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

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### Article

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

49

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

62

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

70

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

76 much of the East Atlantic (Davenport et al. 2011). In order to conduct this research, a small  
77 number of anemones were sacrificed. However, this organism is abundant, and not of  
78 conservation concern (Kipson et al. 2015). Further, the scientific use of a small number of  
79 these organisms is of negligible impact when compared to the habitat damage and animal  
80 fatalities associated with established marine surveying techniques, such as the use of nets,  
81 traps, dredges and grabs. *A. equina* are generalist feeders and opportunistic omnivores  
82 (Davenport et al., 2011), and their prevalence in intertidal zones makes them readily  
83 accessible for eDNA metabarcoding analysis. As sedentary organisms (although capable of  
84 moving slowly), species detections through nsDNA should reflect the sampling environment.  
85

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

101

## 102 **2.0 Materials and Methods**

### 103 **2.1 Field Collection**

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

109

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

117

## 118 **2.2 Laboratory procedures**

119 DNA extraction from Sterivex filters followed the mu-DNA protocol for water, while  
120 anemone DNA extraction followed the mu-DNA extraction protocol for tissue (Sellers et al.  
121 2018). For full procedures see Supplementary Material S1.1 and S1.2. To extract DNA from  
122 beadlet anemones, sections from the entire organism (including stomach, tissue, and  
123 tentacles) were dried by blotting the ethanol used for preservation. The dried material was  
124 then cut into small pieces, and 500mg of the resulting dry weight from the whole organism's  
125 tissue was used for DNA extraction.

126

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

134

## 135 **2.4 Bioinformatics and Downstream Analysis**

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

141

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

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

149

150 To visualise temporal differences (May vs. October) between anemone nsDNA samples from  
151 New Brighton, we used nonmetric multidimensional scaling (NMDS) based on Jaccard  
152 distances using presence/absence data. These differences were tested using permutational  
153 multivariate analysis of variance (PERMANOVA, 999 permutations) on binary pairwise  
154 distance matrices using the function *adonis* in VEGAN (Oksanen et al. 2013).

155 PERMANOVA was also used to test for differences between the two sampling sites in  
156 October. Finally, to identify whether there were any species significantly associated with  
157 certain months, we used an indicator species analysis in R using the INDICSPECIES  
158 package, after Hellinger-transforming read count data.

159

### 160 **3.0 Results**

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

168

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

175

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

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

184  
185 Only three taxa (12.5%) were shared between May and October samples (Fig. 3). Six  
186 MOTUs (25%) were detected only in May, while 15 (62.5%) were found only in October.  
187 This results in a strong separation of these temporal samples (Fig. 3A), which is also  
188 supported statistically (PERMANOVA  $F= 5.8614$ ,  $df= 1.0$   $p= 0.001$ ). Indicator species  
189 analysis shows significant abundances in May for shanny *Lipophrys pholis* (stat = 0.733,  
190  $p=0.004$ ), and Common sole *Solea solea* (stat = 0.382,  $p=0.0358$ ) and significantly higher  
191 abundance in October for megrim *Lepidorhombus wiffiagonis* (stat =0.676,  $p=0.0056$ ),  
192 Common goby (stat = 0.573,  $p=0.0434$ ), and ruddy turnstone (stat = 0.676,  $p= 0.0056$ ).

193

#### 194 **4.0 Discussion**

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

206

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

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

225

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

240

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



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

250

## 251 **Acknowledgments**

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255 that improved the manuscript

256

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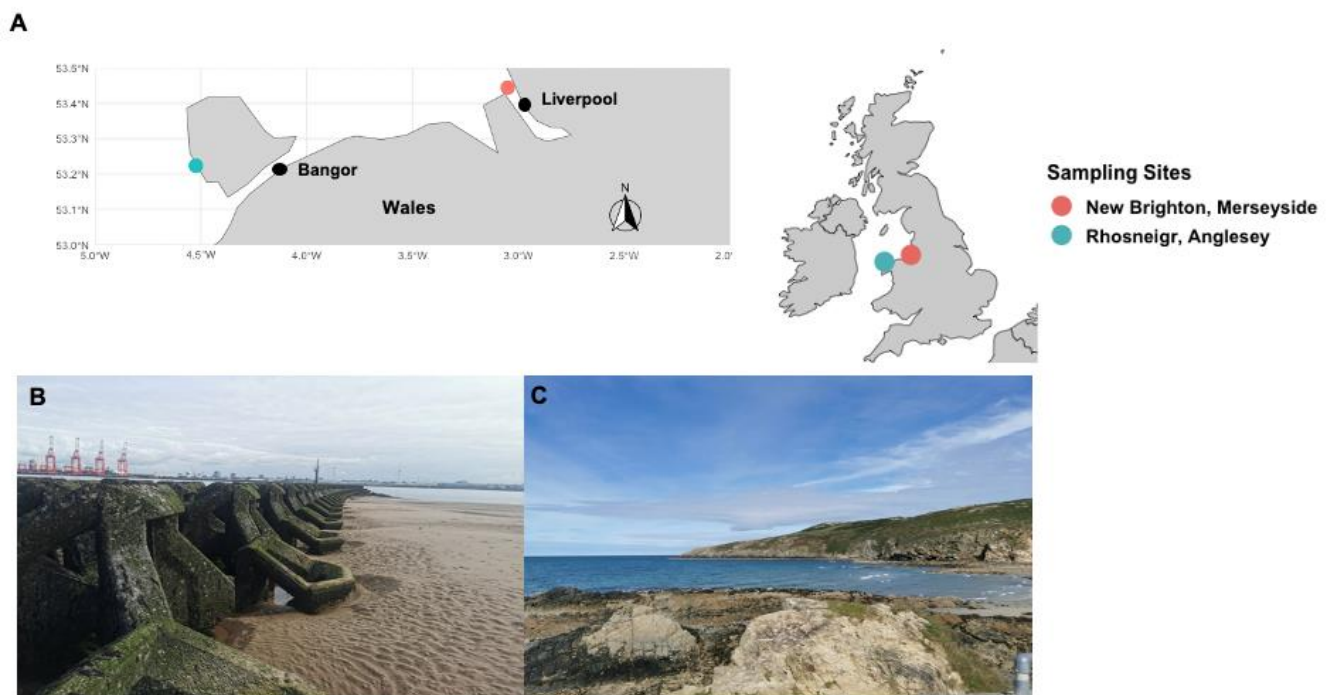
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## Anemones as eDNA samplers

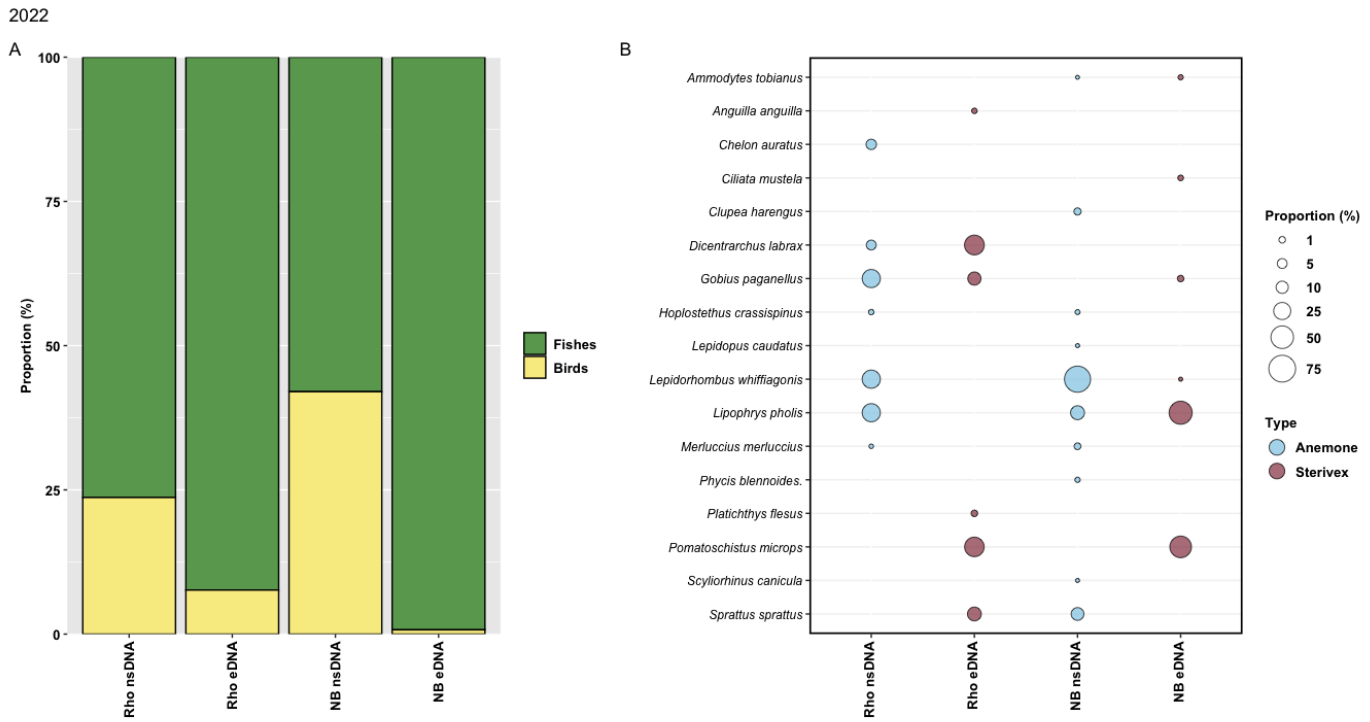
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328 insights into the diverse diet of a dominant suspension feeder, the giant plumose  
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330

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

334



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336

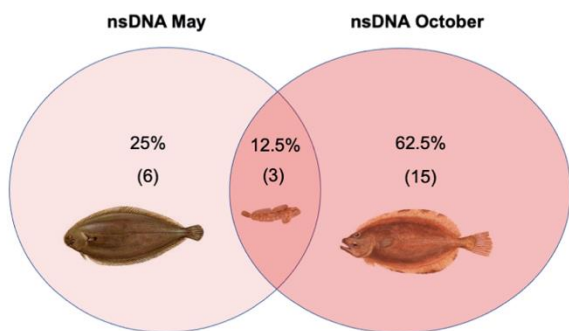
337 **Figure 2:** A Stacked bar chart showing percent proportions for fish and birds between  
 338 nsDNA and eDNA between the two sites in October only. Bubble plot showing the percent  
 339 proportions per species per sampling site between nsDNA and eDNA for October only.

340

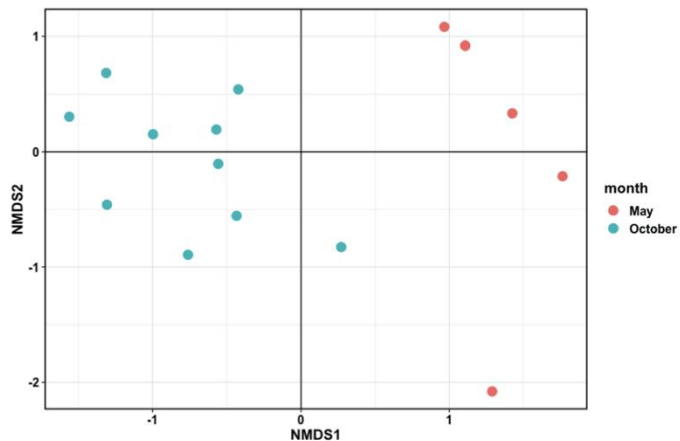
341

2022

A



B



342

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

349

350