Background to Natural England

Natural England (NE) is the government’s advisor on the natural environment. We provide practical advice, grounded in science, on how best to safeguard England’s natural wealth for the benefit of everyone. Our remit is to ensure sustainable stewardship of the land and sea so that people and nature can thrive. It is our responsibility to see that England’s rich natural environment can adapt and survive intact for future generations to enjoy.

### **Understanding the effectiveness of environmental DNA (eDNA) techniques for detection of marine mammals**

Background to the specific work area relevant to this purchase

The marine Natural Capital and Ecosystems Approach (mNCEA) mission is to “Transform and innovate the way our evidence-base is captured, analysed and brought together to ensure science meets the needs of policy / decision makers to embed a natural capital approach, allowing us to leave our marine environment in a better state than we found it, achieving clean, productive, healthy and biologically diverse seas, and a sustainable blue economy.”

The mNCEA will provide a holistic, accurate and robust set of evidence and data for DEFRA to make informed policy decisions about the state of our natural capital assets in high profile policy areas (e.g. future fisheries, offshore wind etc), and lead to better outcomes for the environment. It will also identify innovative and transformative ways of collecting, analysing and distributing the data.

This project will review methods for the use of innovative environmental DNA (eDNA) techniques for marine mammals as part of the NE mNCEA programme.

Monitoring of marine mammals using conventional visual or acoustic methods from vessels and aircraft is associated with high costs. However, the rapidly developing field of eDNA is a cost-effective and non-invasive method for monitoring marine species, and their application to monitoring marine mammals is an emerging field of research.

The objectives from this project are;

* To understand the efficiency of passive sampling techniques.
* To understand the effectiveness of open-source primer sets for the detection of marine mammals.

NE seeks a lab partner for the analysis of marine eDNA samples for the understanding of this technique for marine mammal surveying. Samples will be collected in late summer 2024 and provided to the lab for metabarcoding analysis, with expected reporting delivery in February 2025.

Requirement

NE requires the delivery of lab analysis and reporting of eDNA samples. The samples will be collected by a separate contractor following NE specified sampling methodologies. The samples will be collected in the UK marine environment in September-October 2024.

Samples

The samples provided will be;

1. filtered and preserved water samples (45 x 0.45µm sterivex filters for analysis, plus 4 x 0.45µm sterivex filters as field negative controls)
2. preserved gauze samples (15 x gauze samples to be divided into 3 each, total 45 for analysis, plus 1 x gauze sample to be divided into 3 as field negative controls)

“Active” sampling: Water samples will be obtained by 5ltr niskin bottle samples taken in triplicate at each sampling station (N=15). Water samples will be stored at room temperature (or below) out of direct sunlight, in foil lined bags (to reduce degradation) until filtration, and filtered as soon as possible (maximum 12 hours) n 36 hours. The water samples will be filtered into 0.45µm sterivex filters (minimum 2ltrs filtrate), and preserved using 1.2-1.5 ml DNA/RNA Later. Preserved filters will be stored at room temperature out of direct sunlight.

“Passive sampling”: Gauze samples will be obtained by the towing of a metaprobe (Maiello *et al.* 2022). The start point of each tow will be the sampling station for each water sample (above). Gauze rolls will be preserved in approx. 150ml 99% ethanol and stored at room temperature out of direct sunlight.

Transportation of all samples will be within a cool box with ice packs. Full sampling protocols can be provided to the successful contractor upon award.

Tenderers are required to provide a full method statement for all lab analysis.

Extraction

In your response, please outline the methods you intend to use to extract and analyse the DNA. Protocols used must be open source. Gauze extraction protocols are required to follow those outlined within Maiello *et al.* 2022.

Please include details on the measures taken to avoid sample contamination in the lab.

Use of a positive control (Venison, *Cervidae sp*.) is required, to be supplied by the successful lab contractor, to evaluate the performance of the primer sets with DNA samples from known species.

PCR & Primers

The following primers are required for metabarcoding analysis for marine mammals;

1. MarVer1: Valsecchi, *et al.* (2020).
2. MarVer3: Valsecchi, *et al.* (2020).
3. MiMammal: Ushio *et al.* (2017).
4. MiFish: Miya *et al.* (2015).

Sequencing, data analysis and reference libraries

Only publicly available reference libraries to be used.

If a sequence is unable to be matched to a species, then it should be assigned it to the lowest possible taxonomic rank (for example, genus or family).

Outputs: Reporting

Two final reports are required as outputs from this project.

* A sampling comparison report, reviewing the data from both “active” and “passive” sampling techniques.
* A survey report, detailing the animals detected and discussing the efficiency of each metabarcoding primer for their detection.

Each final report must contain the following sections (further details below);

* Executive summary
* Introduction
* Methods
* Results
* Discussion

The methods section of the final report should include detailed methodology. Suggested sections are in the table below:

|  |  |
| --- | --- |
| Sampling  | State how samples were collected, number of samples and locations of sampling. State how sites were selected (NE to provide), and the dates and times of sample collection. State the volume of material sampled, and storage and processing of samples prior to DNA extraction.  |
| DNA extraction methods  | State any kits if used. State how the DNA was quantified and discuss the quality of DNA extracted. Discuss how sample contamination was controlled for and avoided.  |
| PCR amplification  | Specify the primers, PCR cycle conditions, reagents, volumes, and number of replicates per sample used. Contractors may only reference another publication without providing these details if the protocol is followed as written, including the same primers, PCR cycle conditions, reagents, volumes, and number of replicates per sample. Describe the indexing process. Describe the positive and negative controls used, and whether these behaved as expected.  |
| Sequencing  | State how the DNA products were prepared for sequencing including reagents, primers and conditions. State how the DNA was quantified, and the model of the sequencing machine used. The methods should allow the reader to understand confidence in the sequences obtained.   |
| Bioinformatic processing  | State in detail how the bioinformatic processing was completed, by specifying the steps taken, must be from open access pipelines. State any programs and models that were used and any thresholds set. Where sequences are being used for taxonomic assignment, explain the methods used to assign a species and why any reads may have been discarded. State all cut-off thresholds and state whether OTUs or ASVs are used.  |
| Reference Libraries  | Name all reference libraries used, and any rules used. State all cut-off thresholds, such as for % identity for taxonomic assignment.    |
| QA  | Explain the QA checks that have been undertaken on the results, including thresholds that may have been set.  |

The contractor should provide a detailed results section, explaining the results generated, which should include:

* The efficiency of DNA extraction and correct amplification of expected PCR products at each stage.
* Number of sequencing reads generated, quality of sequencing reads, (which should be assessed using [FastQC](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) or similar software e.g. MultiQC), proportion of reads discarded and proportion which were assigned to species.
* Full list of species identified.
* Visualizations of marine mammal species detections by primer set.
* Statistical analysis of the data to determine the most efficient survey methodology (Sampling comparison report only).
* Statistical analysis of the data to determine the most efficient metabarcoding primer (Survey report only).

The contractor should include a detailed discussion explaining the results and confidence levels in the bioinformatic processing.  Discussion should include:

* A discussion of any problems and how they were resolved, such as issues with PCR leading to a change of reagents or amplification conditions.
* Quality of the sequencing reads obtained
* Discussion of the pros and cons of the primers and barcode(s) used. Has the expected species diversity been detected? Have non-target taxa been detected? Is species bias expected? Can the assay distinguish between closely related species?
* Discussion of any unexpected species detections.  If any non-native or unexpected species have been detected, the confidence in this result.
* Discussion of any reference database or barcode issues which may have led to sequences being wrongly assigned or not assigned to species level.
* Discussion of whether the data can give information on species abundance or not.
* Discussion of the effectiveness of passive sampling techniques.
* Recommendations for further study, based on the results of the current study.

Reports to be provided as electronic copies in MS Word.

NE will publish this report in accordance with our Technical Publication Guidance. Please refer to available guidance for writing Natural England Technical publications (<http://publications.naturalengland.org.uk/publication/5790636781600768>)

There may be an opportunity to publish findings through a manuscript for a relevant scientific journal. If this is of interest to the awarded party the production of which will be possible through the Open Government Licence <https://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/> and in partnership with Natural England.

Outputs: Data

The follow data sets are required as outputs of a metabarcoding project:

* A Microsoft Excel spreadsheet listing the species identified, the sample location if applicable and the reference library and sequence ID used to identify the species. If a species was not assigned, the sequence should be identified to the lowest possible taxonomic level, e.g. genus or family.
* All raw sequence data generated in the FASTQ format. The sequences obtained should be post sequence cleaning, but prior to more detailed bioinformatic processing. Two FASTQ files (with the extensions R1.fastq and R2.fastq) per sample should be provided, i.e. demultiplexed data.
* Associated metadata (template sheet provided by NE)

Data ownership: Natural England will own all data generated in the project.

Outputs: Physical samples

Samples provided should be stored appropriately for 18 months to allow the opportunity for re-analysis.  Extracted DNA should be stored in solution at –80°C, or dried and stored at room temperature.

References

Maiello, G., Talarico, L., Carpentieri, P., De Angelis, F., Franceschini, S., Harper, L.R., Neave, E.F., Rickards, O., Sbrana, A., Shum, P., Veltre, V., Mariani, S., Russo, T. (2022) Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring. Fisheries Research, Vol 249, ISSN 0165-7836, doi: j.fishres.2022.106259.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society Open Science, 2(7). doi: 10.1098/RSOS.150088

Ushio M, Fukuda H, Inoue T, Makoto K, Kishida O, Sato K, Murata K, Nikaido M, Sado T, Sato Y, Takeshita M, Iwasaki W, Yamanaka H, Kondoh M, & Miya M. (2017) Environmental DNA enables detection of terrestrial mammals from forest pond water. Mol Ecol Resour. 17(6). doi: 10.1111/1755-0998.12690

Valsecchi, E., Bylemans, J., Goodman, S. J., Lombardi, R., Carr, I., Castellano, L., Galimberti, A., & Galli, P. (2020). Novel universal primers for metabarcoding environmental DNA surveys of marine mammals and other marine vertebrates. Environmental DNA. doi: 10.1002/edn3.72

Sustainability

Natural England protects and improves the environment and is committed to reducing the sustainability impacts of its activities directly and through its supply chains. We expect the Contractor to share this commitment and adopt a sound, proactive sustainable approach in keeping with the 25 year environmental plan/our commitments compliant with all applicable legislation. This includes understanding and reducing direct and indirect sustainability impacts and realising opportunities, including but not restricted to; resilience to climate change, reducing greenhouse gas emissions, water use and quality, biosecurity, resource efficiency and waste, reducing the risk of pollution, biodiversity, modern slavery and equality, diversity & inclusion, negative community impacts.

As a delivery partner, the successful contractor is expected to pursue sustainability in their operations, thereby ensuring the Contracting Authority is not contracting with a supplier whose operational outputs run contrary to the Contracting Authority’s objectives. The successful contractor will need to approach the project with a focus on the entire life cycle of the project.

Outputs and Contract Management

The following table has been provided to outline to expected project timelines for milestones and deliverables.

|  |  |  |  |
| --- | --- | --- | --- |
| Reference | Deliverable | Responsible Party | Date of completion |
| A. Milestone 1 | Start-Up meeting | All | Within 2 weeks of contract signing |
| B. Milestone 2 | Sample provision | Natural England | By end of October 2024 |
| C. Deliverable 1 | Deliverables sent (draft reports with FASTQ and Excel files) | Contractor | 17/02/25 |
| D. | Draft report review | Natural England | 10-14/02/25 |
| E. | Comments sent to Contractor | Natural England | 14/02/24 |
| F. | Contractor report updated | Contractor | 17-21/02/25 |
| G. Deliverable 2 | Final report sent | Contractor | 21/02/25 |
| H. | Final report review | Natural England | 24-27/02/25 |
| I. Milestone 3 | Final report signed off | Natural England | 28/02/25 |
| J. Milestone 4 | Contract completion date | All | 28/02/25 |

Fortnightly progress calls are expected for the duration of the contract.

Payment will be made in full on project completion.