

## **Text S1. Supplementary Methods and Materials.**

### **Text S1.1. eDNA Sterivex filters**

DNA extraction from Sterivex filters was based on the mu-DNA protocol by Sellers et al. (2018). Firstly, the eDNA filters were removed using pincer pliers and the filter paper removed using forceps. Filters were cut up into small, ~5mm pieces and half of the filter was used in DNA extraction with the other half stored in a -20°C freezer. 750µL Lysis solution, 250µL lysis additive, 20ul Proteinase K (concentration 100µg/ml) per sample were combined in a 1.5ml Eppendorf tube to make the lysis master mix, which was added to each sample. These were placed on a thermomixer for 12 hours at 55°C at 650rpm and then centrifuged at 10,000 x g for 1 minute at room temperature. The supernatant was transferred to new tubes and 0.3x volume flocculant solution per sample added and then placed on ice for 10-30 minutes. Samples were then centrifuged at 10,000 x g for 1 minute and the supernatant transferred to 2ml Eppendorf tubes. 2x volume of the tissue binding buffer was added and vortexed. The sample was then transferred to a spin column and centrifuged at 10,000 x g for 1 minute. This step was repeated until the entire volume had passed through the filter. 500µL of wash solution was added to each sample and centrifuged. This was repeated twice. 100µL of elution buffer was added directly to the spin column which was then incubated at room temperature for 5 minutes. The sample was then centrifuged 10,000 x g for 1 minute and the supernatant retained.

### **Text S1.2. Anemone nsDNA**

Extractions were based on the mu-DNA extraction protocol by Sellers et al. (2018) and DNA isolation from marine sponges (Harper et al. 2023). Anemones were preserved in 100% ethanol and stored at -20°C prior to DNA extraction. Each anemone was cut into small ~5mm pieces with residual ethanol removed using Whatman blotting paper. Based on Harper et al (2023), 500mg of tissue was used per extraction with the remaining sample stored at -20°C. 730µL Lysis solution, 230µL tissue lysis additive, 40µL Proteinase K per sample were combined to make the lysis master mix. 1ml of this was added to each sample. This was placed on a thermomixer for 12 hours at 55°C at 650rpm. Samples were then centrifuged at 10,000 x g for 1 minute at room temperature. The supernatant was transferred to new tubes and 0.3x volume flocculant solution per sample was added and this was placed for 10-30 minutes on ice. Samples were then centrifuged 10,000 x g for 1 minute and the supernatant transferred to 2ml Eppendorf tubes. 2x volume of the tissue binding buffer was added and vortexed. The sample was then transferred to the spin columns and centrifuged. This step was repeated until the entire volume had passed through the filter. 500µL of wash solution was added to each sample and centrifuged. This was repeated twice. 100µL of elution buffer was added directly to the spin column, centrifuged and the supernatant was retained.

### **Text S1.3. PCR amplification**

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). Positive controls were put in place for each PCR batch. We used extracts of iridescent shark (*Pangasianodon hypophthalmus*) DNA at 0.05 ng/µl, choosing this organism as it is a tropical freshwater fish, unrelated to fish species in UK waters. Primer pairs were uniquely indexed to enable demultiplexing for downstream bioinformatic analysis. The reaction mix included: 10µL Mifi DNA polymerase mastermix (2x), molecular grade water 5.84µL, BSA 0.16µL, 1µL of each primer (10 µM) and 2 µL of DNA with a thermocycling profile of 95°C for 10 mins, 40 cycles of 95°C 30 seconds, 60°C 45 seconds, 72°C 30 seconds and a final extension of 72°C for 5mins. PCR products were run on 2% agarose gels stained with SYBR safe. PCR replicates were pooled and samples were purified with a 1:1 30µL Mag-Bind® TotalPure NGS magnetic beads and 30µL of pooled PCR product. The concentration of each purified PCR was quantified using Qubit 4

Fluorometer dsDNA HS assay kits and pooled in equimolar amounts. The pooled PCR product was quantified using TapeStation 4200 using the high sensitivity D1000 assay. Then, a further 1x bead clean-up was performed on the pooled PCR product. Illumina libraries were prepared using the Perkin-Elmer NEXTFLEX Rapid DNA-Seq Kit 2.0, using 1 µg as starting concentration of the pooled PCR product following the manufacturers guidelines with library amplification. The library and PhiX control were quantified using qPCR using the NEBNext® Library Quant Kit for Illumina®. The final library and PhiX control were diluted to 85pM (with PhiX 20% of the run) and loaded onto an Illumina® iSeq™ 100 Reagent v2 (300-cycle).

#### **Text S1.4. Bioinformatics and downstream analysis**

FASTQC was used to assess the quality scores of the fastq files, ILLUMINAPAIREDEND was used to align reads, and NGSFILTER was used to de-multiplex samples. We filtered the sequence lengths to 120-200bp in order to remove sequences that were not in the target base pair range by using OBIGREP. Chimeras were then removed using VSEARCH (Rognes et al. 2016). Molecular operational taxonomic unit (MOTU) clustering was implemented using SWARM with d=3 (Mahé et al. 2014). Taxonomic assignment per sample was carried out using ECOTAG with a 12S reference database which was constructed using ‘ecoPCR’ in silico against the EMBL database (Release version r143).

To visualise differences between anemone nsDNA and Sterivex eDNA for both locations in October only, we used nonmetric multidimensional scaling (NMDS) based on Jaccard distances using presence/absence data. We tested differences between the two sampling months using permutational multivariate analysis of variance (PERMANOVA, 999 permutations) on pairwise distance matrices using the function *adonis* in VEGAN (Oksanen et al., 2013). Please see Fig. S3. Total sum scaling was used to calculate the relative abundance which converts number of reads to appropriately scaled ratios (Alberdi & Gilbert 2019). The rarefaction curve was generated using the *rarefy* function from the package VEGAN to assess whether the sequencing depth was adequate for the anemone nsDNA.

#### **Text S1.5. Sequencing and sample information**

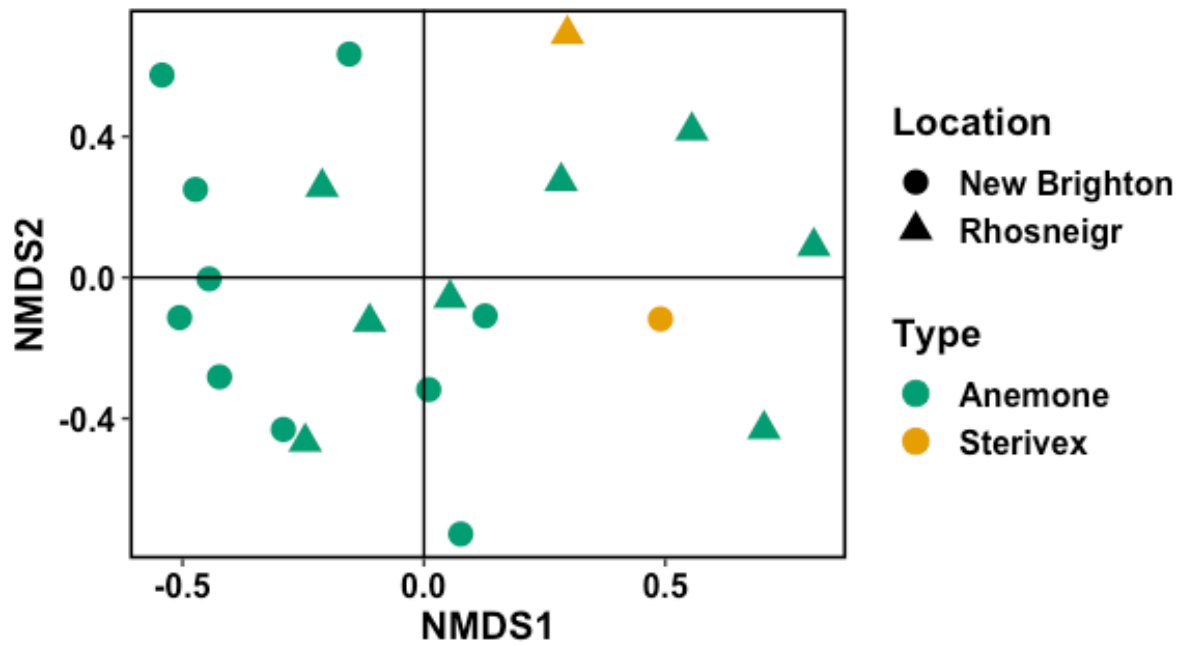
The samples for this study were pooled alongside unrelated libraries for a different eDNA project. 10 anemone samples and 1 water sample from Rhosneigr, 10 anemone samples and 1 water sample as well as 2 extraction blanks, 2 field blanks and positive control made up the 28 samples from the October library. The six anemone samples from May (plus 1 extraction blank and 1 positive control) made up the 8 samples from the May library. Please see Table S1.

**Table S1.** Number of samples for each sample type which make up two separate sequencing runs: May 2022 (8 samples total) and October 2022 (28 samples total).

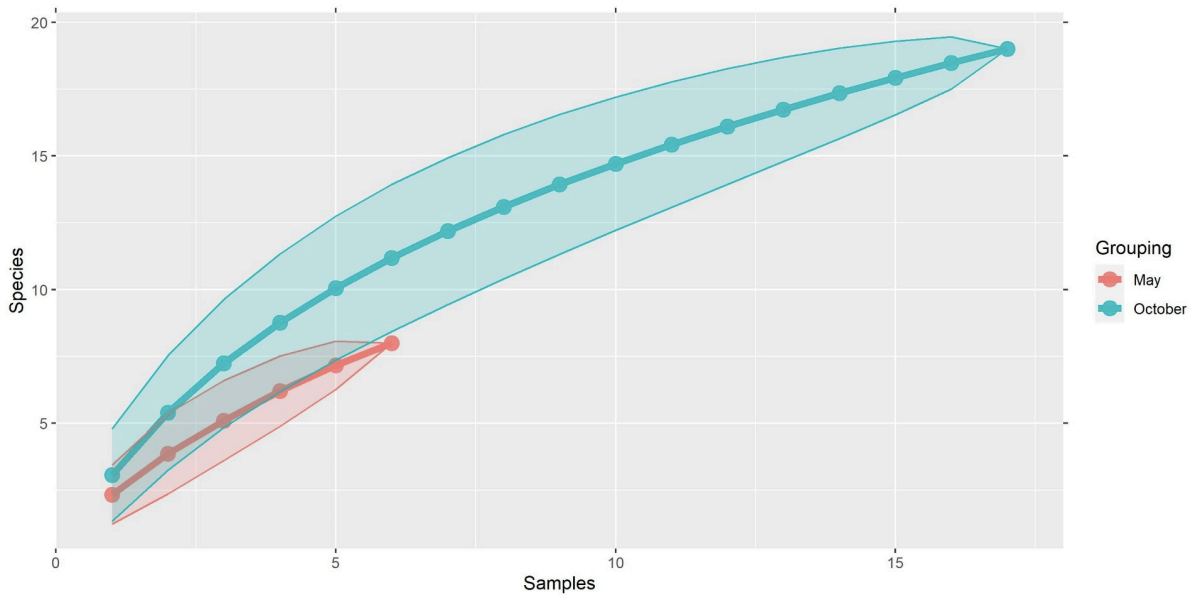
Sample Type	New Brighton		Rhosneigr
	May 2022	October 2022	October 2022
Anemone	6	10	10
Water	0	1	1
Field-blank	0	1	1
Extraction-blank	1	1	1
Positive control	1	1	1

**Table S2.** List of vertebrate species (venn diagram fig. 3A) detected in anemone nsDNA in May 2022, October 2022 or detected in both months. Only at New Brighton.

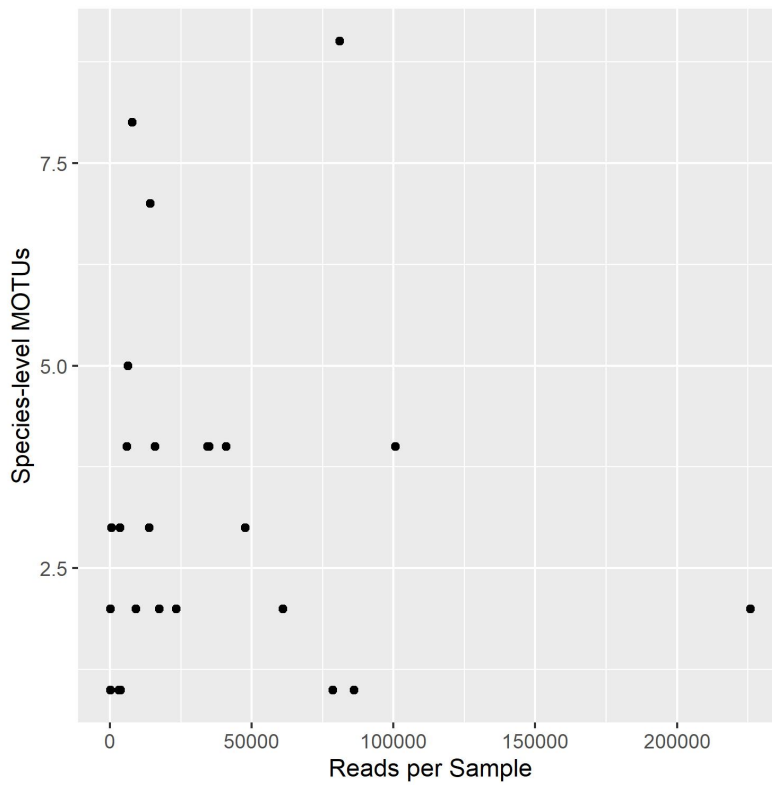
nsDNA May		Shared		nsDNA October	
Scientific name	Common name	Scientific name	Common name	Scientific name	Common name
<i>Solea solea</i>	Common sole	<i>Lipophrys pholis</i>	Shanny	<i>Arenaria interpres</i>	Ruddy turnstone
<i>Thunnus thynnus</i>	Atlantic Bluefin tuna	<i>Larus argentatus</i>	European herring gull	<i>Lepidorhombus whiffiagonis</i>	Megrim
<i>Sarda sarda</i>	Atlantic bonito	<i>Phycis blennoides</i>	Greater forkbeard	<i>Pomatoschistus microps</i>	Common goby
<i>Zeugopterus punctatus</i>	Topknot			<i>Sprattus sprattus</i>	European sprat
<i>Helicolenus dactylopterus</i>	Blackbelly rosefish			<i>Columba livia</i>	Rock dove
<i>Arnoglossus laterna</i>	Mediterranean scaldfish			<i>Clupea harengus</i>	Atlantic herring
				<i>Merluccius merluccius</i>	European hake
				<i>Lepidopus caudatus</i>	Silver scabbardfish
				<i>Scyliorhinus canicula</i>	Small-spotted catshark
				<i>Actitis macularius</i>	Spotted sandpiper
				<i>Chelon auratus</i>	Golden grey mullet
				<i>Ammodytes tobianus</i>	Lesser sand eel
				<i>Gobius paganuellus</i>	Rock goby
				<i>Phoxinus phoxinus</i>	Eurasian minnow
				<i>Salmo trutta</i>	Sea trout



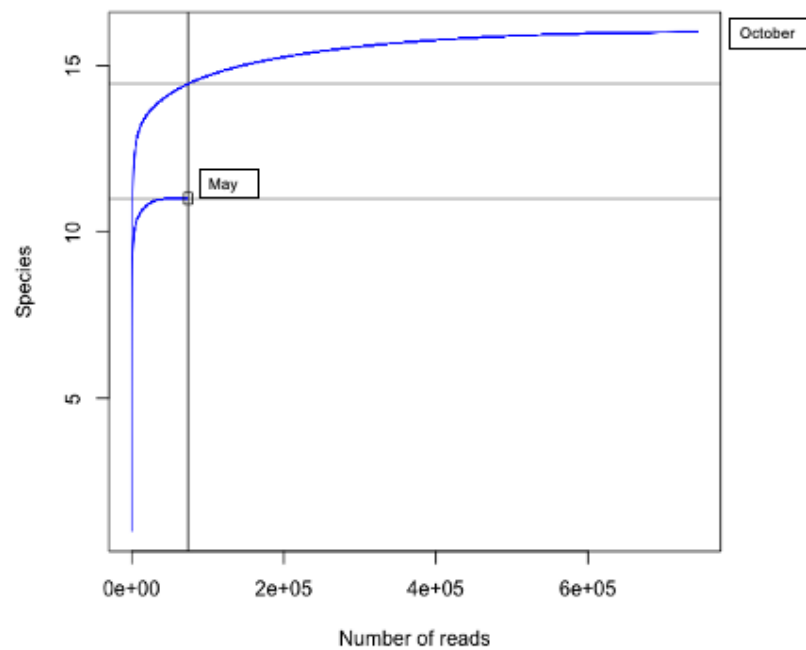
**Fig. S1.** NMDS showing nsDNA and eDNA samples collected from New Brighton and Rhosneigr in October only based on Jaccard distances using binary presence/absence data.



**Fig. S2.** Species accumulation curve showing number of species detected by anemone nsDNA only between May and October.



**Fig. S3.** Showing number of reads per sample for anemone nsDNA only between for both locations and months.



**Fig. S4.** A rarefaction curve showing the number of sequencing reads for anemone nsDNA only, between May and October.

## References

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