# ENVIRONMENTAL DNA (eDNA) PROPOSED APPROACH NOTE

BENTHIC SUBTIDAL ECOLOGY AND FISH AND SHELLFISH ECOLOGY

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# GLOSSARY

| Term | Definition |
|---|---|
| Assay | An investigative procedure in a laboratory to analyse a sample medium for the content of a target species or species group; different assays target different organismal groups (e.g. fish, marine mammals, metazoans/invertebrates or bacteria). |
| Cryptic | Species that are rarely recorded, or present in low numbers or very specific conditions. |
| Physico-chemistry | Term to describe physical and chemical analytical parameters collectively, typically including Particle Size Analysis, Total Organic Carbon, Heavy and Trace Metals and Hydrocarbons, and others, where applicable. |
| Taxonomy | Classification scheme for organisms, based on physical identification. |

# ACRONYMS

| Acronym | Description |
|---|---|
| DNA | Deoxyribonucleic Acid |
| eDNA | Environmental DNA |
| EIA | Environmental Impact Assessment |
| INNS | Invasive and Non-Native Species |
| MD-LOT | Marine Directorate – Licensing Operations Team |
| MPA | Marine Protected Areas |
| MSS | Marine Scotland Science |
| Ossian OWFL | Ossian Offshore Wind Farm Limited |
| PMF | Priority Marine Feature |
| PCR | Polymerase Chain Reaction |
| PSA | Particle Size Analysis |
| SoW | Scope of Work |

# UNITS

| Unit | Description |
|---|---|
| g | Gram |
| km | Kilometre |
| l | Litre |
| m | Metre |
| µm | Micrometre |
| OTU | Operational Taxonomic Units |

# 1. INTRODUCTION

1. NatureScot provided feedback on the Ossian Array Environmental Impact Assessment (EIA) Scoping Report (Ossian Offshore Wind Farm Limited (OWFL), 2023) as part of the Scoping consultation detailed within the formal Scoping Opinion provided on the 14 June 2023 (Marine Directorate – Licensing Operations Team (MD-LOT), 2023).
2. NatureScot recommended undertaking additional data collection to inform the environmental baseline for both benthic subtidal ecology and fish and shellfish ecology. Collection and analysis of environmental Deoxyribonucleic Acid (eDNA) samples to support baseline characterisation within both the Array and along the Proposed offshore export cable corridor (which is subject to a separate Application) was proposed. This approach was recommended to support characterisation of cryptic or scarce species, Scottish Priority Marine Feature (PMF) species and prey fish species.
3. This Note outlines Ossian OWFL's proposed approach to address feedback regarding the recommendation to incorporate eDNA data into the baseline characterisation for the Array EIA Report.
4. The Ossian Array is located off the east coast of Scotland, approximately 80 km south-east of Aberdeen from the nearest point (Figure 1.1).

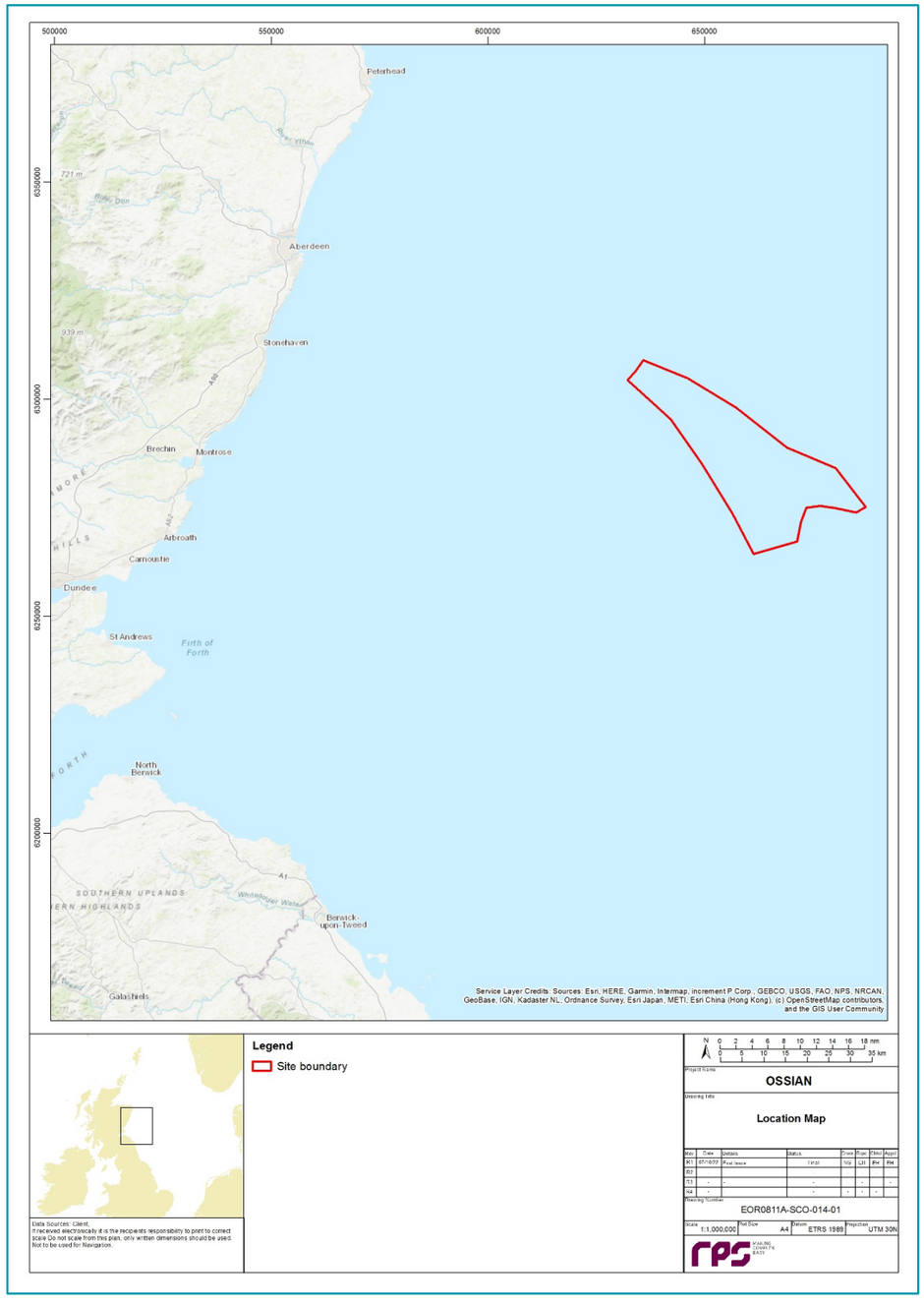


**Figure 1.1 Location of the Ossian Array**

# 2. BASELINE CHARACTERISATION APPROACH

## 2.1. BENTHIC SUBTIDAL ECOLOGY

5. A combination of desktop data sources and site-specific survey data from the Array have been used to characterise the benthic subtidal ecology baseline environment within the Array.

6. The established baseline for the Array as presented in the Ossian Array EIA Scoping Report (Ossian OWFL, 2023) is accepted by MD-LOT, NatureScot and other stakeholders. This baseline will be further developed for the Array EIA Report through desktop study.

7. Literature sources were used to support designing the benthic subtidal ecology site-specific survey, and the survey scope of work (SoW) was submitted to MD-LOT on 20 December 2021 for review. The survey SoW included standard acquisition methods:

   - drop-down camera (stills images and video);
   - grab sampling for sediment biology (standard macrofaunal taxonomy) and physico-chemistry; and
   - epibenthic beam trawling.

8. Feedback from MD-LOT, including responses from NatureScot and Marine Scotland Science (MSS), on the benthic subtidal survey SoW was received via email on 24 January 2022 (MD-LOT, *pers. comms.* 2022), with the following comments raised:

   - *"The proposed methodology follows standard protocols for benthic surveys and analysis."*
   - *"The review of existing benthic data is comprehensive. Very few of the existing data points fall directly within E1 (east), but they will give regional context."*
   - *"The number and distribution of sampling locations (Figure 4.2) look appropriate for the size of the site (including a 2 km buffer)."*
   - *"There is no overlap with any Nature Conservation MPAs* [Marine Protected Areas]*."*
   - *"In section 5.3.1 Habitats/Species of Conservation Importance – we advise that they should also consider for further assessment any Priority Marine Features identified in the drop-down video footage. If the PMFs are sensitive to damage/disturbance, grab samples should be avoided at that location, as proposed for Annex 1 features."*

9. The following gaps were noted in the benthic subtidal survey SoW by MD-LOT as part of their review, although it was acknowledged that these were likely to come later in the project programme:

   - geophysical surveys which will be used to inform the benthic surveys;
   - benthic surveys of the Proposed offshore export cable corridor(s); and
   - intertidal surveys at the Proposed landfall location(s).

10. Based on the above feedback on the benthic subtidal survey SoW, the approach to the site-specific survey was considered accepted by MD-LOT, noting the absence of reference to eDNA.

11. During survey execution in the Ossian Array this advice was actioned, whereby the geophysical survey data was acquired prior to benthic sampling, to enable benthic site selection to be informed by the geophysical return (i.e. the side scan sonar and multibeam echosounder/bathymetric data).

## 2.2. FISH AND SHELLFISH ECOLOGY

12. A detailed review of desktop data sources has been used to characterise the fish and shellfish ecology baseline environment for the Array EIA Report, with the inclusion of site-specific benthic subtidal ecology survey results to support the following elements:

   - substrate suitability assessment for herring *Clupea harengus* spawning and for sandeel Ammodytidae spp., based on particle size analysis (PSA) results;
   - contextual habitat information to support general fish and shellfish assemblage characterisation;
   - opportunistic observations of fish and shellfish species in both grab samples and underwater stills images and video footage; and
   - fish and shellfish catches from epibenthic beam trawls.

13. Desktop data sources used for baseline characterisation for the Ossian Array EIA Scoping Report (Ossian OWFL, 2023) include regional scientific and stock assessment surveys, surveys undertaken for other offshore wind projects within the project region, off the coast of the Firth of Forth, and published literature regarding species ecology, including spawning and nursery ecology.

14. The use of desktop data covering a broad number of years, which demonstrates relative consistency year-to-year, is considered sufficient to provide a robust characterisation, in combination with site-specific substrate suitability classification for key species.

15. The feedback received from MD-LOT (including responses from NatureScot and MSS) on the SoW for the site-specific benthic subtidal ecology survey (i.e. not as part of the Scoping Opinion for the Ossian Array EIA Scoping Report; Ossian OWFL, 2023) is outlined in section 2.1, and the SoW was considered accepted by MD-LOT. The incorporation of eDNA sampling was not recommended by MD-LOT at the time of review of the survey SoW.

16. The established baseline for the Array presented in the Ossian Array EIA Scoping Report (Ossian OWFL, 2023) is accepted by MD-LOT, NatureScot and other stakeholders, in line with the Scoping Opinion (MD-LOT, 2023).

# 3. eDNA

## 3.1. BENEFITS AND CHALLENGES

17. Whilst eDNA analysis using Polymerase Chain Reaction (PCR) amplification and sequencing is a rapidly developing technique, it is acknowledged that species libraries (such as Illumina MiSeq) are currently a work in progress and these can be highly subject to errors. This also leads to a high proportion of results being identified to higher taxonomic levels due to low confidence in genus and species categories. In line with this, some more cryptic or rare species may not yet be recognised using Deoxyribonucleic Acid (DNA) techniques due to low confidence in genus or species assignment.

18. eDNA can however be a useful tool to detect the presence of cryptic or rare species or invasive and non-native species (INNS), where such species' DNA are already mapped and available within their respective libraries. The majority of research applications for eDNA have been associated with fish species in support of stock assessment and similar applications, however, mor recently the North Sea has been subject to a reasonable degree of sampling for eDNA for invertebrates (e.g. BP Exploration Operating Company Limited, 2023, for the Northern Endurance Partnership) therefore whilst species libraries cannot be considered complete and will be continually evolving, key species or PMFs such as horse mussel *Modiolus modiolus* may be detected if present.

19. DNA samples can be obtained through both sediment sampling and seawater sampling, and the most appropriate method is dependent upon the overall objectives of the campaign.

20. Sediment eDNA sampling can present challenges due to the increased longevity of shed or residual DNA within the sediment, as opposed to free-floating in the water column with seawater sampling. For example, presence records in sediment samples can be indicative of species which have occurred in the location over a long period of time (potentially years) and may not be reflective of the current environment and associated communities. Distribution of sediment bound DNA can also be subject to resuspension and hydrodynamic transport away from the location where the DNA material was shed, meaning spatial results should be interpreted with caution (Turner *et al.*, 2015).

21. Seawater DNA samples are considered more reflective of the contemporary environment, and are typically considered to represent a single tidal cycle, although free-floating DNA material can last up to a few days

before becoming degraded (the rate of degradation is based upon multiple variables, such as temperature, salinity, weather, hydrodynamics etc.). Based on this degradation time, water samples are considered to reflect the local area, assuming a non-extreme hydrodynamic regime (Cristescu and Herbert, 2018). However, it is worth noting that eDNA in offshore environments is reported to degrade at a slower pace than inshore environments, with seasonality having a minor effect on degradation rates, showing slightly higher rates of DNA degradation in winter as opposed to summer (Collins *et al.*, 2018). Port *et al.* (2016) analysed a series of seawater samples for DNA collected in a highly dynamic environment from 2 m to 70 m water depth in an area of kelp forest and deeper rocky reef. This study found that spatial resolution of vertebrate community results compared favourably to visual surveys, within a range of 60 m to 100 m, showing relatively accurate and reliable spatial data relating to the distribution of those communities identified.

22. Due to eDNA within the marine water column being relatively dilute (when compared to freshwater systems), larger water samples tend to be required to obtain sufficient material for analysis (Cristescu and Herbert, 2018). Seawater samples typically require a minimum volume of 5 litres of seawater to be collected per depth sampled, whereas for sediments, only a small sample of <100 g is typically required. The use of eDNA can also introduce occurrences of false positives, where the species is not actually present (DNA material may have been released into the water column following mortality (including within predator faecal deposits), or have been transported from elsewhere; Roussel *et al.*, 2015). Conversely, the absence of a detection does not necessarily mean that the organism is not present, however this caveat can also be applied to standard macrofaunal sampling (Roussel *et al.*, 2015).

23. Quantitative analysis is not possible using DNA techniques due the number of Operational Taxonomic Units (OTU) being linked to the number of positive detections of a particular DNA sequence, rather than the number of individual organisms within a species group. Organisms can contain huge amounts of DNA, with many replicates of the same sequence present, although, high level comparisons of the amount of DNA present (relative abundance) can be made, however this may reflect the presence of different sized specimens, or behavioural anomalies where organisms shed higher amounts of DNA during particular events. The technique is therefore applicable only as a presence-absence tool at the current stage of development and should always be used alongside standard macrofaunal techniques for benthic ecology, for example, to enable quantitative analysis.

24. The benefits and challenges associated with eDNA are summarised in Table 3.3.

**Table 3.1: Summary of the Benefits and Challenges Associated with eDNA Analysis**

| Item | Benefits | Challenges |
|---|---|---|
| Species detection | Can be a useful tool to detect the presence of cryptic or rare species, or INNS.<br><br>Highly sensitive detection, where species are present within the DNA library.<br><br>Studies for the detection of marine vertebrates (particularly fish) have shown promising results when investigating biodiversity within an environment (Shu *et al.*, 2020). | Species must have previously been sequenced to an appropriate confidence level to allow detection to genus or species within a sample.<br><br>False positives can occur, and are hard to rule out, due to the possibility that DNA is transported from other areas (including through aquaculture feed, predation, persistence of shed DNA in the sample matrix etc.).<br><br>DNA persistence is longer in a sediment matrix than free-floating in the water column, with slightly higher persistence in offshore water columns when compared to inshore areas. |
| Sample quantification | eDNA allows for relative abundance assessment, based upon the quantities of eDNA material present. | Quantitative analysis is not possible using eDNA. Quantities of eDNA present can be reflective of organisms of the same species of differing sizes, species-specifics or life cycle stage specifics in terms of how much DNA is shed into the environment. eDNA alone cannot be used to provide an informed quantitative assessment of marine communities. |
| Sample size | For sediment eDNA, smaller sample sizes are required than for standard macrofaunal taxonomy. | eDNA is more dilute in the marine water column, requiring larger volumes to be processed to obtain sufficient DNA material for detection. |
| Spatial resolution | A study by Port *et al.* (2016) suggested that eDNA results showed spatial resolution of marine vertebrate communities to within 60 m to 100 m, and results compared favourably to visual surveys. This likely to be dependent upon the sampling environment, which in this case was high energy and nearshore (i.e. within 2 km of the coast).<br><br>Seawater samples are generally considered to reflect the contemporary environment at the time of sampling. | Due to its persistence in the environment (which varies based on the site-specific conditions), eDNA can be transferred from other locations to the sampling location by various means in both sediment and seawater matrices. This can lead to false positives, and poor spatial resolution of results. |

25. Based on the above, eDNA analysis can provide information to support biodiversity assessment, but should be conducted alongside other techniques, particularly for marine benthos to allow for quantification of results.

26. eDNA analysis can be a useful tool for identifying the presence of multiple species within a defined assay (or assays), although results should be interpreted with caution surrounding false positives and the potential for identification to higher taxonomic levels only, due to low confidence in genus and species data based upon the available reference library. The absence of sequenced data for some cryptic or rare species may lead to false negatives, where species are present, but are not detected due to the absence of sufficient data within the library to provide species level identification. Where used, eDNA results should be interpreted with consideration of the caveats outlined earlier within the section.

# 4. PROPOSED APPROACH

27. For the Ossian Array subtidal benthic ecology characterisation, standard macrofaunal sampling, along with drop-down camera and epifaunal trawling was undertaken. The laboratory processing of macrofaunal and epifaunal samples allowed identification of specimens to the lowest taxonomic level possible, with most organisms identified to genus and species level. This approach, combined with substrate determination using both physical sampling and geophysical data allowed for the exact species within a sample at an exact location to be identified and used to inform the habitat description at that specific location. The geophysical data, combined with results from multiple sediment sampling stations then allowed for this information to be extrapolated in line with the seabed textural data to provide a detailed habitat chart of the Array. eDNA sampling from sediment samples may lead to false positives, in terms of identifying the presence of DNA from historic occurrences which are not representative of the current environment. eDNA is therefore considered more supportive of broadscale characterisation than targeted site-specific

characterisation to inform habitat mapping. The approach undertaken for the Ossian Array is considered sufficient to produce a detailed habitat map, and to support robust characterisation of the benthic communities present in combination with available desktop data sources, allowing for confident identification of Important Ecological Features to carry through to assessment.

28. The baseline characterisation developed for fish for the Ossian Array is based on varied data sources, collated over a long time period and at a regional spatial scale. As such, the species that may interact with the Ossian Array is fully captured in this data, encompassing seasonality and annual variation. The list of species scoped in is therefore considered precautionary, and extensive, and it's considered unlikely that eDNA samples would identify additional species. The approach undertaken for the Ossian Array is considered sufficient to provide a robust characterisation of the fish and shellfish assemblages likely to occur within the regional fish and shellfish study area, and within the Ossian Array.

29. In summary, whilst eDNA is a useful tool alongside more standard sampling practices, it is important to note that the baseline characterisation provided for the Array benthic subtidal ecology and fish and shellfish ecology study areas have not been contested by MD-LOT or other stakeholders. Further, the site-specific survey data collection methodology to inform the baseline characterisation was developed in consultation with MD-LOT, and all advisory responses to such consultation were taken forwards into the final subtidal benthic ecology survey design.

30. Based on the accepted baseline presented within the Ossian Array EIA Scoping Report (Ossian OWFL, 2023), the Scoping Opinion received (MD-LOT, 2023), expert judgement and understanding of the baseline environment within the region and the application timelines, it is not considered necessary to supplement the baseline with additional eDNA data for the Array, as sufficient information has been drawn together to allow for robust characterisation and understanding of the baseline environment.

31. For the Proposed offshore export cable corridor subtidal benthic ecology survey, collection of seawater and sediment samples will be considered for eDNA analysis at a representative number of stations to enhance the baseline characterisation for fish and shellfish ecology and benthic subtidal ecology, alongside standard drop-down camera and grab sampling operations. Due to the anticipated length of the offshore export cable corridor it is anticipated that this area is likely to reflect a wider range of subtidal habitats than the Array, based upon broadscale habitat mapping (EMODnet, 2021). Further consultation on future benthic surveys will be undertaken following identification of the proposed offshore export cable corridor.

## 4.1. REFERENCES

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