County Wicklow.

On Friday 6th September 2024 CWPL (referred to hereafter as the 'Applicant') applied for planning permission to An Coimisiún Pleanála (ACP) (referred to hereafter as the 'Commission') under Section 291 of the Planning and Development Act (PDA) 2000, as amended, for the construction, operation and decommissioning of the CWP Project.

On 1st August 2025, having reviewed the application documentation, including the Environmental Impact Assessment Report (EIAR) and the Natura Impact Statement (NIS), the Commission issued a Further Information Request (FIR) in relation to the CWP Project.

Natural Power Consultants Ltd (Natural Power) has been appointed to manage and execute the delivery of an environmental deoxyribonucleic acid (eDNA) survey to support the Applicant's FIR response.

This document is intended to support **Section 9** of the **EIAR Addendum**, to provide a validation of the baseline characterisation provided in **Volume 3**, **Chapter 9 Fish, Shellfish and Turtle Ecology** of the EIAR, and to inform future monitoring.

1.2. Document Purpose

This report has been produced to present the findings of the eDNA survey. Main objectives of the survey were:

- To provide information on migratory salmon (*Salmo salar*) in and around the Codling Wind Park (CWP) Project area; and
- To provide additional baseline validation information on fish and marine mammal species present in the Codling Wind Park (CWP) Project area.

eDNA samples were analysed using both a fish specific and a general vertebrate assay, as such, information on vertebrates, including marine mammals and sea bird species are also included in this report.

2. Sampling Design & Methodology

2.1. Survey Design

Two eDNA surveys were caried out at eighteen sampling stations located to cover the area within the CWP Project area including two stations within the River Liffey (**Figure A**), and areas outside of the predicted Zone of Influence.

The surveys took place in June and October 2025, within the following pre-defined seasonal sampling windows:

- June - Smolt migration (to the sea); when juvenile salmon, called smolts, migrate out of the freshwater rivers to the Atlantic Ocean; and
- October - Summer run (grilse); grilse are salmon returning after only one winter at sea and they comprise the bulk of the Irish salmon stock.

2.2. Sampling Methodology

2.2.1. eDNA field sampling

At each of the sampling locations outlined below (Appendix A, **Table AA**), three 5 L replicate water samples for eDNA analysis were collected using a Niskin bottle, from approximately 1 meter (m) above the seabed in accordance with the Marine Invasive Species Ireland (MSI) Project eDNA Toolkit (EMFF Operational Programme 2014-2020) and the Proof of Concept White Paper (Natural Power, 2023). The vessel was positioned relative to the wind/tide/current to avoid the cable leading under the vessel and the Niskin bottle was deployed from the side of the vessel, avoiding the propellor. There was no vessel transiting during the deployment.

The bottle was lowered on a Dyneema cable, moved up and down a few times to flush the inside and the sample taken *c.* 1 m above the seabed. The correct length of cable was payed out using the depth on the vessel sounder and meter markings on the cable. The coordinates and time (Greenwich Mean Time - GMT) when the sample was taken was recorded using a handheld Global Positioning System (GPS). Depth of water (m) was recorded from the vessels system.

A clean set of gloves was worn between each sample to prevent cross contamination. Once on board the water sample was transferred from the Niskin bottle to a single-use sterile sample bag for filtering each replicate.

A peristatic pump (vampire sampler) was used to filter the water samples. One end of a length of tubing was connected to the filter and the tubing was fed through the pump. The other end of the tubing was then placed into the sample bag and the pump was turned on to filter the sample. Once the sample bag was empty, any water remaining in the tubing was pumped through to ensure the complete sample had passed through the filter. A preservation buffer was then added to the filter and caps secured to both ends of the filter. The filter and completed data sheet were stored in the corresponding specimen bag labelled with the station and sample number for analysis in the laboratory.

The Niskin bottle and buckets were cleaned with spray bleach and flushed with deionized water between each sample station.

During each survey, a field control sample was taken (towards the end of the survey) to test for contamination that may have occurred during sampling. Field controls were collected by filling the cleaned Niskin bottle with deionized water. The deionized water was then emptied into a sample bag and the water was filtered following the above procedure.

2.2.2. eDNA laboratory analysis

Technical details of the eDNA laboratory analysis have been provided in Appendix B, with an overview summarised below.

DNA Extraction, Amplification & Sequencing

In the laboratory, DNA was extracted from each filter and a DNA extraction blank was processed with each batch to assess potential contamination in the extraction process. DNA was then purified and quantified.

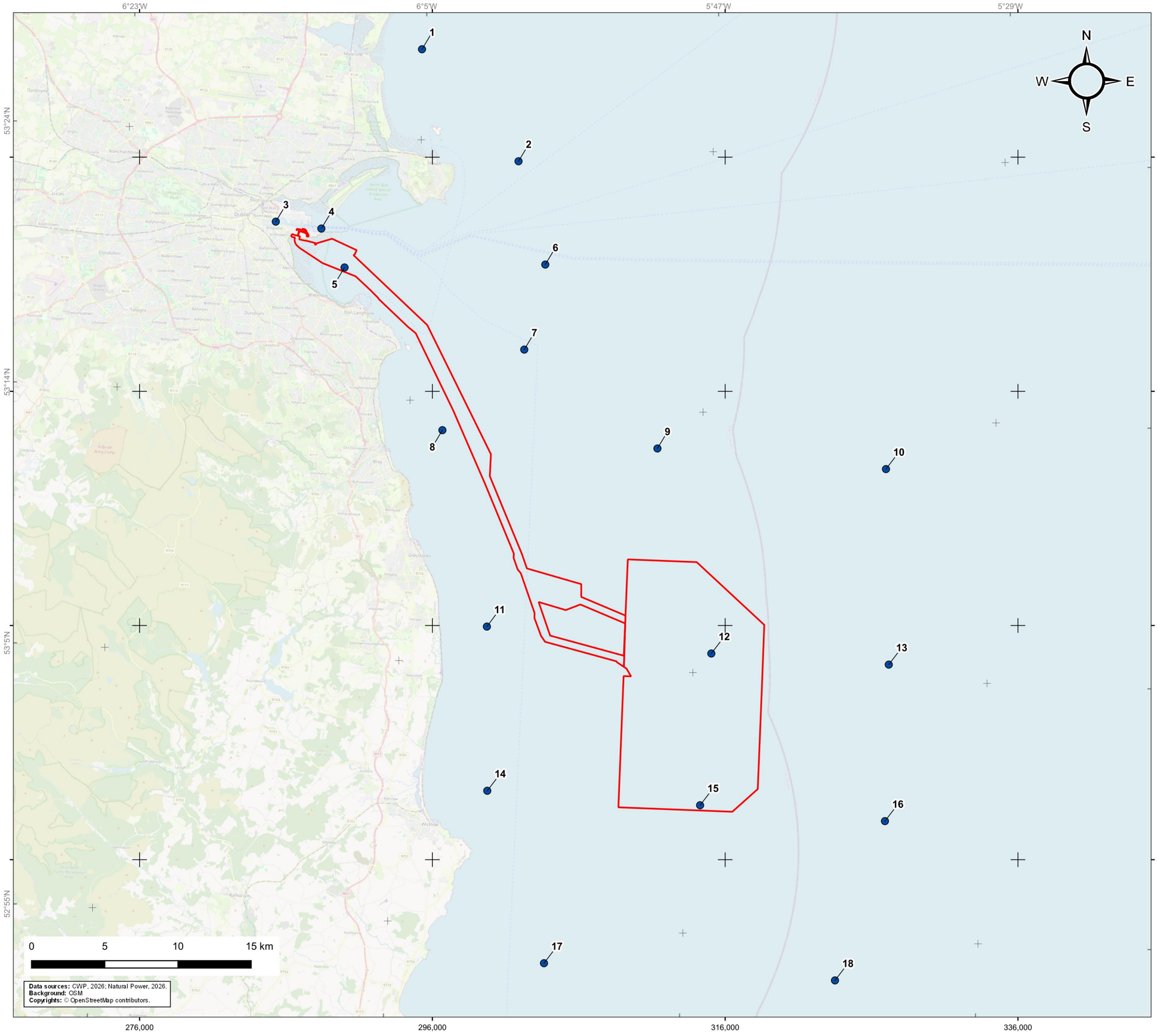
Samples were analysed using both a fish assay and a vertebrate assay. A genetic barcode region was amplified using primers specific to the assay for each sample. The amplified DNA was then sequenced to identify unique genetic sequences.

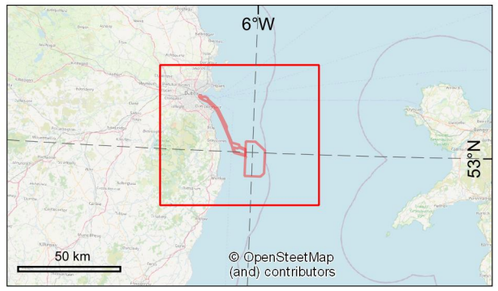
Bioinformatics

The raw sequence data was processed and compared to genetic reference databases of species through a bioinformatics process to generate an output list of taxa detected in each sample for ecological analysis.

For each assay, assignments were made to the lowest possible taxonomic level, using similarity thresholds >90%. A country-based sense-checking step was also implemented in line with the Global Biodiversity Information Facility (GBIF) occurrence records for the United Kingdom. All taxonomic units with species-level identifications were queried against the International Union for Conservation of Nature (IUCN) Red List to obtain global threat status.

The number of reads assigned to each species per sample during the taxonomic assignment against the reference database (i.e., read count) (as in Muri *et al*., 2016) was used for down-stream analysis. While read counts do not directly infer on abundance and biomass, they can provide useful indication of relative signal strength and can reliably indicate patterns in community composition, differences between sites, broad spatiotemporal trends and relative abundance (Muri *et al*., 2016; Carvalho *et al.,* 2022; Stoeckle *et al.,* 2021). Evidence from the Proof of Concept White Paper (Natural Power, 2023) demonstrated that eDNA detected a higher number of species overall (including cryptic and benthic species under-sampled in trawl surveys) and that seasonal and spatial patterns were consistent between eDNA and conventional methods. The overlap in community patterns between methods also supports the use of eDNA data in standard ecological diversity analyses.





Legend

- Planning Application Boundary (PAB)
- Migratory fish eDNA sampling station

codling wind park

Project: Codling Wind Park

Contractor: natural power www.naturalpower.com

Figure A
eDNA water sampling stations

CWP doc. number: CWP-NPC-CON-09-MAP-2143

Internal descriptive code:	*Size:*	*CRS:*
N/A	A3	EPSG 25830
	Scale: 1:250,000	

Rev.	*Updates*	*Date*	*By*	*Chk'd*	*App'd*
01	For FIR submission	2026/04/15	AC	AK/EA	LJ

3. Data Analysis

3.1. Data Processing

For eDNA analysis, two typical assays used by the subcontractor NatureMetrics for ‘fish’ and ‘vertebrate’ were tested. Comparative analysis of the fish and vertebrate assays have been presented in this report. The vertebrate assay also provided results on marine mammal species and bird species which are included in this report. Fish and vertebrate assays may differ in their detection due to primer specificity, amplification efficiency and taxonomic resolution (Zhang *et al.,* 2020). Fish assays provide a higher sensitivity for fish detection (e.g. salmonids) whereas vertebrate assays are designed to amplify wider range of vertebrate groups, therefore leading to possible differences in results.

Contaminants were cross-referenced between field samples and field blanks (control samples) by the analysing laboratory, and therefore no decontamination was required as part of the data processing stage. A probability threshold of 0.1 was applied; taxa exceeding this threshold were removed from the dataset for the respective survey. A low-read filtering threshold was applied to remove the detections likely caused by sequencing noise or stochastic artefacts. Operational Taxonomic Units (OTUs) contributing to fewer than 20 reads per samples were excluded (see Appendix B). This ensures that the number presented in the results reflect biological detection rather than raw sequencing noise.

The common and Latin binomial names of species were checked against the World Register of Marine Species (WoRMS) database, to ensure that the most up to date taxonomic classification was used. Only taxa identified to species or genus level were used in the analysis. Where a species was identified to genus level, but a different DNA sequence was identified (meaning that it may be two different species), these were named genus and sp. 1, genus sp. 2. For example, if in a given assay and month combination, the freshwater stone loach *Barbatula barbatula* was identified, but there was also *Barbatula* identified to genus level only, this was named *Barbatula sp. 1*, and if another unique DNA sequence was identified to genus level only, this would be *Barbatula sp. 2*.

Read counts were used directly to calculate diversity metrics, and were transformed as the fourth root for multivariate analysis.

3.2. Spatio-temporal Maps of Occurrence

The spatio-temporal distribution of salmon, herring and sandeel, were of interest and therefore mean read count maps were created for these species for both assays and surveys. While mean read counts do not provide direct quantitative abundance, these can be used as an indicator of DNA signal strength and be an indicator of relative abundance (Carvalho *et al*., 2022; Stoeckle *et al*., 2021). As such, several diversity metrics were computed and are listed in the section below. Through the report read counts represent the strength of the DNA signal recovered at each station, representing indicative detection level.

3.3. Diversity Metrics.

Diversity metrics were calculated for each survey and assay used separately.

- Number of Species (S) (Taxa): provides the number of species present in a sample, based on detection and non-detection, with no indication of relative abundances;
- Effective species: the number of equally abundant species needed to obtain the same mean proportional species abundance as that observed in the survey data, based on the proportional distribution of read counts;

- Number of read counts (n): provides the total number read counts counted assigned to taxa within a sample;
- Species Diversity - Shannon-Wiener index (H'): measures the uncertainty in predicting the identity of the next species withdrawn from a sample. Typically, between 1.5 and 3.5, a lower value shows lower diversity, calculated here using the proportional read counts;
- Species Richness - Margalef's index (d): measures the number of species present relative to the total amount of sequence data generated for that sample. The higher the index, the greater the diversity;
- Pielou's evenness (J'): shows how evenly the read counts are distributed among species in a sample. J' is a range of zero to one, with higher values indicating more even community structure.

These univariate indices reduce complex eDNA datasets into statistics that describe differences in species richness, diversity, evenness across samples and describe community structures.

3.4. Multivariate Analysis

Multivariate analysis is an effective method for detecting subtle changes in species community datasets. Multivariate analyses were calculated in R using the vegan package (Oksanen *et al*., 2022). Due to the partially skewed nature of species data, and its varying abundances, a fourth root transformation was applied to normalise the eDNA data distribution - reducing dominant effects of highly abundant taxa. A Bray-Curtis resemblance matrix was applied to the transformed infauna data. To cluster stations based on the similarity profiles (SIMPROF) of community composition, hierarchical clustering and permutation testing were utilized to identify the coherence of groups of stations.

Non-metric Multi-Dimensional Scaling (nMDS - a distance-based ordination technique) was used to visualize multiple dimensions of community composition in a simplified way to depict the similarity of stations / samples based on their SIMPROF-identified cluster, or survey. This essentially plots similar samples near one another, and dissimilar sample units far away from one another. As nMDSs are three-dimensional (at least), two-dimensional representations usefulness will depend on the orientation of the view; but it does a good job of representing complex multi-dimensional data in a small number of dimensions. The 'stress' reported indicates whether the nMDS is valuable to interpret, calculated by comparing the ranked distances in the original matrix to those in the nMDS. A stress of >0.35 is where samples are placed essentially at random, with little relation to original ranked distances; >0.20 could be dangerous to interpret; <0.20 is useful but has potential to mislead; <0.10 is a good ordination; <0.05 is an excellent representation. These thresholds are a guide only, and in reality will be influenced by sample size and the nature of the data.

Multivariate analysis was repeated for each survey and assay used, as well as testing for effects within an assay between surveys.

3.5. Marine mammals and Seabirds

Species of mammal and bird that were identified using eDNA are reported on for each survey, using the detection from vertebrate assay only.

4. Results

4.1. Spatio-temporal Maps of Occurrence

4.1.1. Atlantic salmon

Table D presents the mean read counts of Atlantic salmon DNA per station, month and assay. No Atlantic salmon DNA was detected in October using the vertebrate assay. **Figure B** below shows the read counts map for Atlantic salmon.

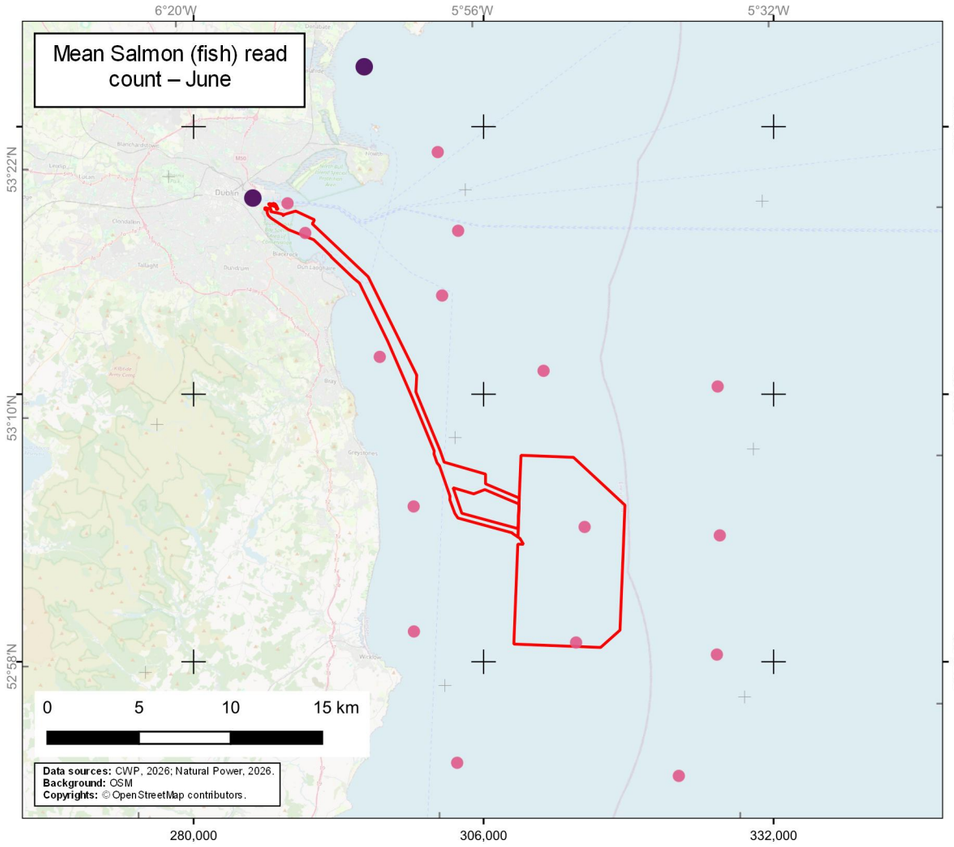
E

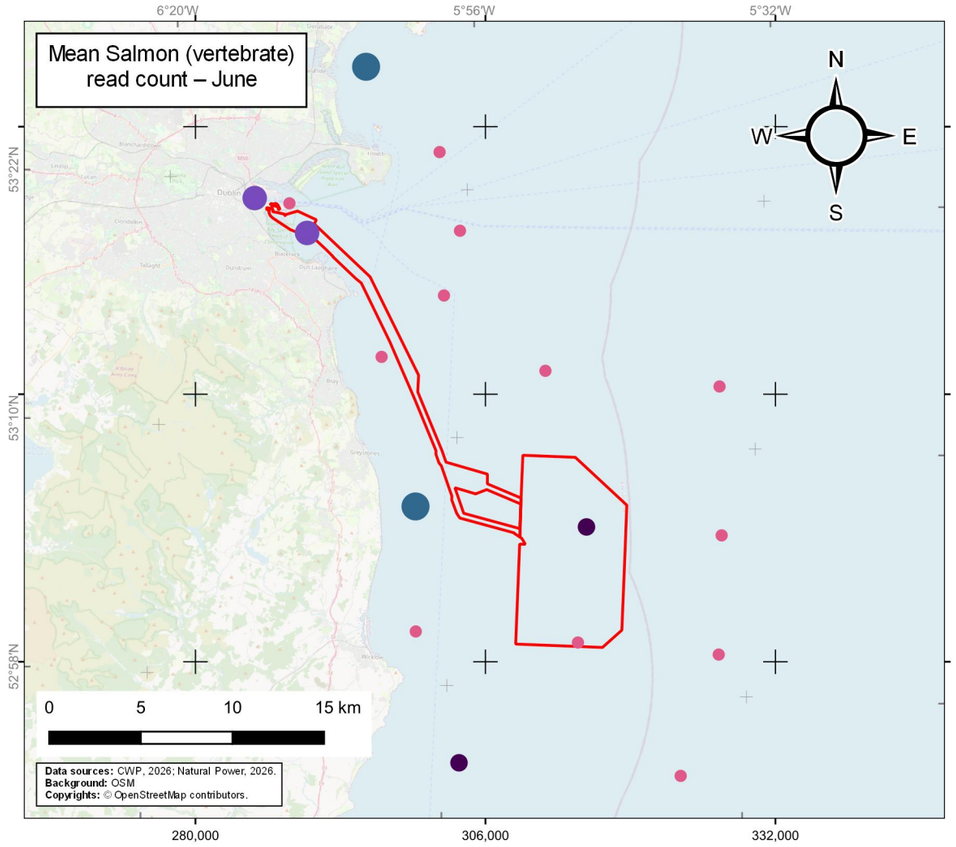
Table D: Mean read counts of salmon per station, month and assay, representing relative eDNA signal strength detected at each location

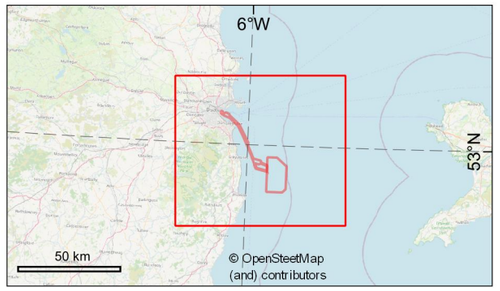
Station	June		October	
	Fish Assay	Vertebrate Assay	Fish Assay	Vertebrate Assay
1	212	1510	0	0
2	0	0	0	0
3	181	786	0	0
4	0	0	0	0
5	0	751	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	44	0
9	0	0	0	0
10	0	0	0	0
11	0	2016	0	0
12	0	117	213	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	112	0
17	0	132	0	0
18	0	0	0	0

Atlantic salmon were detected at six of 18 stations in the June survey when analysed using the vertebrate assay and detected in two of those stations using the fish assay. In October, Atlantic salmon were detected in three of 18 stations via the fish assay and at no stations using the vertebrate assay (**Figure B**). In the June survey Atlantic salmon were present at Station 1, east of Malahide estuary, Station 3 in the River Liffey and Station 5 in the ECC just outside of South Dublin Bay, Station 11 southeast of Greystones, Station 12 in the array site and Station 17 southeast of Wicklow. Of these, Station 11 followed by Station 1 had the highest read counts. In general salmon

were located at stations further from shore in the October survey and at lower read counts than in the June survey. In the October survey Atlantic salmon were present at Station 8 nearshore east of Bray, Station 12 in the array site and Station 16 offshore east of the array site (**Figure B**).





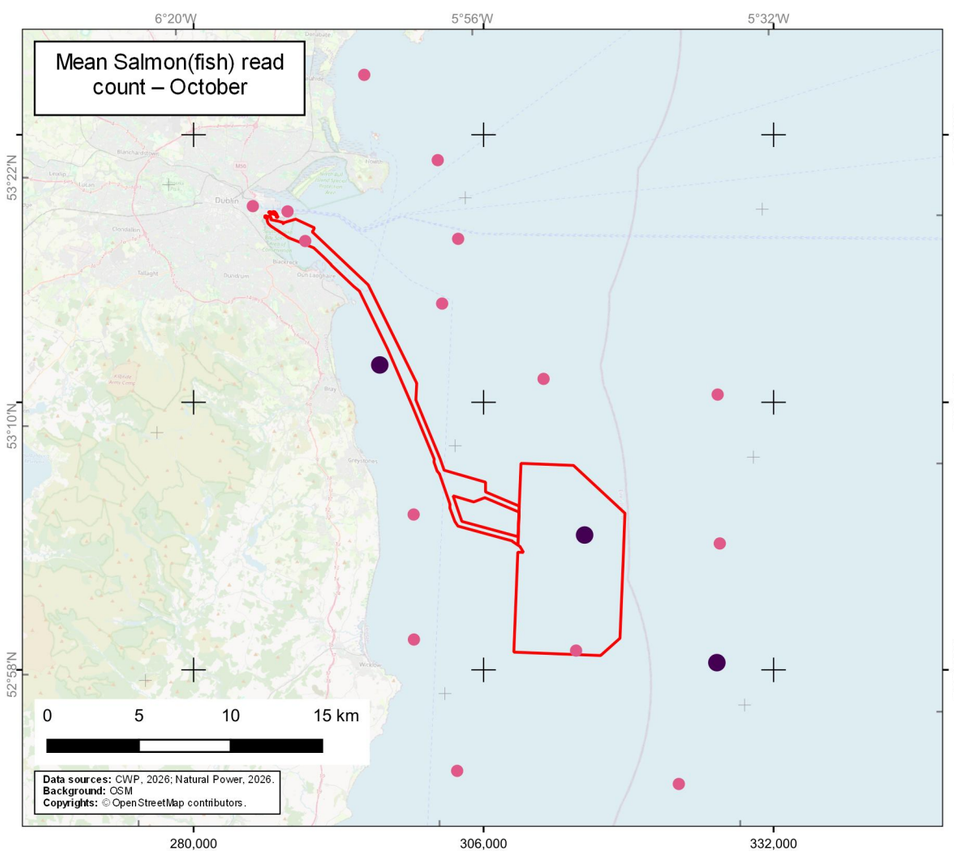


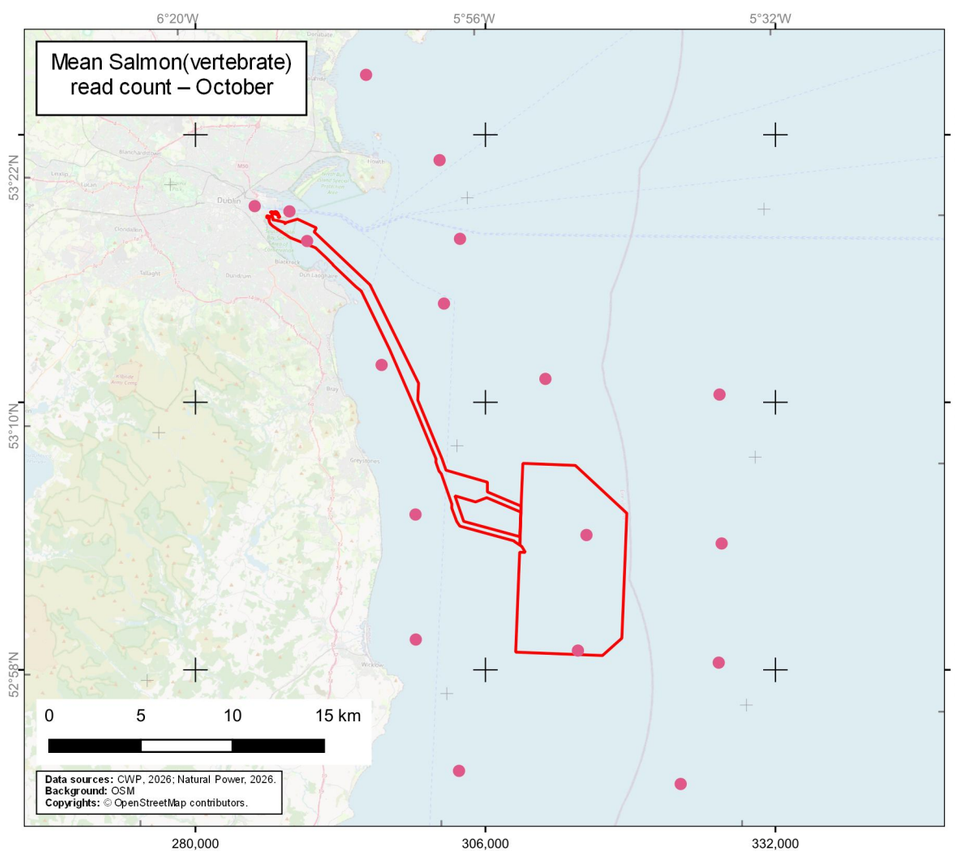
Legend

Planning Application Boundary (PAB)

Mean Salmon read count

- < 1
- 1 - 500
- 500 - 1,000
- 1,000 - 2,500
- 2,500 - 3,500
- 3,500 - 4,500
- 4,500 - 7,500
- > 7,500





codling wind park

Project: Codling Wind Park

Contractor: natural power www.naturalpower.com

Figure B
Salmonl read counts map

CWP doc. number: CWP-NPC-CON-09-MAP-2144

Internal descriptive code: N/A

Size: A3

Scale: 1:550,000

CRS: EPSG 25830

Rev.	Updates	Date	By	Chk'd	App'd
00	For FIR submission	2026/04/15	AC	ME/EA	LJ

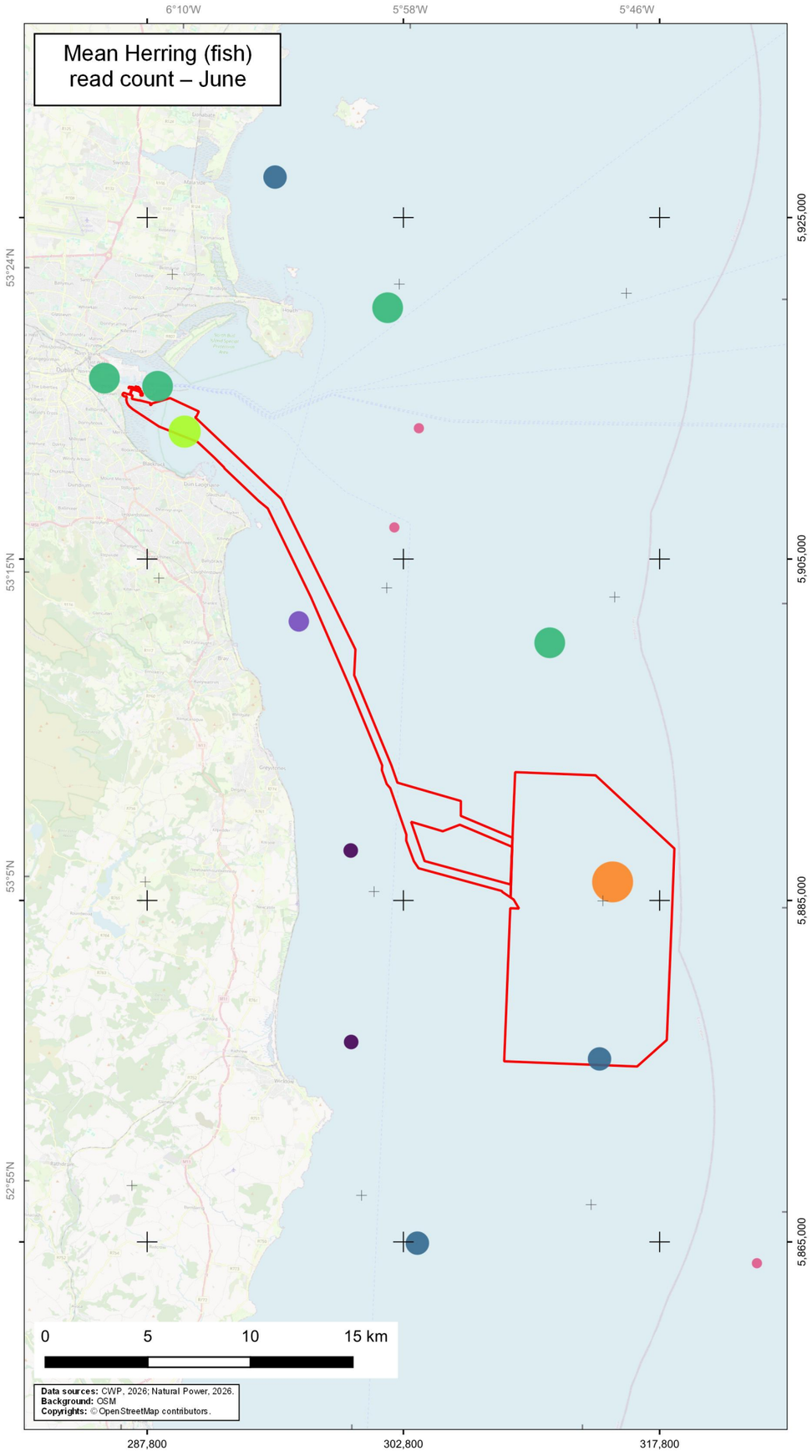
4.1.2. Atlantic herring

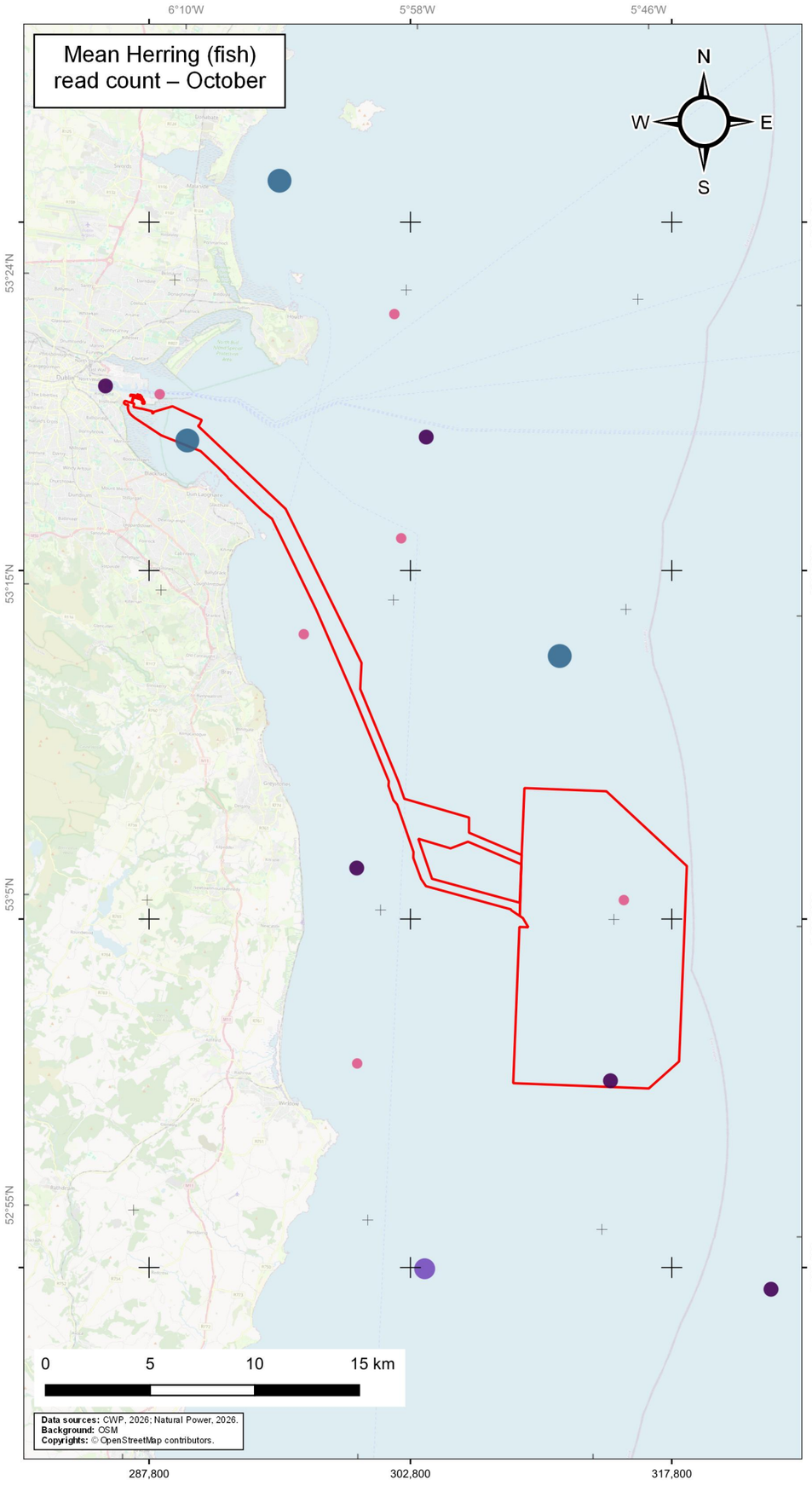
Atlantic herring (*Clupea harengus*) was detected to species level, by the fish assay only. **Table E** present the mean read counts of Atlantic herring per station and per month using the fish assay. **Figure C** below shows the read counts map for Atlantic herring.

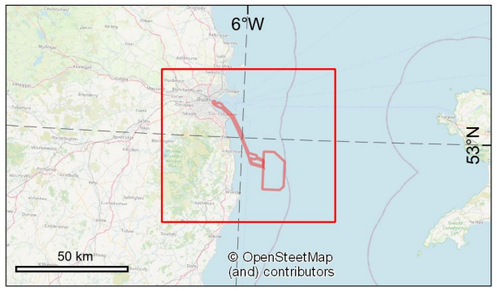
Table E: Read counts of herring per station and per month, using the fish assay, representing relative eDNA signal strength detected at each location

Station	June	October
1	1914	1628
2	3082	0
3	2573	384
4	2989	0
5	3509	1755
6	0	476
7	0	0
8	994	0
9	3201	1152
10	3932	3824
11	183	362
12	7750	0
13	14011	0
14	478	0
15	2449	293
16	14463	850
17	2119	971
18	0	352

Atlantic herring were detected at more stations, and at higher read counts, in June than October (15 stations and 11 stations respectively) (**Figure C**). Atlantic herring were present in the array site and the ECC on the approach to South Dublin Bay in the June survey and in the ECC on the approach to South Dublin Bay in the October survey. In June, read counts of Atlantic herring were highest at Stations 16 and 13 located offshore, east of the array site, followed by Station 12 in the array site. In October, Atlantic herring were present at highest read counts at Station 10, located offshore northeast of the array site, followed by Station 5, near South Dublin Bay, and Station 1 east of Malahide.







Legend

Planning Application Boundary (PAB)

Mean Herring read count

- < 1
- 1 - 500
- 500 - 1,000
- 1,000 - 2,500
- 2,500 - 3,500
- 3,500 - 4,500
- 4,500 - 7,500
- > 7,500

codling wind park

Project: Codling Wind Park

Contractor: natural power www.naturalpower.com

Figure C
Herring read counts map

CWP doc. number: CWP-NPC-CON-09-MAP-2145

Internal descriptive code:	*Size:*	*CRS:*
N/A	A3	EPSG 25830
	Scale: 1:300,000	

Rev.	*Updates*	*Date*	*By*	*Chk'd*	*App'd*
00	For FIR submission	2026/04/15	AC	ME/EA	LJ

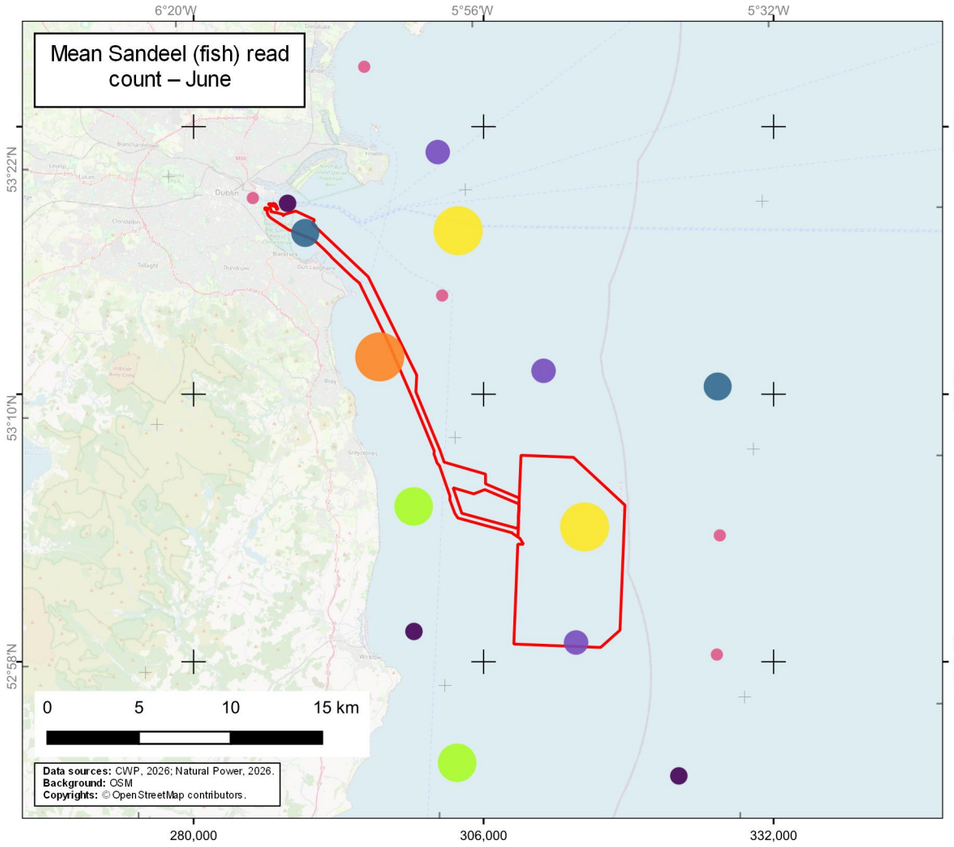
4.1.3. Sandeel

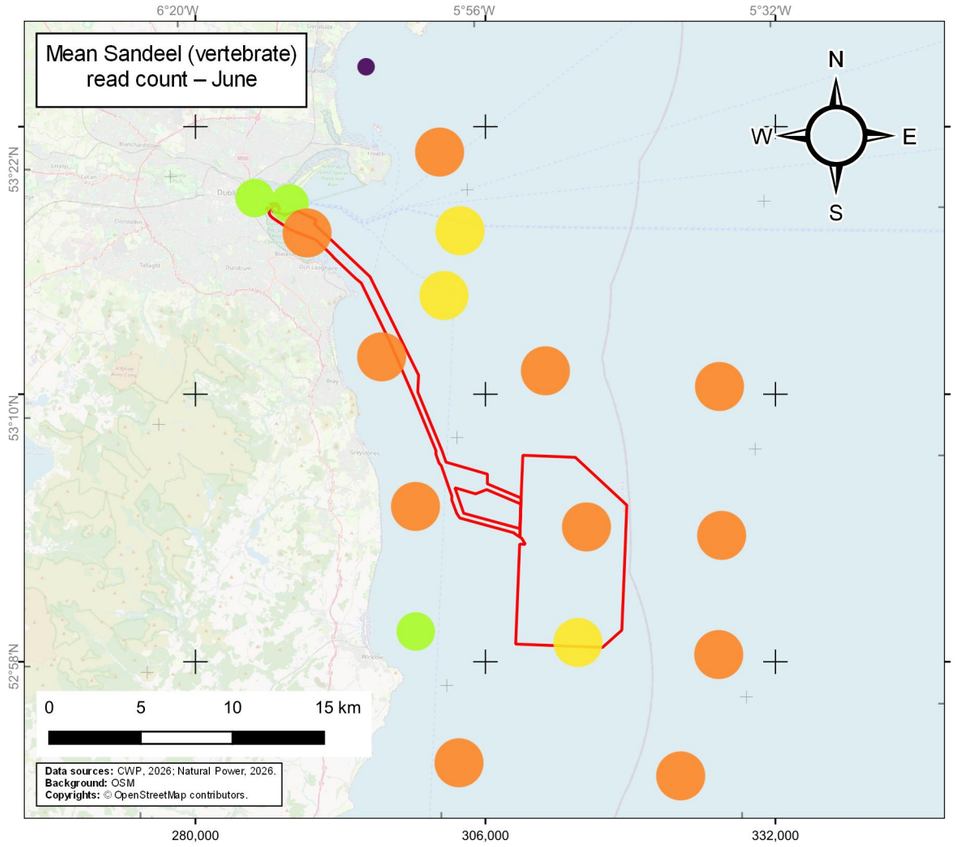
Sandeel DNA was detected in greater quantity by the vertebrate assay compared to the fish assay. In **Table F**, mean read counts of sandeel DNA are per station, month and assay. **Figure D** below shows the read counts map for sandeel.

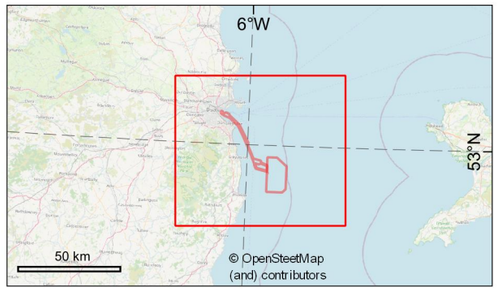
Table F: Read counts of sandeel per station, month and assay, representing relative eDNA signal strength detected at each location

Station	June		October	
	Fish Assay	Vertebrate Assay	Fish Assay	Vertebrate Assay
1	0	304	0	0
2	942	23858	0	584
3	0	3625	0	0
4	44	3884	0	0
5	2253	154323	0	0
6	7444	5879	0	0
7	0	5875	0	0
8	12586	16277	0	0
9	891	72883	0	0
10	2270	31841	0	1286
11	3975	114398	343	0
12	4868	13346	432	3293
13	0	37728	0	3528
14	200	3800	0	0
15	612	7435	0	0
16	0	53984	0	244
17	3550	16119	2550	0
18	466	54866	68	24

Sandeel were recorded at all stations in June via the vertebrate assay and 15 stations via the fish assay. In October sandeel were recorded at six stations via the vertebrate assay and four stations using the fish assay. Read counts were typically higher in June than October. In June sandeel were recorded at the highest read counts at Station 5 in the ECC just outside of South Dublin Bay and Station 11 southeast of Greystones. Whereas, in October sandeel were recorded at highest read counts at Station 12 in the array site and Station 13 located further offshore east of the array site.





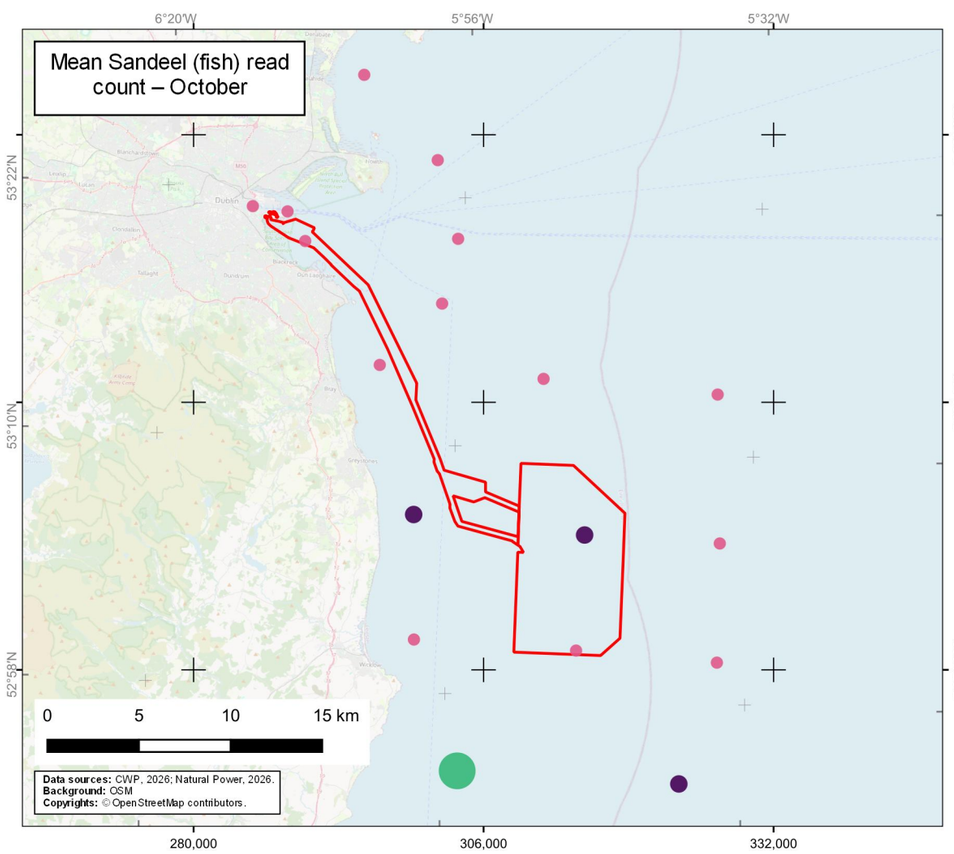


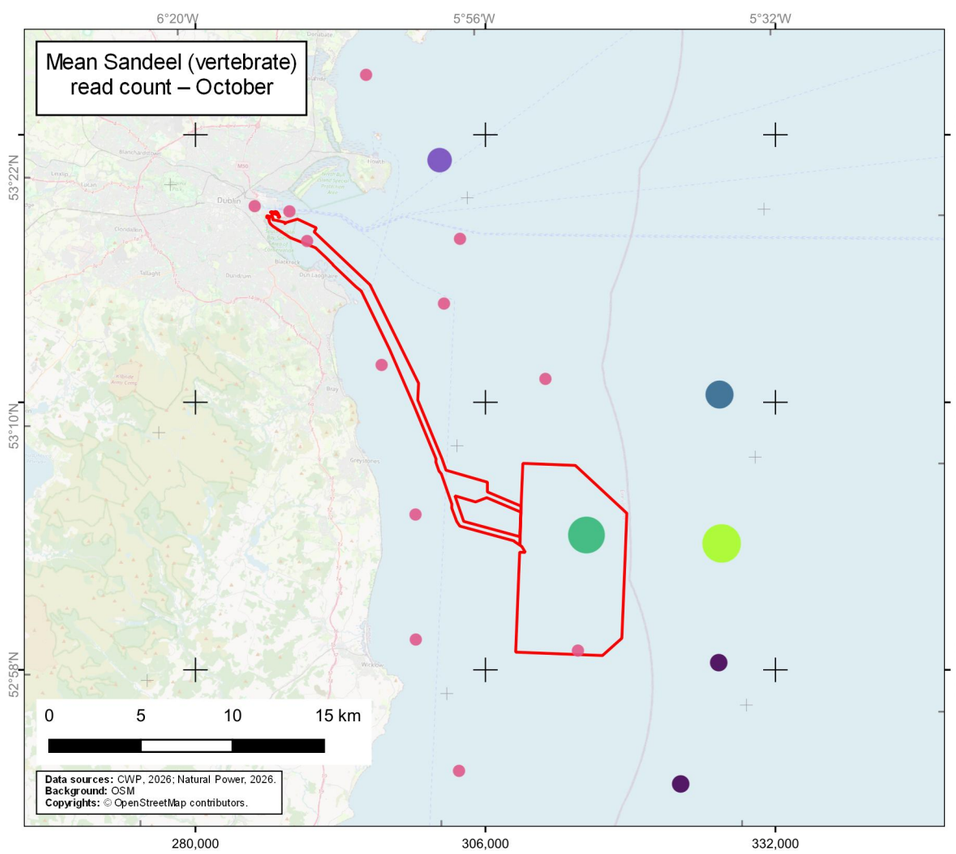
Legend

Planning Application Boundary (PAB)

Mean Sandeel read count

- < 1
- 1 - 500
- 500 - 1,000
- 1,000 - 2,500
- 2,500 - 3,500
- 3,500 - 4,500
- 4,500 - 7,500
- > 7,500





codling wind park

Project: Codling Wind Park

Contractor: natural power www.naturalpower.com

Figure D
Sandeel read counts map

CWP doc. number: CWP-NPC-CON-09-MAP-2146

Internal descriptive code:	*Size:*	*CRS:*
N/A	A3	EPSG 25830
	Scale: 1:550,000	

Rev.	*Updates*	*Date*	*By*	*Chk'd*	*App'd*
00	For FIR submission	2026/04/15	AC	ME/EA	LJ

4.1.4. All fish species detected

A number of fish species were detected using both fish and vertebrate assays, identifying 112 taxa in total. **Table G** below shows the total number of reads per assay and per month, and the number of stations these taxa were present at. Fish species recorded at the highest read counts were *Ammodytes* sp. (a species of the sand lance genus which includes some sandeel species), European sprat (*Sprattus sprattus*), sand goby (*Pomatoschistus minutus*) and common dragonet (*Callionymus lyra*). Fish species recorded at the greatest number of stations were whiting (*Merlangius merlangus*), European sprat (*Sprattus sprattus*), common dragonet (*Callionymus lyra*), poor cod (*Trisopterus minutus*) and Atlantic mackerel (*Scomber scombrus*) in both June and October and *Ammodytes* sp. in June.

Table G: All fish species detected by both fish and vertebrate assays, per month

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Agonus cataphractus	Pogge	1960	1482	2657	600	4	4	2	3
Alosa sp. 1	-	0	2089	0	2332	0	1	0	4
Ammodytes sp. 1	-	56056	0	1240850	11686	12	0	18	6
Ammodytes tobianus	Lesser sand eel	12073	3393	0	0	6	4	0	0
Anguilla anguilla	European eel	1979	4401	5351	4455	2	1	3	4
Anguilla sp. 2	-	0	0	373	0	0	0	3	0
Aphia minuta	Transparent goby	53	0	0	0	1	0	0	0
Atherina presbyter	Sand smelt	2897	692	7680	786	2	1	3	3
Barbatula barbatula	Stone loach	0	0	0	133	0	0	0	1
Barbatula sp. 1	-	0	799	0	0	0	1	0	0
Belone belone	Garfish	0	640	966	0	0	2	1	0
Blennius ocellaris	Butterfly blenny	423	0	994	0	3	0	3	0
Buglossidium luteum	Solenette	9146	1152	581	263	6	1	2	2
Buglossidium sp. 2	-	1114	0	246	0	2	0	1	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Callionymus lyra	Common dragonet	78360	38296	151425	153187	16	10	15	12
Callionymus maculatus	Spotted dragonet	103	0	0	0	1	0	0	0
Callionymus reticulatus	Reticulated dragonet	1101	367	0	0	2	1	0	0
Callionymus sp. 2	-	0	0	0	8674	0	0	0	1
Callionymus sp. 3	-	0	782	0	0	0	2	0	0
Callionymus sp. 4	-	1226	0	0	0	1	0	0	0
Centrolabrus exoletus	Rock cook	0	0	0	463	0	0	0	1
Centrolabrus sp. 1	-	0	0	0	66	0	0	0	1
Chelon sp. 1	-	1100	1324	3302	0	2	2	3	0
Chirolophis ascanii	Yarrell's blenny	0	0	633	0	0	0	1	0
Ciliata mustela	Fivebeard rockling	598	59	7271	3279	3	1	7	7
Ciliata sp. 1	-	402	1227	0	0	2	2	0	0
Clupea harengus	Atlantic herring	63647	10849	0	0	15	11	0	0
Clupea sp. 1	-	0	1198	0	0	0	1	0	0
Conger conger	European conger	119	375	0	0	2	2	0	0
Crystallogobius linearis	Crystal goby	211	872	0	0	1	1	0	0
Crystallogobius sp. 1	-	0	379	0	0	0	1	0	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Ctenolabrus rupestris	Goldsinny wrasse	3807	338	1192	2453	2	2	1	2
Dicentrarchus labrax	European seabass	6184	4148	673	0	4	5	3	0
Dicentrarchus sp. 2	-	0	138	0	0	0	1	0	0
Diplecogaster bimaculata	Two-spotted clingfish	117527	3255	0	0	9	5	0	0
Diplecogaster sp. 2	-	0	185	0	0	0	1	0	0
Echiichthys vipera	Lesser weever	13929	3088	21897	11421	6	3	10	2
Esox sp. 1	-	0	0	0	207	0	0	0	1
Gadus morhua	Atlantic cod	7667	405	0	0	2	1	0	0
Gadus sp. 2	-	2064	0	0	0	1	0	0	0
Gadus sp. 3	-	3098	0	0	0	1	0	0	0
Gaidropsarus vulgaris	Three-bearded rockling	0	0	1654	0	0	0	2	0
Galeorhinus galeus	Tope shark	64	0	0	0	1	0	0	0
Gasterosteus aculeatus	Three-spined stickleback	416	400	1920	5283	3	2	4	1
Gasterosteus sp. 1	-	0	1413	0	0	0	1	0	0
Gobio gobio	Gudgeon	0	0	31	0	0	0	1	0
Gobius paganellus	Rock goby	407	1354	0	0	2	2	0	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Gobius sp. 1	-	0	0	3368	2829	0	0	4	2
Hyperoplus sp. 1	-	0	0	0	3116	0	0	0	1
Labrus bergylta	Ballan wrasse	559	508	2201	3388	1	1	2	4
Limanda limanda	Common dab	56900	20908	0	3256	15	8	0	3
Liparis liparis	Common sea snail	126	49	1687	11448	1	1	2	1
Liparis sp. 1	-	0	0	0	8963	0	0	0	2
Liparis sp. 2	-	0	424	0	0	0	3	0	0
Lipophrys pholis	Shanny	5611	0	1107	0	2	0	3	0
Lophius piscatorius	Angler	519	0	0	0	1	0	0	0
Melanogrammus aeglefinus	Haddock	19760	5770	0	0	7	4	0	0
Merlangius merlangus	Whiting	79903	75588	0	0	18	15	0	0
Merluccius merluccius	European hake	1244	185	0	0	1	1	0	0
Microchirus variegatus	Thickback sole	0	777	0	0	0	1	0	0
Micromesistius poutassou	Blue whiting	0	435	0	0	0	1	0	0
Microstomus kitt	Lemon sole	5167	4037	5592	1141	6	2	4	1
Microstomus sp. 2	-	1153	0	0	0	4	0	0	0
Mullus surmuletus	Striped red mullet	0	315	0	0	0	1	0	0
Mustelus asterias	Starry smooth-hound	0	0	0	26	0	0	0	1
Myoxocephalus scorpius	Shorthorn sculpin	831	239	0	0	3	1	0	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Oncorhynchus mykiss	Rainbow trout	858	722	3042	10572	1	1	1	1
Parablennius gattorugine	Tompot blenny	1507	265	592	179	2	2	4	3
Parablennius sp. 1	-	153	0	0	0	1	0	0	0
Perca fluviatilis	European perch	500	559	226	2318	1	1	1	1
Petromyzon	Lamprey	97	0	0	0	1	0	0	0
Pholis gunnellus	Rock gunnel	78985	21022	111610	49924	11	8	10	8
Pholis sp. 1	-	0	1571	0	0	0	1	0	0
Phoxinus phoxinus	Eurasian minnow	12463	5107	20996	78283	4	1	3	2
Phrynorhombus norvegicus	Norwegian topknot	78	2220	0	1345	1	2	0	1
Platichthys flesus	European flounder	9536	1257	0	0	8	5	0	0
Pleuronectes platessa	European plaice	21448	5523	0	0	10	6	0	0
Pollachius pollachius	Pollack	532	374	0	0	3	2	0	0
Pollachius sp. 1	-	138	0	0	0	1	0	0	0
Pomatoschistus flavescens	Two-spotted goby	0	0	12259	4285	0	0	7	7
Pomatoschistus microps	Common goby	6619	1282	5531	1875	3	1	3	1
Pomatoschistus minutus	Sand goby	126821	20164	189831	68020	5	2	7	10
Pomatoschistus pictus	Painted goby	1037	2471	0	0	4	5	0	0
Pomatoschistus sp. 1	-	0	6197	0	0	0	4	0	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Raniceps raninus	Tadpole fish	556	0	3233	0	1	0	2	0
Rutilus rutilus	Common roach	301	249	2219	1525	2	1	2	1
Salmo salar	Atlantic salmon	393	369	5312	0	2	3	6	0
Salmo sp. 2	-	0	117	0	0	0	1	0	0
Salmo trutta	Trout	1346	2600	3075	3163	2	4	4	2
Sardina pilchardus	European pilchard	414	2265	10973	13515	1	3	5	3
Scomber scombrus	Atlantic mackerel	37827	24682	1880	11135	12	13	4	5
Scophthalmus maximus	Turbot	0	524	175	0	0	1	1	0
Scophthalmus rhombus	Brill	3332	0	5336	168	4	0	2	1
Scyliorhinus canicula	Small-spotted catshark	0	57	0	0	0	1	0	0
Solea solea	Common sole	353	2061	1851	15732	2	4	5	8
Solea sp. 2	-	632	0	0	0	1	0	0	0
Sprattus sprattus	European sprat	651263	574324	0	0	18	15	0	0
Symphodus melops	Corkwing wrasse	2062	3098	5929	15527	3	2	4	7
Symphodus sp. 2	-	0	0	0	90	0	0	0	1
Syngnathus acus	Greater pipefish	0	31	0	0	0	1	0	0
Syngnathus rostellatus	Lesser pipefish	1495	65	3439	448	2	1	3	2
Taurulus bubalis	Long-spined sea scorpion	2370	1354	0	0	4	2	0	0

		Total read counts				Number of stations detected at (maximum 18)			
		Fish assay		Vertebrate assay		Fish assay		Vertebrate assay	
Taxa	Common name	June	October	June	October	June	October	June	October
Thunnus sp. 1	-	0	0	0	2102	0	0	0	1
Trachurus sp. 2	-	861	555	0	0	2	1	0	0
Trachurus trachurus	Atlantic horse mackerel	7704	15608	17383	68513	3	11	7	14
Trisopterus esmarkii	Norway pout	689	0	0	0	1	0	0	0
Trisopterus luscus	Pouting	963	1622	0	0	3	6	0	0
Trisopterus minutus	Poor cod	12954	53240	28675	130188	12	11	10	14
Trisopterus sp. 2	-	0	0	156	19671	0	0	1	6
Trisopterus sp. 3	-	0	1974	1032	0	0	3	1	0
Zeus faber	John Dory	0	906	0	0	0	1	0	0
Zeus sp. 1	-	0	0	0	160	0	0	0	1

4.2. Diversity Metrics

4.2.1. June

The number of taxa identified per station, using the vertebrate assay, ranged from 4 (Station 16) to 26 (Station 3). The read counts ranged from 1764 (Station 8) to 11739 (Station 6). Richness ranged from 0.33 (Station 16) to 3.11 (Station 4). Diversity metrics results are shown in **Figure E** and Appendix C, **Table CA**.

The number of taxa identified per station, using the fish assay, ranged from 7 (Station 18) to 31 (Stations 3 and 4). The read counts ranged from 688.06 (Station 5) to 5845.57 (Station 15). Richness ranged from 0.71 (Station 18) to 4.44 (Station 5). Diversity metrics results are shown in **Figure F** and Appendix C, **Table CB**.

Overall, a higher number of fish species were detected using the specialised fish assay than the vertebrate assay in June, reflecting the difference in primer sensitivity and coverage of the assays as described in Section 3.1. Station 3, located in the River Liffey recorded the highest number of fish species in both assays with Station 4, located at the mouth of the River Liffey having an equal number of species when using the fish assay. Station 16, offshore east of the array site, and Station 18, offshore southeast of the array site, had the lowest number of taxa (vertebrate assay and fish assay respectively). However, there is no other obvious spatial trend in diversity metrics in and around the offshore development area in June.

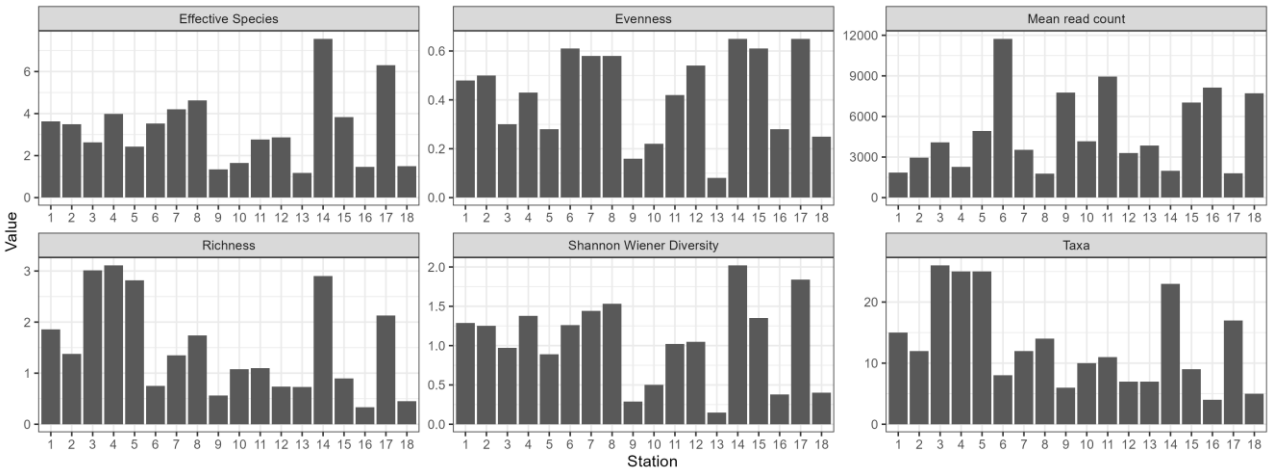


Figure E: Diversity metrics at eDNA sampling stations for June, using the vertebrate assay

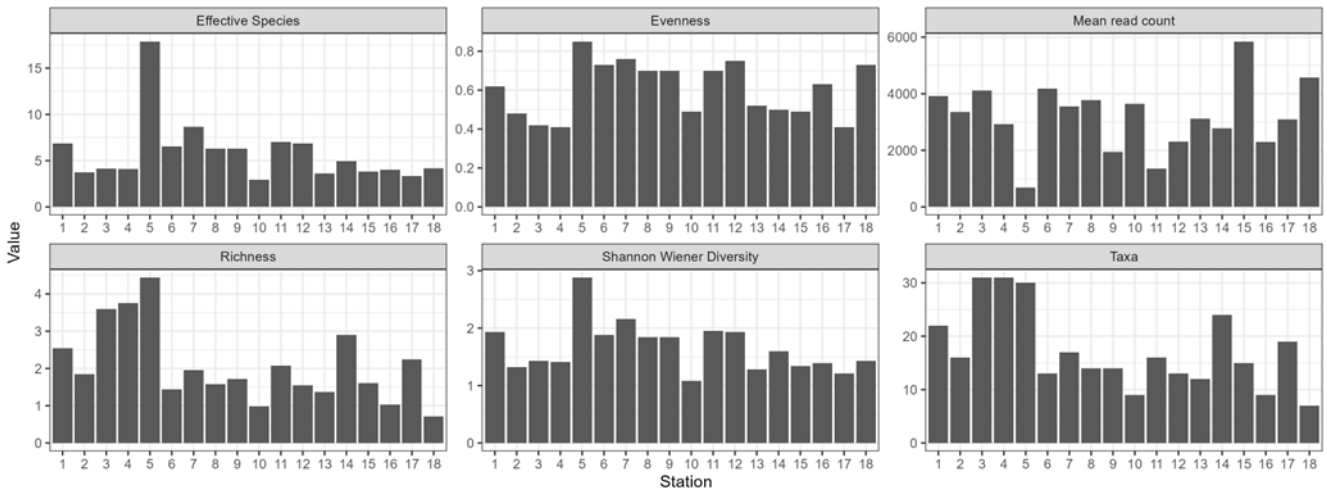


Figure F: Diversity metrics at eDNA sampling stations for June, using the fish assay

4.2.2. October

The number of taxa identified per station, using the vertebrate assay, ranged from 3 (Station 2) to 20 (Station 3). The read counts ranged from 899.25 (Station 16) to 6863.78 (Station 10). Richness ranged from 0.23 (Station 2) to 2.19 (Station 3). Diversity metrics results are shown in **Figure G** and Appendix C, **Table CC**.

The number of taxa identified per station, using the fish assay, ranged from 7 (Station 2) to 32 (Station 5). The read counts ranged from 1470.77 (Station 5) to 5314.67 (Station 2). Richness ranged from 0.70 (Station 2) to 4.25 (Station 5). Diversity metrics results are shown in **Figure H** and Appendix C, **Table CD**.

Overall, a higher number of fish species were detected using the fish assay than the vertebrate assay in October. Station 3, located in the River Liffey recorded the highest number of fish species under the invertebrate assay whilst Station 5, located just outside South Dublin Bay, had the highest number of fish species under the fish assay. Station 2, located east of Howth, had the lowest number of taxa using both the vertebrate assay and fish assay. There is no obvious spatial trend in diversity metrics in and around the offshore development area in October.

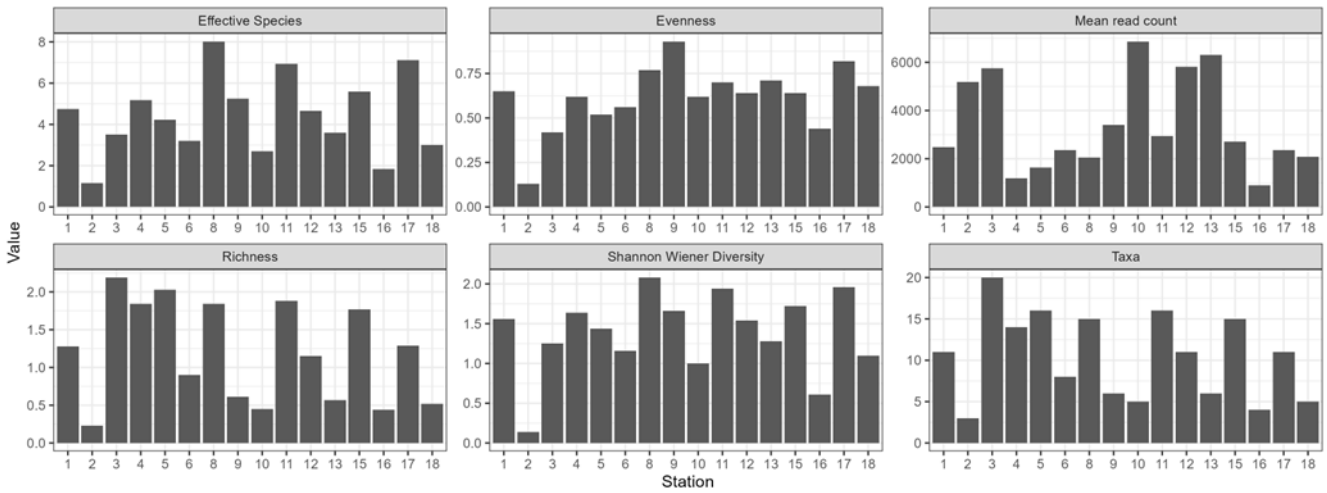


Figure G: Diversity metrics at eDNA sampling stations for October, using the vertebrate assay

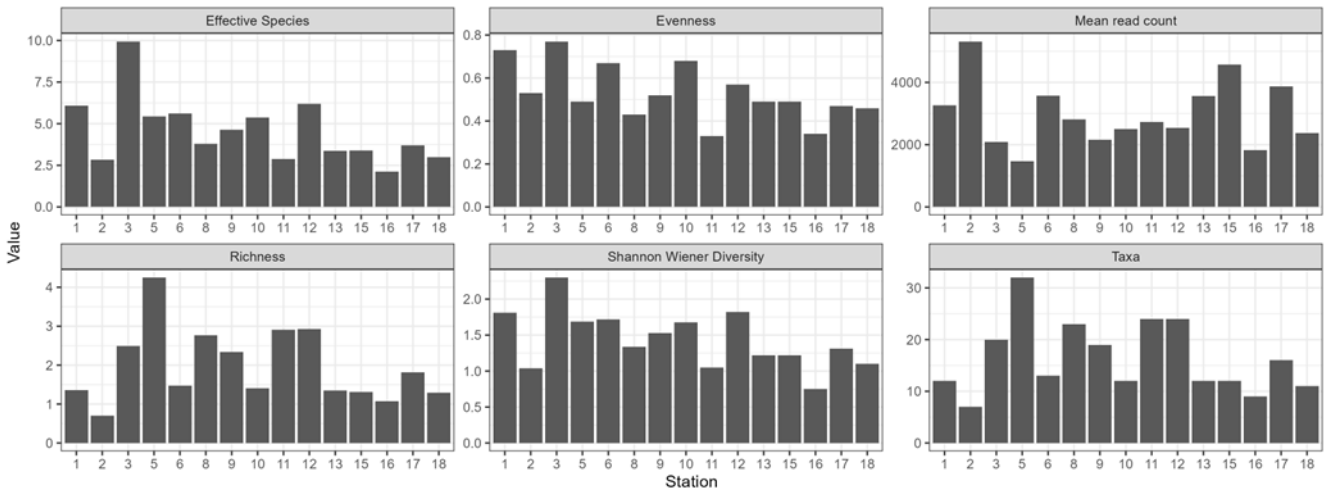


Figure H: Diversity metrics at eDNA sampling stations for October, using the fish assay

4.3. Multivariate Analysis

4.3.1. June

SIMPROF found 5 statistically significant groups of stations, using the vertebrate assay (**Figure I**), based on relatedness of species composition (based on mean read counts per species and stations).

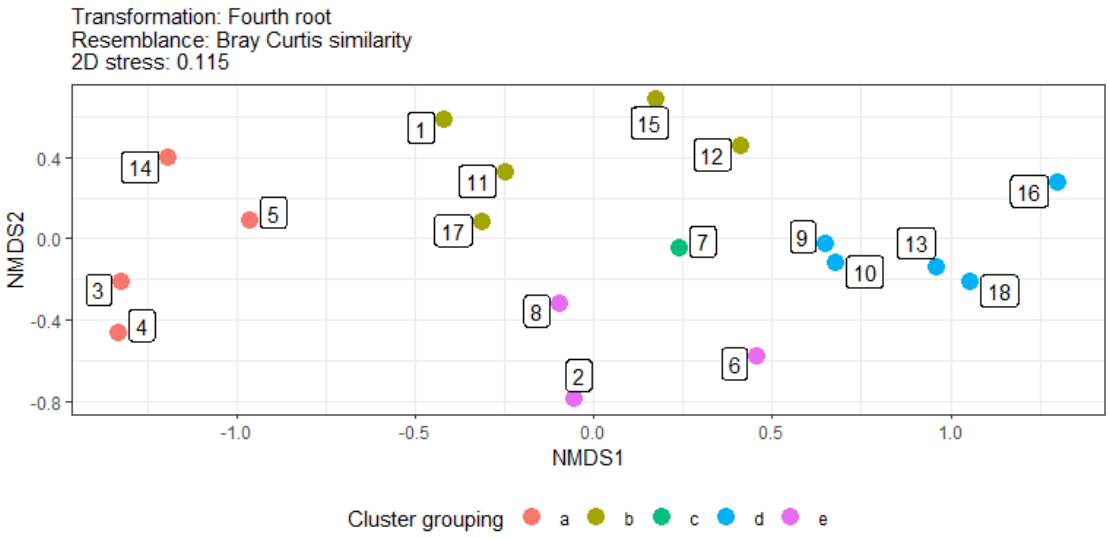


Figure I: nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the vertebrate assay

SIMPROF found 4 statistically significant groups of stations using the fish assay (**Figure J**), based on relatedness of species composition (based on mean read counts per species and stations).

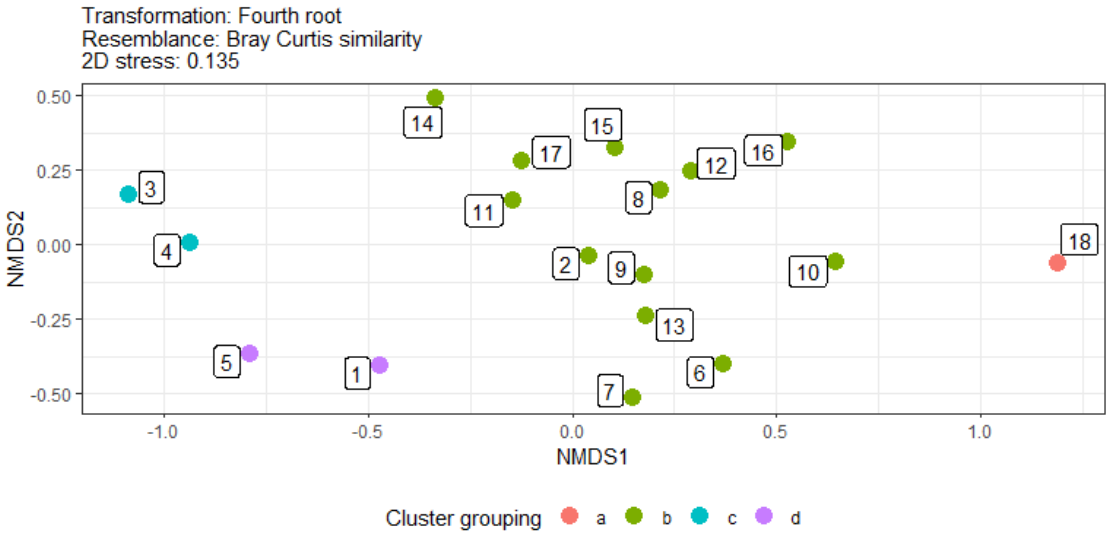


Figure J: nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for June, using the fish assay

Whilst there are differences in station groupings between assays, **Figure I** and **Figure J** show that the majority of sampling stations are grouped around the centre of the plot with Stations 3, 4 and 5 to the left and station 18 to the right of the plot. Stations 3, 4 and 5, showing a similarity to species composition to each other are located furthest inshore whilst station 18 is offshore to the east of the array site. Using the vertebrate assay, station 18 groups with Stations 10, 13 and 16 which are also located offshore to the east of the array site in deeper water alongside Station 18.

4.3.2. October

For the survey carried out in October, SIMPROF found 3 statistically significant groups of stations, using the vertebrate assay and 5 using the fish assay (**Figure K** and **Figure L**), based on relatedness of species composition (based on mean read counts per species and stations).

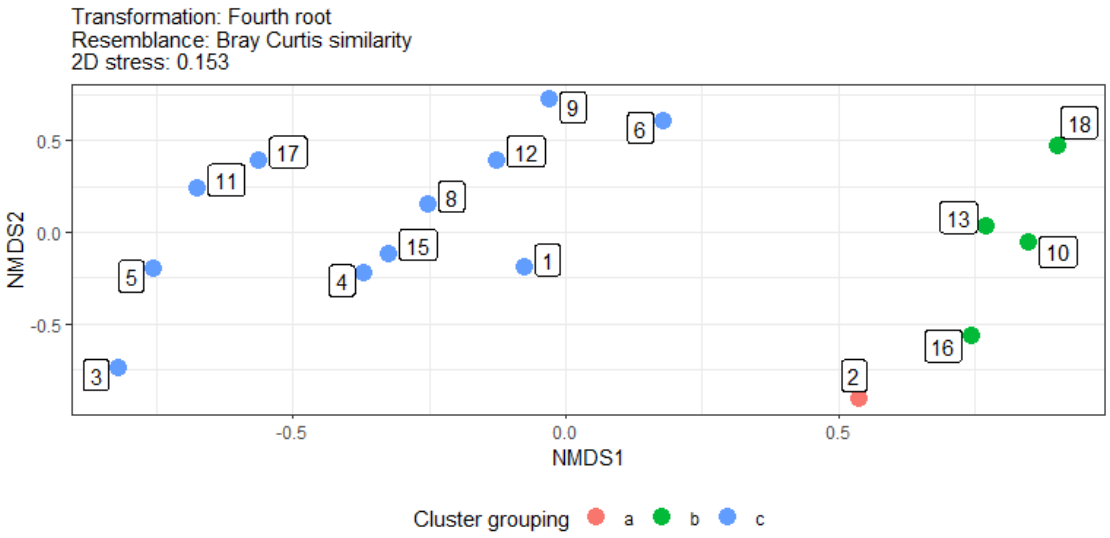


Figure K: nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for October, using the vertebrate assay

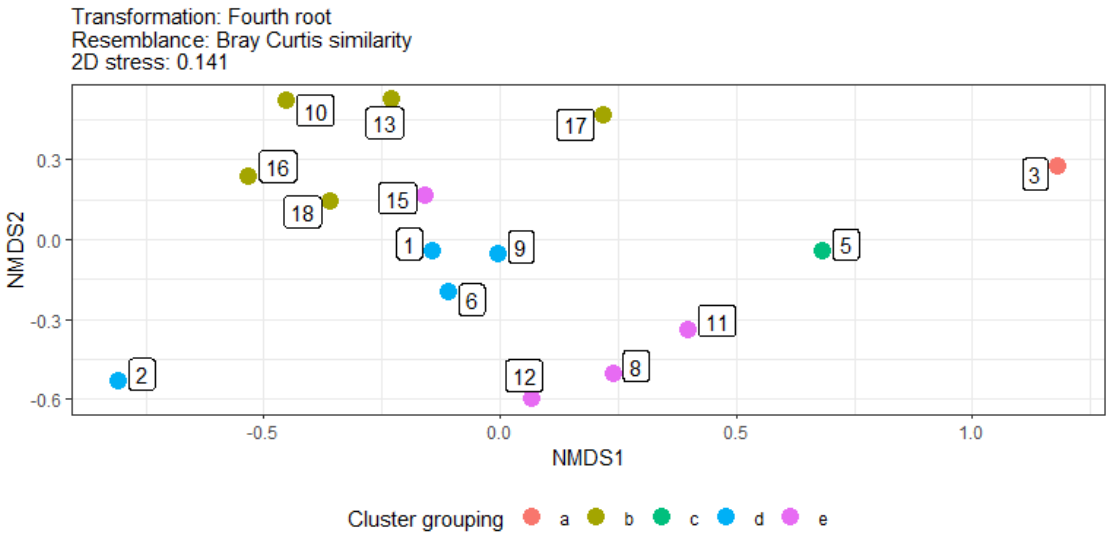


Figure L: nMDS plot showing similarity of stations based on community composition, coloured by SIMPROF identified station clusters for October, using the fish assay

There are no obvious similarities between station groupings from the vertebrate and fish assays for October, though **Figure K** (vertebrate assay) shows the further offshore stations to the east of the array site (Stations 10, 13, 16 and 18) clustering together and away from the other stations.

4.4. Marine Mammal and Seabird Occurrence

eDNA samples analysed using vertebrate assay also provided information on mammal and bird species present.

Three marine mammal species were detected in the samples, common dolphin and harbour porpoise were recorded in both June and October and minke whale recorded in June only (**Table H**, **Table I**). Harbour porpoise were recorded at 12 stations throughout the survey area while common dolphin and minke whale were each recorded at three sampling stations (Stations 1, 10 and 13 and Stations 2, 13 and 18 respectively). Stations 1 and 2 are located north of the OECC while Stations 10, 13 and 18 are east of the array site.

Table H: Total read counts showing marine mammal occurrence. Read counts represent the relative eDNA signal strength detected

Species	June read count	June no. of stations present	October read count	October no. of stations present
Common dolphin	5219	3	4845	3
Common minke whale	2290	3	0	0
Harbour porpoise	53228	12	57715	14

Table I: Read counts showing marine mammal occurrence across stations, in June and October. Read counts represent the relative eDNA signal strength detected

Station	June			October		
	Common dolphin	Common minke whale	Harbour porpoise	Common dolphin	Common minke whale	Harbour porpoise
1	3712	0	0	0	0	181
2	0	21	6040	0	0	0
3	0	0	0	0	0	50
4	0	0	0	0	0	169
5	0	0	0	0	0	124
6	0	0	5167	0	0	12134
7	0	0	10016	0	0	0
8	0	0	239	0	0	7152
9	0	0	274	92	0	419
10	1416	0	8460	4723	0	17090
11	0	0	0	0	0	3885
12	0	0	3326	30	0	3717
13	91	1431	2001	0	0	4438
14	0	0	4580	0	0	0
15	0	0	3771	0	0	1236
16	0	0	3041	0	0	0
17	0	0	6313	0	0	6094
18	0	838	0	0	0	1026

Five seabird species and one species of wader were detected across both surveys and 12 different stations (**Table J**, **Table K**), of which common guillemot, great cormorant and European shag were detected in June and October whilst northern gannet was only recorded in June and razorbill only in October. The Eurasian oystercatcher was detected at Station 4, located at the mouth of the River Liffey, in the June survey only.

Table J: Total read counts showing seabird / wading bird occurrence. Read count represent the relative eDNA signal strength detected

Species	June read count	June no. of stations present	October read count	October no. of stations present
Common guillemot	4718	6	4794	3
Great cormorant	565	1	2308	2
Northern gannet	452	1	0	0
Razorbill	0	0	4441	5
European Shag	2633	1	3721	3
Eurasian oystercatcher	94	1	0	0

Table K: Read counts showing seabird / wading bird occurrence across stations in June and October. Read count represent the relative eDNA signal strength detected.

Station	June					October				
	Common guillemot	Eurasian oystercatcher	Great cormorant	Northern gannet	Razorbill	Common guillemot	Eurasian oystercatcher	Great cormorant	Northern gannet	Razorbill
1	237	0	0	452	0	0	0	0	0	203
2	122	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	81
4	0	94	0	0	0	0	0	0	0	129
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	777	0	0	0	0	0	0	0	0	0
8	2224	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	1596	0	0
10	1101	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	4138	0	712	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	240	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	1184
16	0	0	0	0	0	0	0	0	0	0
17	257	0	565	0	0	416	0	0	0	2844
18	0	0	0	0	0	0	0	0	0	0

5. Discussion

The main aims of the surveys were to provide additional baseline information on fish and marine mammal species present, particularly on migratory salmon in and around the CWP Project area. As such, two surveys were conducted with survey timings principally chosen to reflect the migratory seasons of Atlantic salmon.

Atlantic salmon were detected in relatively low read counts in both surveys and at less than half of sampling stations in the June survey and only a sixth of sampling stations in the October survey. Atlantic salmon were present at stations closer to the shore in June, including at a sampling station within the River Liffey, than in the October survey. This likely reflects the greater abundance of Atlantic salmon during the smolt migration from rivers to the sea.

Other fish species of interest were Atlantic herring and sandeel. Atlantic herring were more prevalent in June across the survey area than in October. Sandeel were also more prevalent in June than October, recorded at all stations and at higher read counts in June. This temporal difference may be due to sandeel hibernation which occurs between September and February. Although sandeel were detected at stations within the array site, the sediment type is coarser in this area than typical sandeel habitat and it is considered unlikely that sandeel utilise sediments in this area.

Overall, 112 fish species were detected across both surveys, indicating a diverse fish community is present in the survey area, which covered the CWP Project area, and extends into the EIAR ICES regional study area, including a station in the River Liffey. Fish species recorded at the greatest number of stations were whiting (*Merlangius merlangus*), European sprat (*Sprattus sprattus*), common dragonet (*Callionymus lyra*), poor cod (*Trisopterus minutus*) and Atlantic mackerel (*Scomber scombrus*) in both June and October, and sandeel (*Ammodytes* sp.) in June. Whiting and European sprat were ubiquitous across the survey area, being present at all stations in June and 15 stations in October, Common dragonet were present at the majority of stations in June and October (16 and 12 stations respectively) and Atlantic mackerel were more common in June, than October (13 and 5 stations respectively). Poor cold was recorded at the majority of stations in both June and October (12 and 14 stations respectively). Diversity scores ranged across the survey area with few discernible patterns however, stations offshore tended to have fewer taxa and lower diversity scores than nearshore sampling stations in both surveys, however in October the station with the fewest taxa and lowest diversity scores lies closer to the coastline, east of Howth.

Analysis conducted to look for spatial differences in fish community composition found that the majority of sampling stations were similar to one another with in June, though in general the furthest inshore stations were least similar to the furthest offshore stations.

Multi-variate analysis showed that fish community composition was similar across much of the survey area, with the most inshore stations and furthest offshore stations having a slightly different community composition to each other. This is likely due to the difference in water depths and salinity levels supporting different types of species.

The surveys also provided information on marine mammal and seabird species in and around CWP Project area. Three marine mammal species were detected across the 18 stations, of which harbour porpoise was most frequently detected species occurring at the majority of stations in both June and October (12 and 14 respectively).Common dolphin was detected at three stations in June and October and Common minke whale at three stations in June and was not recorded at any stations in October. Five seabird species and one wading bird species were recording across the June and October surveys across 12 of the sampling stations, of which common guillemot was most commonly detected, occurring at six stations in June and three station in October.

6. References

Alfaro-Cordova E, Ortiz-Alvarez C, Alfaro-Shigueto J, Mangel JC, García O, Velez-Zuazo X (2022) What lies beneath? Revealing biodiversity through eDNA analysis in Lobos de Afuera Islands, Peru. Latin american journal of aquatic research, 50(4), pp.642-659. https://url.uk.m.mimecastprotect.com/s/_KMkCNx73UE2oo2SJSKHycGbi?domain=dx.doi.org

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic Local Alignment Search Tool. Journal of Molecular Biology 215, 403-410.

Calvignac-Spencer, S., Merkel, K., Kutzner, N., Kühl, H., Boesch, C., Kappeler, P.M., Metzger, S., Schubert, G. and Leendertz, F.H., 2013. Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. Molecular ecology, 22(4), pp.915-924.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. BMC Bioinformatics, 10, 421. https://url.uk.m.mimecastprotect.com/s/zbcWCO7J3h06336SZTZHGRD0y?domain=doi.org

Carvalho, C. S., De Oliveira, M. E., Rodriguez-Castro, K. G., Saranholi, B. H., & Galetti Jr, P. M. (2022). Efficiency of eDNA and iDNA in assessing vertebrate diversity and its abundance. *Molecular Ecology Resources*, *22*(4), 1262-1273. https://onlinelibrary.wiley.com/doi/abs/10.1111/1755-0998.13543

Chamberlain S, Barve V, Mcglinn D, Oldoni D, Desmet P, Geffert L, Ram K (2023) rgbif: Interface to the Global Biodiversity Information Facility API. https://url.uk.m.mimecastprotect.com/s/czr4CP163Co7QQ7SoUOHx0o1P?domain=cran.r-project.org

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-2461. https://url.uk.m.mimecastprotect.com/s/michCQ1A9C35775H4cVHGPbj1?domain=doi.org

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods, 10(10):996-8. doi: 10.1038/nmeth.2604

Edgar RC (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv 081257. https://url.uk.m.mimecastprotect.com/s/MdnPCRg83CRANNAh8fKH1BMQG?domain=doi.org

EMFF Operational Programme (2014-2020). Marine Invasive Species Ireland (MISI) PROJECT eDNA TOOLKIT. Available online: Title

Gearty W, Chamberlain S (2023) rredlist: 'IUCN' Red List Client. https://url.uk.m.mimecastprotect.com/s/fxSECVmJ3T5DMMDSKhvHEzsyp?domain=github.com

Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. PloS one, 9(1), p.e86175. https://url.uk.m.mimecastprotect.com/s/6fPHC3lYLUG5ZZ5SNHpHQZxsM?domain=doi.org

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17, 10.

Natural Power (2023). Assessing Fish Ecology Around OWFs Using eDNA. Proof of Concept White Paper. Available online: Assessing fish ecology around OWFs using eDNA

Di Muri C., Handley L.L., Bean C.W., Li J.3,4, Peirson G., Sellers G.S., Walsh K., Watson H.V., Winfield I.J.7 & Hänfling B. (2016). Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. *Metabarcoding and Metagenomics* doi: 10.3897/mbmg.4.56959.

Oksanen, Jari & Simpson, Gavin & Blanchet, F. Guillaume & Kindt, Roeland & Legendre, Pierre & Minchin, Peter & hara, R & Solymos, Peter & STEVENS, HENRY & Szöcs, Eduard & Wagner, Helene & Barbour, Matt & Bedward, Michael & Bolker, Ben & Borcard, Daniel & Carvalho, Gustavo & Chirico, Michael & De Cáceres, Miquel & Durand, Sesbastien & Weedon, James. (2022). vegan community ecology package version 2.6-2 April 2022.

Spens J, Evans AR, Halfmaerten D, Knudsen SW, Sengupta ME, Mak SST, Sigsgaard EE, Hellström M (2017) Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods in Ecology and Evolution 8, 635-645. https://url.uk.m.mimecastprotect.com/s/Qy5OCWnW3fPA00AS5i2HoFfBJ?domain=doi.org

Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., & VanMorter, S. M. (2021). Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, *78*(1), 293-304. https://doi.org/10.1093/icesjms/fsaa225

Zhang, S., Zhao, J., & Yao, M. (2020). A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. *Methods in Ecology and Evolution*, *11*(12), 1609-1625. https://doi.org/10.1111/2041-210X.13485

Appendices

A. Locations of Sampling Stations

Table AA: Migratory fish eDNA Sampling Stations

Station number	Latitude	Longitude
1	53.45552	-6.08336
2	53.38933	-5.9794
3	53.3475	-6.24937
4	53.34284	-6.17854
5	53.31961	-6.15301
6	53.32665	-5.94756
7	53.27395	-5.96547
8	53.22241	-6.04571
9	53.2166	-5.82521
10	53.20928	-5.59087
11	53.10309	-5.99185
12	53.0921	-5.76205
13	53.08936	-5.5808
14	53.00239	-5.9845
15	52.99873	-5.76751
16	52.99324	-5.57897
17	52.89804	-5.91967
18	52.89437	-5.62382

B. eDNA Laboratory Analysis

Water Extraction (freshwater and marine)

Samples were processed in dedicated clean rooms, designed for the handling of eDNA samples, at NatureMetrics with all work undertaken in class II biosafety cabinets and all workstations decontaminated with a chemical disinfectant and UV irradiated before and after use.

Samples were collected with 0.8 μm Polyethersulfone (PES) filters with Longmire's solution added to the filter housing to preserve DNA prior to extraction. DNA was extracted from the 0.8 μm PES filters using a Mag-Bind Environmental DNA Kit (Omega Bio-Tek) following Spens *et al*., 2017 method for disc filters in buffer, with proteinase K added directly to the filter housing to minimise the risk of contamination arising from handling of the filter. To remove known PCR inhibitors, an inhibition removal step is performed as part of the DNA extraction. Negative controls, consisting of molecular grade water, were processed with each batch of samples to monitor for exogenous DNA contamination through both the extraction and inhibition removal steps. Extraction yields were checked by measuring DNA concentration using a Qubit fluorometer with the Qubit dsDNA broad range assay kit (Thermo Fisher Scientific).

DNA Amplification

Marine water fish and vertebrates

Replicate PCRs for each sample and extraction blank were amplified via a two-step PCR process, with tails added to the 5' end of taxon specific primers to complement downstream adapter and index primer sequences.

For the marine water fish, amplification was performed with a commercially available High-fidelity DNA polymerase following manufacturers guidelines using the MiFish-U-F MiLamprey_F MiCatfish_F 5'-GCCGGTAAAACTCGTGCCAGC GCTGGTAAACCTCGTGCCAGC GTCGGTAAAATTCGTGCCAGC-3' and MiFish-U-R MiLAmprey_R 5'- CATAGTGGGGTATCTAATCCCAGTTTG CATAGCGGGGTATCTAATCCCGGTTTG-3' primers (Alfaro-Cordova *et al*, 2022) targeting the mitochondrially encoded 12S ribosomal RNA (12S rRNA) gene for the target taxa.

For the marine water vertebrates, amplification was performed with a commercially available Hot Start DNA polymerase following manufacturers guidelines using the F1 5'- ACTGGGATTAGATACCCC-3' and R1 5'-TAGAACAGGCTCCTCTAG-3' primers (Kelly *et al*, 2014) targeting the mitochondrially encoded 12S ribosomal RNA (12S rRNA) gene for the target taxa. As these primers are known to amplify primate DNA, a blocking primer was used during first round PCR to limit the amplification of non-target human DNA (12S_V5_blkhum; 5'-TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATC-spacerC3-3'; Calvignac-Spencer *et al*.,2013).

See the Report Interpretation Guide (https://url.uk.m.mimecastprotect.com/s/KjIbCLgA3CmJLLJfMHYHyyQpy?domain=naturemetrics.com) for information on target and non-target taxa.

Positive and negative controls, consisting of proprietary synthetic sequences (that do not match known biological records) and PCR-grade water, respectively, were included with every PCR plate to verify amplification performance. PCR amplification success was confirmed visually by gel electrophoresis.

Library Preparation & Sequencing

Successfully amplified first round PCR replicates were pooled per sample and purified using MagBind TotalPure NGS magnetic beads (Omega Biotek). A sequencing library was prepared from the purified amplicons using unique dual indexes, following Illumina 16S Metagenomic Sequencing Library Preparation protocol (https://url.uk.m.mimecastprotect.com/s/gp6LCMj73fRQjjQh0I4H8gdp8?domain=support.illumina.com). Indexed PCR products were subsequently purified, quantified, normalised, and pooled in equal volumes. The final pooled library was sequenced on an Illumina MiSeq system using a V3 600 cycle reagent kit (Illumina).

Bioinformatics

Sequences were demultiplexed with bcl2fastq based on the combination of the i5 and i7 index tags. Paired-end FASTQ reads for each sample were merged with USEARCH (Edgar, 2010) requiring a minimum of 80% agreement in the overlap. Forward and reverse primers were trimmed from the merged sequences using cutadapt (Martin, 2011) with a length filter of 80-120bp (post primer removal). Sequences were quality filtered with USEARCH to retain only those with an expected error rate per base of 0.01 or below and dereplicated by sample, retaining singletons. Unique sequences from all samples were denoised in a single analysis with UNOISE (Edgar, 2016) requiring retained zOTUs (zero-radius Operational Taxonomic Units) to have a minimum abundance of 8 in at least one sample. Consensus taxonomic assignments were made for each zOTU using sequence similarity searches against

NCBI nucleotide (NCBI nt). Searches against databases were made using blastn (Altschul *et al*.,1990; Camacho *et al*.,2009) and required hits to have a minimum e-score of 1e-20 and cover at least 90% of the query sequence. The taxonomic identification associated with all hits was converted to match the GBIF taxonomic backbone. Assignments were made to the lowest possible taxonomic level where there was consistency in the matches, with minimum similarity thresholds of 100%, 97% and 95% for species, genus, and higher-level assignments respectively.

Automated identifications were sense-checked against GBIF occurrence records for presence in the sampling country, and elevated to higher taxonomic levels where required (rgbif; Chamberlain *et al*., 2023). In countries where species are poorly documented and have limited occurrence records, the occurrence search may be expanded to include records from surrounding countries.

In cases where there were hits to multiple species at the top similarity, GBIF occurrence records were used to resolve the conflicts and further improve taxonomic resolution where possible.

Following taxonomic assignment, zOTUs were clustered into OTUs to minimise the number of sequence variants for a species (that may be present due to intra-specific variations, or amplification or sequencing artefacts). Supervised clustering was done using a combination of USEARCH UPARSE (Edgar, 2013) and a custom pipeline that takes into account sequence similarity, co-occurrence patterns, abundance profiles, and taxonomy to prevent the over-clustering of distinct, closely related species. Chimeric sequences were excluded, and an OTU-by-sample table was generated by mapping all dereplicated reads for each sample to the OTU representative sequences with USEARCH at an identity threshold of 97%.

All OTUs with species-level identifications were queried against the IUCN Red List (rredlist; Gearty & Chamberlain, 2023) to obtain global threat status, and the Global Register of Introduced and Invasive Species (GRIIS) to obtain their invasive status in the sampling country.

The OTU table was filtered to remove low abundance OTUs from each sample. We aimed to keep the minimum read count for a detection of an OTU within a sample at approximately 20 reads. To do this we identified the percentage threshold across all samples within the dataset that most closely achieved this and applied this threshold across all samples. Unassigned OTUs, and OTUs identified to human and domesticated mammals, were removed from the dataset for subsequent analyses.

C. Diversity Metrics Results

Table CA: Diversity Metrics Results for June, using the vertebrate assay

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	15	1853.45	1.29	1.86	0.48	3.63
2	12	2949.93	1.25	1.38	0.50	3.50
3	26	4083.26	0.97	3.01	0.30	2.63
4	25	2265.69	1.38	3.11	0.43	3.99
5	25	4934.58	0.89	2.82	0.28	2.43
6	8	11739.75	1.26	0.75	0.61	3.53
7	12	3532.06	1.44	1.35	0.58	4.21
8	14	1764.14	1.53	1.74	0.58	4.62
9	6	7772.90	0.29	0.56	0.16	1.34
10	10	4158.17	0.50	1.08	0.22	1.65
11	11	8955.11	1.02	1.10	0.42	2.76
12	7	3292.07	1.05	0.74	0.54	2.86
13	7	3858.27	0.15	0.73	0.08	1.17
14	23	1990.65	2.02	2.90	0.65	7.56
15	9	7023.00	1.35	0.90	0.61	3.84
16	4	8120.38	0.38	0.33	0.28	1.47
17	17	1801.37	1.84	2.13	0.65	6.30
18	5	7712.50	0.40	0.45	0.25	1.49

Table CB: Diversity Metrics Results for June, using the fish assay

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	22	3919.00	1.93	2.54	0.62	6.86
2	16	3358.91	1.32	1.85	0.48	3.76
3	31	4117.44	1.43	3.60	0.42	4.16
4	31	2930.11	1.41	3.76	0.41	4.10
5	30	688.06	2.88	4.44	0.85	17.88
6	13	4182.85	1.88	1.44	0.73	6.56
7	17	3547.43	2.16	1.96	0.76	8.68
8	14	3774.50	1.84	1.58	0.70	6.31
9	14	1937.58	1.84	1.72	0.70	6.32
10	9	3646.50	1.08	0.98	0.49	2.96
11	16	1357.00	1.95	2.08	0.70	7.02
12	13	2315.33	1.93	1.55	0.75	6.88
13	12	3122.95	1.28	1.37	0.52	3.61
14	24	2783.93	1.60	2.90	0.50	4.94
15	15	5845.57	1.34	1.61	0.49	3.82

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
16	9	2297.71	1.39	1.03	0.63	4.02
17	19	3087.97	1.21	2.24	0.41	3.35
18	7	4571.60	1.43	0.71	0.73	4.17

Table CC: Diversity Metrics Results for October, using the vertebrate assay

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	11	2482.73	1.56	1.28	0.65	4.74
2	3	5178.75	0.14	0.23	0.13	1.15
3	20	5753.19	1.25	2.19	0.42	3.51
4	14	1190.00	1.64	1.84	0.62	5.17
5	16	1638.45	1.44	2.03	0.52	4.23
6	8	2357.22	1.16	0.90	0.56	3.20
8	15	2053.48	2.08	1.84	0.77	8.02
9	6	3397.30	1.66	0.61	0.93	5.25
10	5	6863.78	1.00	0.45	0.62	2.70
11	16	2946.42	1.94	1.88	0.70	6.94
12	11	5819.13	1.54	1.15	0.64	4.66
13	6	6310.55	1.28	0.57	0.71	3.59
15	15	2710.85	1.72	1.77	0.64	5.59
16	4	899.25	0.61	0.44	0.44	1.84
17	11	2352.43	1.96	1.29	0.82	7.11
18	5	2080.83	1.10	0.52	0.68	3.00

Table CD: Diversity Metrics Results for October, using the fish assay

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
1	12	3271.00	1.81	1.36	0.73	6.09
2	7	5314.67	1.04	0.70	0.53	2.83
3	20	2083.00	2.30	2.49	0.77	9.94
5	32	1470.77	1.69	4.25	0.49	5.43
6	13	3569.29	1.72	1.47	0.67	5.61
8	23	2808.59	1.34	2.77	0.43	3.80
9	19	2158.73	1.53	2.34	0.52	4.64
10	12	2500.63	1.68	1.41	0.68	5.37
11	24	2725.40	1.05	2.91	0.33	2.87
12	24	2541.71	1.82	2.93	0.57	6.19
13	12	3562.67	1.22	1.35	0.49	3.38
15	12	4570.80	1.22	1.31	0.49	3.39

Station	No. Taxa	No. Read Counts	Shannon-Wiener Diversity	Richness	Evenness	Effective Species Number
16	9	1820.78	0.75	1.07	0.34	2.13
17	16	3870.12	1.31	1.82	0.47	3.70
18	11	2369.29	1.10	1.29	0.46	3.00

natural power

Creating a better environment

naturalpower.com
sayhello@naturalpower.com

25 years

For full details on our ISO and other certifications, please visit our website.