Exploring intertidal sea anemones *(Actinia equina)* as natural eDNA samplers for coastal biodiversity assessment

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Abstract

Biodiversity in coastal marine environments is under unprecedented threat from anthropogenic impacts, calling for cost-effective and expedient survey methods. The analysis of environmental DNA (eDNA) – typically obtained through artificially filtered water samples – can paint a detailed picture of fish diversity in marine coastal environments. More recently, the analysis of natural sampler DNA (nsDNA, based on filter-feeding invertebrates that naturally trap eDNA in their tissues) has emerged as a potential alternative to water filtering. In this study, we investigate the widely distributed beadlet anemone (*Actinia equina*) as a potential natural eDNA sampler. Anemone samples were collected from two coastal locations in the United Kingdom: Rhosneigr – Anglesey, North Wales, and New Brighton – Wirral, North-western England. Sampling took place over two different months, and samples were compared to concomitantly sampled water. DNA metabarcoding via 12S Tele02 fish-specific primers revealed successful detection of a range of fish and other vertebrate species. We observed differences in species detected between conventional eDNA and sea anemone nsDNA samples, as well as a significant difference in seasonality detected through nsDNA. Our results indicate that the beadlet anemone can be a successful natural eDNA sampler, but that its value is more likely to reside in its complementarity alongside established eDNA methods.

Keywords: Metabarcoding, intertidal environments, Environmental DNA, Natural sampler DNA, beadlet anemone, coastal fishes, Irish Sea

1.0 Introduction

The escalating severity of the global biodiversity crisis affecting terrestrial and aquatic life should be at the forefront of conservation biology (Sutherland et al. 2023). Coastal environments are vibrant hotspots of biodiversity that play a pivotal role in securing a range of important ecosystem services (Jones et al. 2020). These habitats are being increasingly threatened by a variety of stressors - including urbanisation, pollution and climate change, underscoring the urgent need to enhance and refine methods of monitoring and measuring biodiversity changes and their impacts on ecosystem functions and services.

In coastal ecosystems, observational methods, such as underwater visual surveys or baited remote underwater videos, and capture-based netting and trapping are used widely to estimate fish biodiversity (Jovanovic et al. 2007). These techniques can be time consuming, expensive, often inaccurate, and sometimes destructive (Baker et al. 2016). Recent advances in high throughput, non-invasive molecular methods, particularly environmental DNA (eDNA) metabarcoding, hold substantial promise for enhancing the precision and scope of biodiversity surveys (Aglieri et al. 2021). At present, aquatic eDNA is typically obtained through passing water through an artificial filter with the aid of a range of manual or automated water pumping devices. As the field of eDNA metabarcoding advances, more cost-effective and accessible sampling methods are emerging, such as passive sampling techniques (Bessey et al. 2021), which utilise various artificial materials and objects to trap and accumulate eDNA from the surrounding environment.

Natural eDNA samplers present a compelling alternative to artificial filters, offering a more elegant avenue to the retrieval of eDNA fragments. The scope of natural eDNA samplers is extensive, spanning from natural substrates such as cobbles (Shum et al. 2019) and spider-webs (Gregorič et al., 2022) to the gut contents of aquatic generalist feeders (Siegenthaler et al. 2019), and extending to sponges (Porifera), the taxon that best epitomises aquatic filter-feeding (Mariani et al. 2019). These organisms prompted further exploration of eDNA sampling properties in other aquatic invertebrates, such as mussels (Weber at al. 2022).

In this paper we explore whether sea anemones are viable natural eDNA samplers for detection of fish biodiversity in benthic intertidal habitats. Anemones are abundant and distributed in both deep oceans and coastal zones globally (Steinberg et al. 2020). Here we focus on the common and widespread suspension feeding beadlet anemone (*Actinia equina*), a common species distributed along the coasts of the United Kingdom, Western Europe and

much of the East Atlantic (Davenport et al. 2011). In order to conduct this research, a small number of anemones were sacrificed. However, this organism is abundant, and not of conservation concern (Kipson et al. 2015). Further, the scientific use of a small number of these organisms is of negligible impact when compared to the habitat damage and animal fatalities associated with established marine surveying techniques, such as the use of nets, traps, dredges and grabs. *A. equina* are generalist feeders and opportunistic omnivores (Davenport et al., 2011), and their prevalence in intertidal zones makes them readily accessible for eDNA metabarcoding analysis. As sedentary organisms (although capable of moving slowly), species detections through nsDNA should reflect the sampling environment.

To test whether *A. equina* can be an effective natural eDNA sampler, we used a fish specific primer pair. To broaden our understanding of taxa detected by the anemones, we used a conventional water eDNA approach as a reference point. As we extracted DNA from the whole body of the anemone (including the gut), using a fish specific primer enabled us to focus on taxa that are unlikely to be primary targets of the anemone's diet, making the nsDNA detections most comparable to aqueous eDNA data for vertebrate biodiversity monitoring. In this context, it is important to consider the digestion time of the anemones and the influence this might have on eDNA degradation. This is likely to be in the same order of magnitude as the known degradation time of eDNA in seawater, which is between 24 and 72 hours (Collins et al. 2018): Kruger & Griffiths (1997) report a gut retention time in *A. equina* between 12 and 23 hours, when feeding on planktonic crustaceans, with longer digestion times for of 40-60 hours in the case of shelled prey (Shick et al. 1991). These digestion times indicate that aqueous eDNA and anemone nsDNA approaches are comparable, allowing us to consider the merits of these candidate natural eDNA samplers in the context of coastal biodiversity assessments.

2.0 Materials and Methods

2.1 Field Collection

In an initial experiment, six beadlet anemones were collected in May 2022 from rockpools at New Brighton, Wirral (Figure 1B). Subsequently, ten anemones and 3L of water samples were collected in October 2022 from both New Brighton and Rhosneigr, Anglesey, North Wales (Figure 1C). Rhosneigr is an exposed rocky coastal site, whilst the rockpools at New Brighton are situated under man-made concrete groynes (Fig. 1) on a sandy beach.

To monitor contamination at each site, purified water was filtered and used as a field blanks, which were treated identically to other samples throughout the collection and extraction process. Anemones were collected using sterile gloves and stored separately in 100% ethanol and placed in a cooler on ice in the field. The three 1-litre water samples (collected from the same rockpool as the sampled anemones) were pushed through 0.45 μm Sterivex filters, with each filter placed inside two sterile bags, and immediately stored on ice. All samples were stored at -20°C in the lab until further processing.

2.2 Laboratory procedures

DNA extraction from Sterivex filters followed the mu-DNA protocol for water, while anemone DNA extraction followed the mu-DNA extraction protocol for tissue (Sellers et al. 2018). For full procedures see Supplementary Material S1.1 and S1.2. To extract DNA from beadlet anemones, sections from the entire organism (including stomach, tissue, and tentacles) were dried by blotting the ethanol used for preservation. The dried material was then cut into small pieces, and 500mg of the resulting dry weight from the whole organism's tissue was used for DNA extraction.

PCR amplification was performed in triplicate for each sample, using the Tele02 fish-specific primers, which target a ~167 bp fragment of the 12S rRNA mitochondrial region (Taberlet et al. 2018). Primer pairs were uniquely indexed to enable demultiplexing for downstream bioinformatic analysis. Iridescent shark catfish (*Pangasianodon hypophthalmus*) was used as positive control. PCR amplicons were pooled in a single library and sequenced on an Illumina iSeq100 using v2 150x2 chemistry. Further details on PCR conditions and library preparation can be found in Supplementary Material S1.3.

2.4 Bioinformatics and Downstream Analysis

Bioinformatic analysis followed the OBITOOLS pipeline. For full protocol see Supplementary material S1.4. Taxonomic assignments were validated by cross-checking non-native and unexpected taxa by manual BLAST against the nucleotide GenBank database. The package DECONTAM in R was used on the raw MOTU output. MOTUs were filtered by removing those that did not reach a sequence identity match of at least 97%.

A Venn diagram was used to visualise fish MOTUs in nsDNA samples from May and October to portray seasonal variation. Samples were grouped per site and nsDNA/eDNA

capture method for October only, and then visualised using a stacked bar chart of proportion percentage per class (fish and birds). To visualise the species proportions, a bubble plot was used for fish species only. Proportions were calculated using the number of sequencing reads of each species/MOTU per sample divided by the total sample reads; this represents the proportional read counts per species and serves as a proxy for relative abundance.

To visualise temporal differences (May vs. October) between anemone nsDNA samples from New Brighton, we used nonmetric multidimensional scaling (NMDS) based on Jaccard distances using presence/absence data. These differences were tested using permutational multivariate analysis of variance (PERMANOVA, 999 permutations) on binary pairwise distance matrices using the function *adonis* in VEGAN (Oksanen et al. 2013). PERMANOVA was also used to test for differences between the two sampling sites in October. Finally, to identify whether there were any species significantly associated with certain months, we used an indicator species analysis in R using the INDICSPECIES package, after Hellinger-transforming read count data.

3.0 Results

Twenty-eight samples from October (Supplementary Table S1) made up one third of an iSeq100 run, yielding 602,127 reads, which after the first filtering step (>97% identity match) were reduced to 535,263 (88.9%). The eight samples from May (Supplementary Table S2) yielded 379,994 reads from an iSeq100 run, of which 374,432 (98.5%) were retained for downstream analyses. Although no contamination was found in the controls, three anemone samples were removed as they contained no sequencing reads. See Supplementary Material S1.5.

We identified 17 fish species across anemone nsDNA and water eDNA from October 2022 samples (Figure 2). Although the DNA marker used is primarily designed to detect vertebrates, *Actinia equina* was also detected, albeit with only 115 reads across all samples, as were five distinct bird species: Spotted sandpiper *Actitis macularius*, Ruddy turnstone *Arenaria interpres*, Rock dove *Columba livia*, European herring gull *Larus argentatus*, and Common redshank *Tringa totanus.*

Higher diversity of fish was detected in New Brighton (Fig. 2); however, no significant difference in community composition was detected between the two sites (PERMANOVA

pseudo-F=1.0165, df= 1.0, p= 0.393). There was no significant difference detected in community composition between nsDNA and eDNA in both locations in October (PERMANOVA pseudo-F=0.5629, df= 1.0, p=0.19) (supplementary figure S3). Three vertebrate species were statistically more abundant in eDNA samples*:* common goby *Pomatoschistus microps* (*p*=0.0063), European eel *Anguilla anguilla* (*p*=0.0167) and common redshank *Tringa totanus* (*p*=0.0110).

Only three taxa (12.5%) were shared between May and October samples (Fig. 3). Six MOTUs (25%) were detected only in May, while 15 (62.5%) were found only in October. This results in a strong separation of these temporal samples (Fig. 3A), which is also supported statistically (PERMANOVA F= 5.8614, df= 1.0 *p*= 0.001). Indicator species analysis shows significant abundances in May for shanny *Lipophrys pholis* (stat = 0.733, p=0.004), and Common sole *Solea solea* (stat = 0.382, p=0.0358) and significantly higher abundance in October for megrim *Lepidorhombus wiffiagonis* (stat =0.676, p=0.0056), Common goby (stat = 0.573, p=0.0434), and ruddy turnstone (stat = 0.676, p= 0.0056).

4.0 Discussion

Easily accessible, sessile, filter-feeding invertebrates make ideal candidates for coastal nsDNA applications, especially if, as in the case of the beadlet anemone, they are widely distributed. With this first attempt to evaluate the role of sea anemones as natural eDNA samplers to aid fish biodiversity assessments, we begin to understand the extent and circumstances in which such an approach may be beneficial. Wells et al. (2021) amplified DNA extracts of gut content from the giant plumose anemone, *Metridium farcimen*, targeting the mitochondrial *COI* region to investigate the diet of the organism. While the use of the *COI* primer pair enabled successful identification of the organisms on which the anemone fed, it also allowed detection of several fish species, indicating that anemones may have potential as successful natural eDNA samplers, assessing biodiversity beyond the organisms that they select for consumption.

Our study demonstrates the effective use of *A. equina* nsDNA in detecting fish species representative of their environment. We found no significant difference in species detected between nsDNA and eDNA, suggesting their similar efficiency; however, this could be due to the relatively small sample size, hence further studies comparing intertidal nsDNA and eDNA should be conducted to establish whether consistent, ecologically relevant differences

exist between these capture methods. We find that while nsDNA can identify a subset of vertebrate species that are also detected by conventional eDNA capture methods, it uniquely identifies vertebrate species that have a minimal presence in aqueous eDNA samples, such as the shorebirds ruddy turnstone (*Arenaria interpres)* and spotted sandpiper (*Actitis macularius)*. These species are closely associated with intertidal zones, with *A. interpre*s known to feed on molluscs, crustaceans, and small invertebrates (Kendall et al. 2004). We speculate that a high proportion of shorebird reads results from predation attempts on beadlet anemones or their mere exposure to wading birds or their guano at low tide, resulting in the anemone nsDNA. This would indicate that in some circumstances this approach could be more effective than conventional eDNA filtration at monitoring rare and endangered coastal shorebirds. The inclusion of non-target species enriches this study and suggests that forthcoming conservation research could employ bird-specific primers to strengthen these findings.

Our study therefore underscores the importance of utilising both nsDNA and eDNA techniques in biodiversity assessments. Relying solely on either method would have resulted in several vertebrate species going undetected. Monitoring seasonal changes in fish assemblages in coastal environments via traditional methods is challenging, but eDNA has been shown to serve this purpose (Sigsgaard et al. 2017). Here, we found a strong temporal signal in anemone nsDNA between May and October, which indicates its sensitivity to detect seasonal variations in vertebrate communities. In cold-temperate intertidal habitats, there is a greater diversity at the end of summer rather than the spring (Jovanovic et al. 2007), with fluctuations in diversity driven by migration and spawning (Connor et al. 2019). Anemone nsDNA between these two months demonstrated strongly divergent patterns of read proportions in some species. In May, shanny (*L. pholis*) and common sole (*S. solea*) contained a higher percentage of reads than in October. This potentially reflects the spawning time of these species (Shackley et al. 1977; Armstrong et al. 2001), thus reinforcing the argument that anemone nsDNA can detect environmental seasonal changes.

With this study being an initial step in exploring anemones as natural eDNA samplers, further research is necessary to optimise laboratory methods, explore variation in nsDNA collection between anemone species, and investigate the influence of feeding behaviour on nsDNA recovery. We also encourage further research to investigate less invasive techniques of obtaining eDNA such as biopsies or swabs to minimise the impact on these invertebrates.

This is a compelling introduction of a new phylum to the field of eDNA and nsDNA analysis and reinforces the potential of this technique, at least in conjunction with conventional eDNA methods, to obtain a more comprehensive picture of species diversity in intertidal environments.

Acknowledgments

The authors would like to thank the faculty of Biological and Environmental Sciences and UKRI-NERC grant NE/T007028/1 for co-funding this project. We also thank Kirstie Scott for her support and advice, and the handling editor and reviewers for their helpful comments that improved the manuscript

6.0 References

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331 **Figure 1** Sampling locations (A). Concrete groynes at New Brighton, Wirral (B) Rocky
332 coastline at Rhosneigr, Anglesey (C).
333

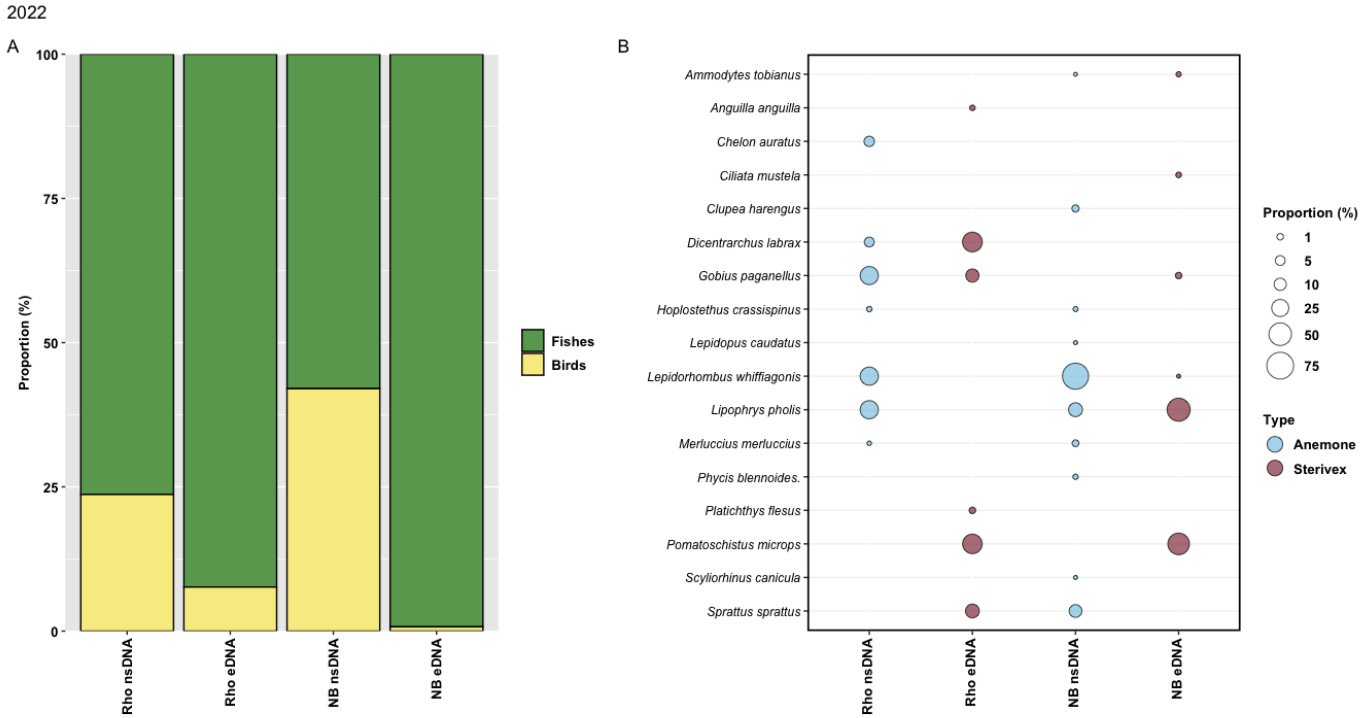

Figure 2: A Stacked bar chart showing percent proportions for fish and birds between nsDNA and eDNA between the two sites in October only. Bubble plot showing the percent proportions per species per sampling site between nsDNA and eDNA for October only.

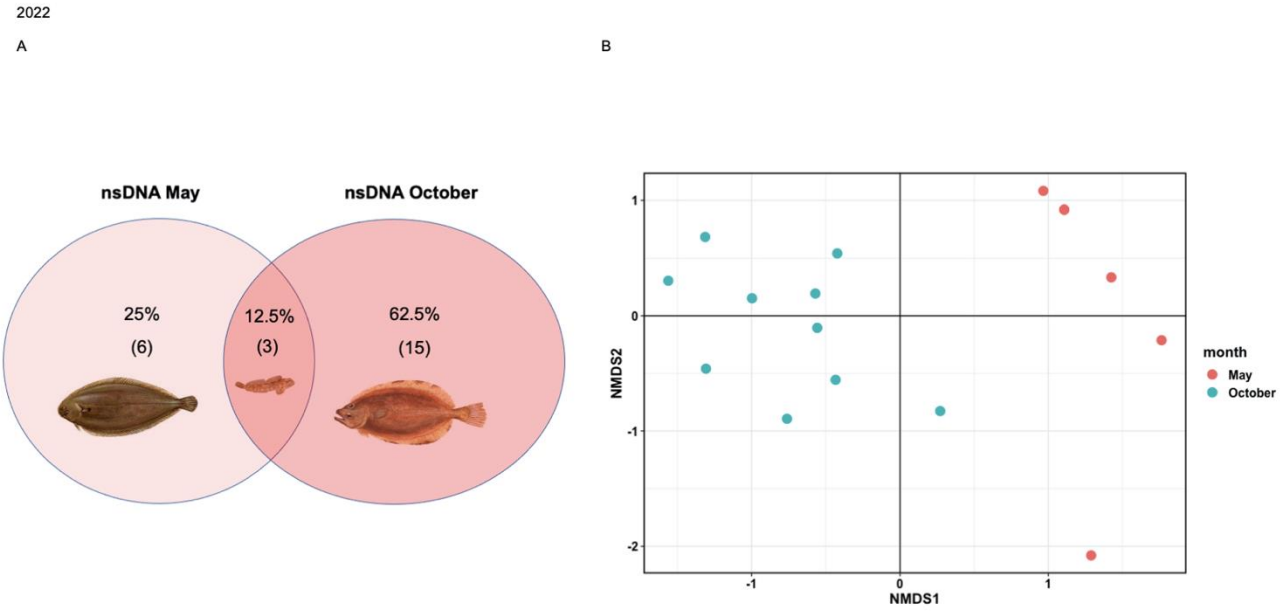

Figure 3: Venn diagram representing the degree of overlap, in terms of nsDNA Operational Taxonomic Units (OTUs), between May and October samples from New Brighton only (A). Pictures show species of fish with the highest read count: May (*Solea solea*), shared (*Lipophrys pholis*) and October (*Lepidorhombus whiffiagonis*). NMDS showing nsDNA samples collected from New Brighton in May and October based on Jaccard distances using binary presence/absence data (B).