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Sponges as natural environmental DNA samplers

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At a time of unprecedented impacts on marine biodiversity, scientists are rapidly becoming persuaded by the potential of screening large swathes of the oceans through the retrieval, amplification and sequencing of trace DNA fragments left behind by marine organisms; an approach known as 'environmental DNA' (eDNA) [1]. In trying to circumvent the many challenges associated with water filtration and DNA isolation from environmental samples, significant investment is being made in high-tech solutions, such as automated underwater vehicles and robots [2]. Here, instead, we explored a simpler, alternative option, based on the recovery of eDNA from sponges (phylum Porifera), the planet's most effective water-filterers. We obtained sponge samples from Mediterranean and Antarctic surveys, extracted total DNA from their tissues, and obtained tens of thousands of fish DNA reads via metabarcoding, which were able to clearly distinguish samples from the two regions. One Antarctic sample yielded hundreds of reads from chinstrap penguin (*Pygoscelis antarcticus*) and Weddell seal (*Leptonychotes weddellii*). We argue that this 'natural sampler DNA' (nsDNA) approach is poised to become a powerful, affordable, universal tool for aquatic biodiversity monitoring globally.

In just a few years, eDNA metabarcoding has surged as a novel, revolutionary approach to biodiversity monitoring [3], and despite the caveats and unknowns inherent to every new method [4], overwhelming evidence indicates greater speed and efficacy in taxon detection, compared with traditional 'catch-and-see' techniques [5]. Most recently, researchers are turning to automation and robotics [2] in the attempt to streamline filtration, DNA isolation and detection, and to screen larger volumes of water, which is paramount in large ecosystems like the ocean. Nevertheless, these systems are expensive to build and run, they cannot be easily deployed in every habitat, and are often designed to monitor a narrow range of taxa.

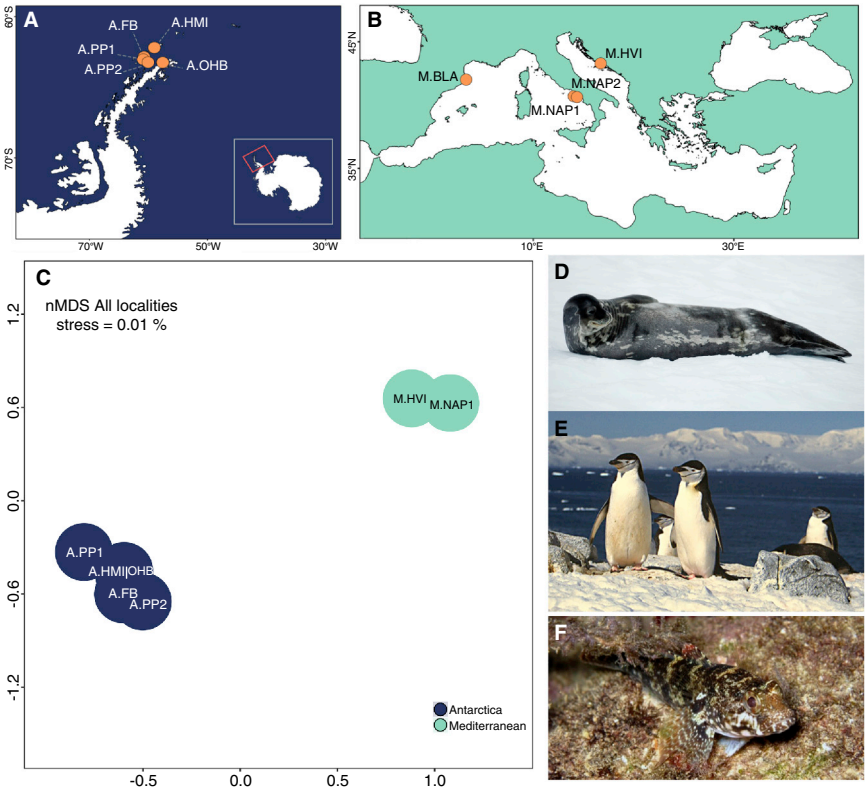

Figure 1. Beta diversity among Antarctic and Mediterranean locations, inferred through DNA sequences retrieved from sponge tissues.
Maps identifying the sample locations from (A) Antarctica and (B) the Mediterranean Sea. (C) Multidimensional scaling plot of the samples based on Jaccard's distances. Some of the organisms identified to the species level at 98% sequence identity are (D) the Weddell seal (*Leptonychotes weddellii*), and (E) chinstrap penguin (*Pygoscelis antarcticus*) from Antarctica; and (F) rock goby (*Gobius paganellus*) from the Mediterranean. Images from Wikimedia Commons: Gotdot13 (D), Gregory "Slobirdr" Smith (E), Roberto Pillon (F).

At the other end of the spectrum lies an attractive, low-tech solution, based on organisms that are naturally predisposed to filter water far more effectively than any artificial device. Sponges can sift through up to 10,000 litres of water in one day [6] — that is about 1000-fold the sampling effort normally achieved through current rosette-based sampling in marine habitats. The sponge tissue naturally traps and concentrates the particles from which environmental DNA is isolated, effectively acting as a natural filter. We hypothesized that by extracting DNA from sponge samples and subjecting it to metabarcoding for a fish-specific 12S mtDNA marker [7], we would be able to recover significant biodiversity information on the vertebrate faunal assemblage of the areas where the sponges were collected.

Five Antarctic and four Mediterranean sponge samples (Figure 1A,B and Supplemental Information) — which were originally sampled for purposes other than eDNA research [8] — were pooled alongside 63 samples from an unrelated environmental DNA project, in a 12S amplicon library, and sequenced in parallel. The nine samples yielded 246,910 reads (around 0.02% of the total run), and recovered at least 31 metazoan taxa, of which 22 could be identified at least to the Family level or below (Supplemental Information). These comprised five typically Antarctic Notothenioid species, including black rockcod (*Notothenia coriiceps*), emerald rockcod (*Trematomus bernacchii*) and Antarctic toothfish (*Dissostichus mawsoni*), as well as

common Mediterranean families, such as Carangidae, Serranidae, Clupeidae and Sparidae (Data S1). After filtering, two samples from the Mediterranean could not be retained for analysis as too few reads/MOTUs passed our filtering criteria (Table S1). In no case was sponge DNA amplified, and taxon assemblages clearly separated the two biogeographic regions (PERMANOVA F=2.28, p=0.04; Figure 1C). Although the DNA marker used has been designed to amplify fish DNA, it is known to also detect other vertebrates: the 1,951 reads of chinstrap penguin and the 344 reads belonging to Weddell seal, recovered from Half Moon Island (sample A.HMI, which is in proximity of a large chinstrap penguin breeding colony), indicate that, with some optimisation, this method has the potential to also be employed in the detection of marine mammals and sea birds of conservation concern. Interestingly, additional 'bonus' findings of the screening (especially in the Antarctic samples; Data S1) included at least seven species of sea stars (Echinodermata: Asteroidea), from two orders, of which one was identified to the species level, *Perknaster aurantiacus*, a typical inhabitant of South Atlantic polar benthic habitats.

There are some analogies between this approach and previous attempts to obtain important biodiversity data from the feeding mode of other invertebrates, such as the detection of rare mammals from DNA extracted from leeches [9], and the reconstruction of estuarine fish communities based on DNA isolated from shrimp stomach contents [10]. Yet, hematophagous invertebrates are relatively specialised and can only be employed over a narrow range of habitats and taxa — benthic scavengers, as generalist as they may be, will also exhibit some selectivity for their food sources.

On the other hand, simple, sessile, filter-feeding organisms such as sponges represent the ideal 'natural sampler' for aquatic biodiversity. Their early-splitting phylogenetic position means that their DNA is not amplified when targeting most metazoan groups, especially vertebrates; their water-filtering efficiency is unparalleled; environmental impacts associated with their collection is minimal; and their ubiquitous, sessile and regenerating nature makes them easy to sample non-lethally in virtually every aquatic habitat.

The advent of high throughput DNA-based assessment and discovery of biodiversity is revolutionising, bolstering and standardising the way we study whole ecosystems. While contributions from engineering will doubtless continue to improve marine exploration, advances in ocean monitoring and conservation will also greatly depend on the availability of large amounts of inexpensive, standardised and tractable samples from across the world.

It should be stressed that the data presented here were generated from opportunistic samples: the *Chondrosia reniformis* specimen from Spain and the *Tethya citrina* sample from Naples were only available as DNA extractions from three and six years before this study, respectively, and generated poor sequencing data, which had to be later discarded. The laboratory protocols used were devised for artificially filtered water, without any attempt to optimise procedures for sponge tissue, and still, the majority of samples returned informative biodiversity data from the sampled areas. Necessary next efforts must go towards examining the influence of sponge phenotype, sponge mass/volume excised, area sampled, and laboratory protocols on DNA yield and sequence information. Biodiversity data obtained through sponge nsDNA should then be directly compared with tried and tested eDNA data obtained from water samples. Eventually, we predict that after this phase of optimisation and validation, sponges will be well positioned to provide a practical, cost-effective, universal 'natural sampler DNA' tool for marine biodiversity studies.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, one table, one data file, experimental procedures and supplemental references, and can be found at https://doi.org/10.1016/j.cub.2019.04.031.

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AUTHOR CONTRIBUTIONS

S.M. conceived the idea for the study and wrote the manuscript; A.R. provided samples and contributed to study design; G.C. conducted lab work; C.B. contributed to study design, oversaw lab activities and analysed the data.

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