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Biofouling sponges as natural eDNA samplers for marine vertebrate biodiversity monitoring

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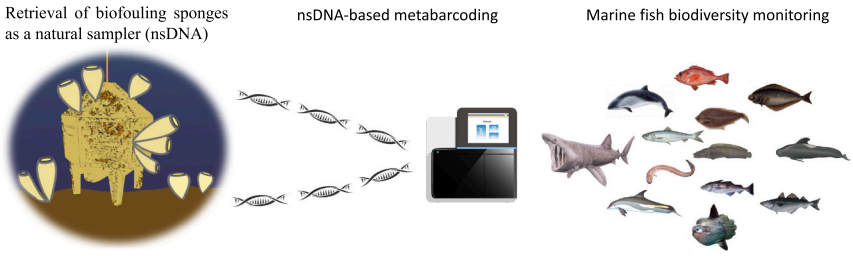
HIGHLIGHTS

- Marine sponges are efficient natural eDNA collectors for detecting marine life.
- The indiscriminate use of living sponges might raise conservation concerns.
- We demonstrate the effectiveness of biofouling sponges as natural eDNA collectors
- We highlight that some biofouling is not just a nuisance, but also a biodiversity recorder

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental DNA (eDNA) analysis has now become a core approach in marine biodiversity research, which typically involves the collection of water or sediment samples. Yet, recently, filter-feeding organisms have received much attention for their potential role as natural eDNA samplers. While the indiscriminate use of living organisms as 'sampling tools' might in some cases raise conservation concerns, there are instances in which highly abundant sessile organisms may become a nuisance as biofouling on artificial marine structures. Here we demonstrate how a sea sponge species that colonizes the moorings of the world's largest curtain of hydroacoustic receivers can become a powerful natural collector of fish biodiversity information. By sequencing eDNA extracted from *Vazella pourtalesii* retrieved from moorings during routine biofouling maintenance, we detected 23 species of marine fish and mammals, compared to 19 and 15 species revealed by surface and bottom water eDNA respectively, and 28 species captured by groundfish survey in the surrounding area, which are more ecologically impactful and involve higher additional costs. Sponge-based species inventories proved at least as informative as those obtained by traditional survey methods, and are also able to detect seasonal differences in fish assemblages. We conclude that opportunistic sampling of marine sponge biofouling may become an efficient way to document and monitor biodiversity in our rapidly changing oceans.

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1. Introduction

Accurate, multi-scale characterization of spatial and temporal patterns of marine biodiversity is an essential step towards mitigating anthropogenic pressures and climate change in the ocean (Canonico et al., 2019) and towards achieving the United Nations (UN) 2030 ocean-related goals for sustainable development (UNESCO, 2020). The identification of essential ocean variables (EOVs) for monitoring the state of the physical and chemical characteristics of the ocean is relatively well developed, and global monitoring programmes are in place, such as the Framework for Ocean Observing (FOO) (Lindstrom et al., 2012). In contrast, efforts to standardize the monitoring of EOVs for marine life are still under development (Danovaro et al., 2020).

A primary obstacle to effective monitoring of marine life is that the marine environment is vast and mostly difficult to access. Marine biodiversity assessment still heavily relies on capture-based methods, such as trawling and dredging, which can have negative impacts on both physical habitats and living assemblages (Jones, 1992; Good et al., 2022). The availability of a wealth of novel monitoring approaches, such as acoustic telemetry (Fregosi et al., 2016; Matley et al., 2022), passive acoustics (Fleishman et al., 2023), camera observations from cabled observatories (Aguzzi et al., 2020a, 2020b), remotely operated vehicles (ROVs) (Button et al., 2021; Guedes and Araújo, 2022), and environmental DNA (eDNA) analysis (Miya, 2022), offer new opportunities to augment the spatial and temporal resolution of data collection and to inform the development of EOVs for biological communities and ecosystems. Further, some of these provide less invasive monitoring tools than capture-based approaches (Freeman and Freeman, 2016; Goldberg et al., 2016). Passive acoustics is a promising strategy, but sound libraries from natural ecosystems are still being developed, and reliable attribution of sound emissions to many species can be problematic (Gibb et al., 2019). Geographic location is also an issue, particularly for species that emit low-frequency sounds that can travel long distances, such as whales (Van Parijs et al., 2021). Acoustic telemetry, in which target animals are equipped with a sound-emitting tag that is detected by networks of receivers at different points in the ocean, is another useful method for studying movement ecology (Matley et al., 2022), but electronic tags are physically invasive (Gahagan and Bailey, 2020) and relatively expensive (Sibert and Nielsen, 2000), which typically makes acoustic tracking appropriate to only a limited number of large, charismatic species. Cabled observatories (networked underwater photographic equipment) require good underwater visibility to obtain images that can identify species (Aguzzi et al., 2020a, 2020b) and have a very limited spatial extent. Evolving robotic systems (ROVs and underwater drones) provide an alternative underwater perspective, but for the time being they are too expensive for large scale biodiversity surveys. Another line of research, eDNA-based biodiversity assessment, is revolutionizing marine and fisheries resource monitoring (Kelly et al., 2014; Stefanni et al., 2022), and although eDNA analysis also has its limitations (Hansen et al., 2018; Takahashi et al., 2023), it requires less investment in sampling equipment, little taxonomic expertise, and can be applied universally to virtually all organisms through the judicious choice of appropriate gene markers (Cristescu, 2014).

Recent studies suggest that filter-feeding organisms can be used as 'natural samplers' to collect and concentrate DNA shed by marine life in the surrounding environment (Mariani et al., 2019; Turon et al., 2020; Brodnicke et al., 2023; Jeunen et al., 2023a, 2023b), an approach hereafter referred to as 'natural sampler DNA' (nsDNA). Due to filter-feeding organisms' inherent ability to filter water, nsDNA eliminates the need for artificial water filtration, which is the most time-consuming aspect of eDNA sampling, and also reduces plastic waste associated with water filtration. Among filter-feeding organisms, sponges (phylum Porifera) appear to be the most efficient natural samplers due to their extraordinary water filtering capabilities (Kahn et al., 2015), which can filter up to 24,000 l of seawater per day for a 1 kg sponge (Vogel, 1977). The rationale for using sponges as natural samplers lies primarily in these organisms' remarkable water-filtering, DNA-trapping characteristics (Jeunen et al., 2023a; Neave et al., 2023), but also in their wide distribution from coastal waters to the deep sea, which provides opportunities to collect eDNA from a huge range of habitats, including, potentially, biodiversity-enhanced habitats engineered by the sponges themselves (Hawkes et al., 2019).

Biofouling – sessile aquatic fauna and flora growing on the surface of underwater man-made objects – is often found in large amounts on various artificial structures (moorings, ship hulls, oil rigs, buoys, cables, piers, etc., Callow and Callow, 2002; Stachowitsch et al., 2002; Walker et al., 2007), and must be regularly removed to maintain the functionality of these maritime structures. Although the removed fouling is usually discarded, the sponge component could represent a valuable reserve of naturally filtered eDNA, potentially offering an excellent opportunity for monitoring marine communities through time.

The glass sponge *Vazella pourtalesii* forms globally unique sponge grounds on the Scotian Shelf off Nova Scotia, Canada, which are considered Vulnerable Marine Ecosystems with special measures for their protection (OTN, 2017). In the same area, the Ocean Tracking Network (OTN) maintains a line of bottom-moored acoustic receivers (known as the "Halifax Line") for tracking marine animals above the sponge grounds. OTN routinely tends the acoustic receivers to remove biofouling and *V. pourtalesii* has been observed to settle on these moorings (Hawkes et al., 2019; Busch et al., 2020). The present study specifically evaluated the potential benefits of using sponge biofouling as a valuable natural collector for vertebrate biodiversity assessment. First, we compared the nsDNA strategy with the widely used water eDNA strategy (artificially filtered water). This was done by simultaneously collecting surface and bottom seawater samples at the same sites where the sponges were collected (Fig. 1), and using the same metabarcoding workflow targeting fish DNA (using Tele02 and Elas02 primers, Taberlet et al., 2018) to generate biodiversity data from both nsDNA and water eDNA. Second, given the unique opportunity provided by the detections of known tagged fish species from the acoustic receiver array, and the annual groundfish surveys conducted in this region by Fisheries and Ocean Canada (DFO), we placed the nsDNA results in the broader context of the known biodiversity features of the area.

2. Materials and methods

2.1. Sampling

The Russian hat sponge (*Vazella pourtalesii*), a member of Hexactinellida ("glass sponges"), is unique to the Scotian Shelf off Nova Scotia (Beazley et al., 2021). The Ocean Tracking Network has maintained a line of benthic acoustic receiver moorings spanning the continental shelf off Halifax, Nova Scotia, Canada, for >10 years, and the Russian hat sponge has been found to settle on these moorings where they pass through the sponge grounds. Both sponges and water samples for this study were collected during routine maintenance of the acoustic receiver moorings. Samples were collected on three occasions: 11 August and 7 October in 2021, and 26 April in 2022.

Sponge samples — During each sampling event, we collected three individual sponge specimens, for each sampling location (mooring). Sponges were sampled from moorings, typically within 3 m or less of the bottom. Three individual sponges were cut from the selected moorings with disposable scalpels (pre-sterilized), and were then placed in separate jars pre-filled with 100 % ethanol. These jars were sealed with tape and then transported ashore and shipped at ambient temperature to Liverpool John Moores University, United Kingdom, for laboratory processing.

Water samples — For each sampling event, 6 l of seawater was collected from the surface (<5 m) and 6 l of seawater was collected from the bottom (near the moorings, range 106–171 m) for each sampling location, as water masses over such a depth range may

contain different mixtures of eDNA (Jeunen et al., 2020; Canals et al., 2021). Water samples were collected using Niskin bottles and then transferred into independent sterile sampling containers. Six containers were used for each sampling location which provided triplicate 2 l sub-samples for each depth (surface and bottom). Sample containers were placed in a − 20 °C freezer onboard the ship. When the ship arrived in port (< 24 h), the containers were transferred to the −20 °C freezer located at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia. During each sampling event, 2 l of deionised water was included as a negative field blank control.

In total, we collected 51 sponge specimens from 17 moorings, 48 surface water samples from 16 mooring sites and 45 bottom water samples from 15 mooring sites. Due to weather conditions on some dates we were unable to collect all three sample types from every sampling sites. In August 2021, we successfully collected 18 sponge samples, 18 surface water samples and 18 bottom water samples from six moorings. In October 2021, 18 sponge samples, 12 surface water samples and 9 bottom water samples were collected from 7 moorings. In April 2022, 15 sponge samples, 18 surface water samples and 18 bottom water samples were collected from 6 moorings. All sampling locations and times are given in Supplementary data 1.

2.2. Laboratory procedures

All workstations and all equipment were cleaned with 10 % bleach followed by 70 % ethanol prior to filtration. All materials were pre-decontaminated before use.

Sponge samples — Approximately 250 mg of dry sponge per specimen was used for nsDNA extraction. Each sponge sample was removed from the storage ethanol, blotted dry on filter paper (42.5 mm, Fisher Scientific) in a Petri dish and then placed in a 1.5 ml microtube for DNA extraction.

Water samples — One Sterivex filter (0.45 μm PES membrane, Merck Millipore, Germany) was used for each subsample (2 l of seawater). We used peristaltic pumps for filtration and once filtration was complete, each Sterivex filter was placed in its individual bag and frozen at −20 °C until shipped. On each day of filtration, there was one filtration blank (2 l of Milli-Q water filtered through the Sterivex filter) which was filtered prior to all field blanks and samples to test the sterility of the filtration equipment. Sterivex filters were shipped on dry ice to Liverpool John Moores University, UK, where they were stored at −20 °C until DNA extraction. Each Sterivex filter was opened using sterile carpenter's pliers. The opened capsule was placed on a disposable Petri dish. The membranes were removed from the inner tube and torn into small pieces using sterilized disposable tweezers. All pieces were placed in a 1.5 ml microtube for DNA extraction.

Both sponge and water samples were processed using a bespoke and modular universal DNA extraction protocol: the Mu-DNA method (Sellers et al., 2018). Extracts were then quantified for DNA concentration using a Qubit™ 4 Fluorometer with a QubitTM dsDNA HS Assay Kit (Thermo Fisher Scientific). A total of 20 out of 51 nsDNA extracts had to be diluted in order to allow PCR amplification (dilution information is provided in Supplementary data 2). Water eDNA extracts were not diluted for PCR amplification due to their low DNA concentration.

PCR amplifications were performed with two sets of primers: the Tele02 pair (forward: 5′-AAACTCGTGCCAGCCACC-3′, reverse: 5′-GGGTATCTAATCCCAGTTTG-3′), designed to be most universal for teleosts, and the Elas02 primers (forward: 5′-GTTGGTHAATCTCGTGCCAGC-3′, reverse: 5′-CATAGTAGGGTATCTAATCCTAGTTTG-3′), designed for elasmobranchs (Taberlet et al., 2018). Both primers target the mitochondrial 12S rRNA gene (168 bp -179 bp). Primers were designed with unique 8 bp dual barcodes for sample identification and to reduce tag jumping (Schnell et al., 2015), with 2–4 leading 'N' bases to increase sequence diversity. PCR amplifications for each sample were performed in triplicate. For Tele02 primers, PCR conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 45 s, 72 °C for 30 s, and finishing at 72 °C for 5 mins. For Elas02 primers, PCR conditions were initial denaturation at 95 °C for 15 min, followed by 40 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and finishing at 72 °C for 5 mins. All PCRs were

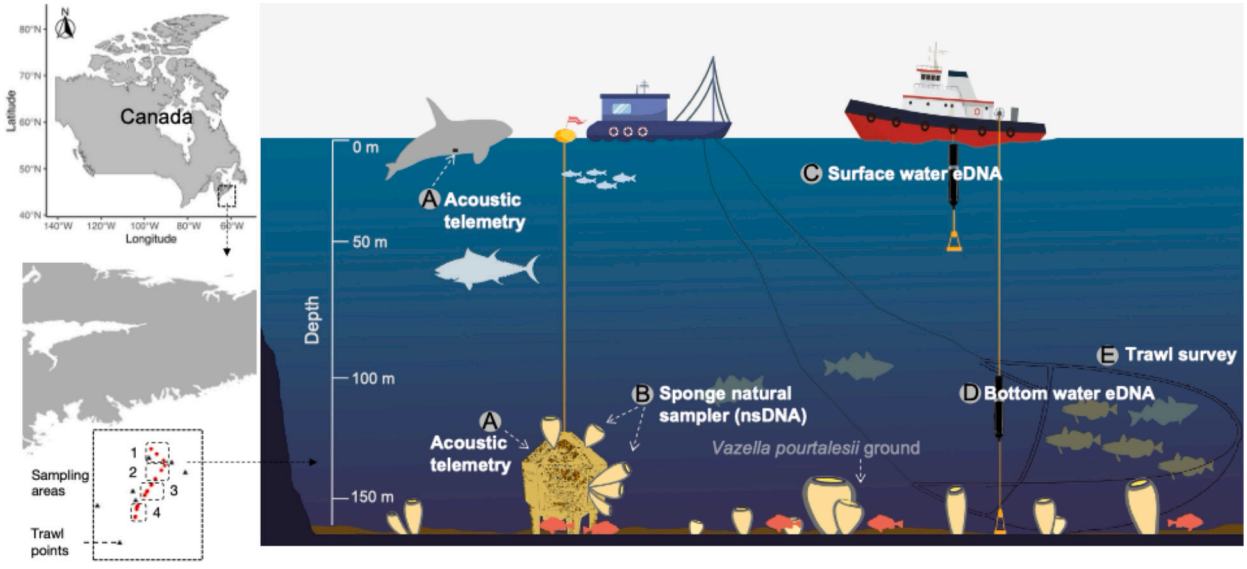

Fig. 1. Survey methods along the Halifax line: acoustic telemetry, sponge (*Vazella pourtalesii*) nsDNA, surface and bottom water eDNA, and bottom trawl survey. Red dots on the map show the sponge and water sampling area along the acoustic receivers. A: Ocean Tracking Network track target animals through the acoustic receiver array off the coast of Nova Scotia. B: *Vazella pourtalesii* grows on the receiver moorings and can be collected during the cleaning of these structures for use as natural samplers. C and D: On each sponge sampling trip, water samples were also collected from the bottom and surface for comparison with sponge nsDNA and acoustic signals. E. Trawling conducted by Fisheries and Oceans Canada (DFO) research vessel surveys (also known as the groundfish biodiversity surveys) on the Scotian Shelf and Bay of Fundy for 2020 as reported in the Global Biodiversity Information Facility (GBIF). Black triangles on the map represent the trawl locations used for the comparative analyses.

performed in 20 μL reactions containing 10 μL 2× MyFi Mix (Meridian Bioscience), 0.5 μM of each primer, 0.04 mg BSA (Bovine Serum Albumin Solution, Thermo Fisher Scientific), 5.84 μL molecular grade water (Invitrogen) and 2 μL DNA template. Two positive and two negative PCR controls were included in each PCR run. We used the DNA extracted from iridescent catfish (*Pangasionodon hypopthalmus* - an Asian freshwater species) as a PCR positive control.

PCR triplicates were pooled and visualised on 2 % agarose gels. The pooled PCR products for each sample were quantified using the Qubit™ 4 fluorometer with the dsDNA HS Assay Kit (Thermo Fisher Scientific). PCR products amplified with both sets of primers were pooled ensuring that each PCR replicate had the same concentration in the final pool. We ended up with two pools for nsDNA and two pools for water eDNA. PCR controls were included in each pool.

Each pool was then purified using Mag-Bind Total Pure NGS magnetic beads (Omega Bio-Tek). Purified pools were prepared for sequencing using the NEXTFLEX Rapid DNA-Seq Kit for Illumina (PerkinElmer) according to the manufacturer's instructions. The final libraries were quantified by quantitative PCR (qPCR) using the NEBNext Library Quant Kit for Illumina (New England Biolabs) and fragment size checked using the Tape Station 4200 (Agilent). Libraries were pooled at equimolar concentrations with a final molarity of 80 pM with 15 % PhiX control, and sequenced on an Illumina iSeq100 using iSeq 100 i1 Reagent v2 (300 cycles) at Liverpool John Moores University.

2.3. Bioinformatics and statistical analysis

Our bioinformatic processing was based on OBITOOLS v1.2.11 (Boyer et al., 2016). First, we trimmed the low-quality ends using 'obicut', then paired-end reads were merged using 'illuminapairedend', after which alignments with low (QC < 30) quality scores were removed. Second, the alignments were demultiplexed using 'ngsfilter' with default parameters. After demultiplexing, we merged the alignments of nsDNA and water eDNA samples amplified by Tele02 into one file, and the alignments of samples amplified by Elas02 into one file. We then performed 'obigrep' to filter out erroneous sequences (keeping sequences between 130 bp and 180 bp without ambiguity), performed 'obiuniq' for dereplication, and performed the de novo chimera search function in vsearch v2.4.3 (Rognes et al., 2016) to remove chimeras. The remaining sequences were clustered using SWARM v2.1.3 (Mahé et al., 2015) with '-d 3'. Taxonomic assignment was performed using 'ecotag'. The reference database used in 'ecotag' was constructed by in silico PCR for Tele02 or Elas02 primers against the EMBL database (release version r143) using 'ecoPCR'. We also performed a second taxonomic assignment using BLAST against the NCBI reference database, manually correcting the species that 'ecotag' assigned to species in the same genus of Atlantic species but distributed in the Pacific, or sequences that could not be assigned to species level (e.g. *Cololabis saira* is corrected to *Scomberesox saurus*, *Etropus microstomus* to *Citharichthys arctifrons*, Gadidae sp. to *Melanogrammus aeglefinus*). Finally, a sample/OTU table with taxon information was formatted using R scripts listed at https://github.com/metabarpark/R_scripts_metabarpark. We then used the R package 'lulu' 0.1.0 (Frøslev et al., 2017) with default parameters to filter out erroneous OTUs based on the calculation of pairwise similarities and co-occurrence patterns of OTUs.

All subsequent statistical analyses were performed with R v4.1.2 (R Core Team, 2022). To achieve high quality detections, we first filtered out low read counts that were likely created by sequencing errors. Read counts smaller than the highest untargeted read counts in the positive controls were removed (read counts smaller than 10 or 5 reads were removed depending on the control in each run). Second, we removed some OTUs that do not exist in the Scotian Shelf off Nova Scotia, Canada, whose sequences derive from background contamination from other sequencing projects being carried out during the same period (e. g. *Gadiculus argenteus* and *Odonus niger*). Finally, we removed the OTUs that represented the most common anthropogenic contaminants, such as from humans, livestock and pets.

For the filtered OTUs, we first pooled the detections from both primer sets, as we did not aim to compare primer efficiency, and then converted the raw read counts to presence/absence scores (1/0), so that a positive/negative detection from either primer set was interpreted as presence/absence. Finally, we pooled the triplicates of each sample; thus for each sampling site we had one sponge sample, one surface water sample and one bottom water sample for subsequent analysis. We pooled the triplicates to obtain a robust, less stochastic result, as the species detected by the triplicate extractions were variable and a single triplicate subsample could not offer an exhaustive picture. The probability of detection of each species for Fig. 4 is calculated by the proportion of the Hellinger-transformed read counts of all nsDNA/water eDNA.

We used the sample-based rarefaction-extrapolation approach in the 'iNEXT' v2.0.12 package (Hsieh et al., 2016) to compare alpha diversity between sampling months and methods (nsDNA, surface water eDNA and bottom water eDNA). Significant differences in estimated alpha diversity were assessed using non-overlapping confidence intervals (MacGregor-Fors and Payton, 2013). To analyse the effect of covariates (sampling months, sampling areas) on community assembly, we used 'mvabund' v3.12.3 (Wang et al., 2012) with the manyglm function (fit glms for each species with binomial error distribution for presence/absence data). We also performed an analysis to partition beta diversity into turnover and nestedness using 'betapart' v1.5.4 (Baselga, 2010) to show the seasonal turnover of the community assembly, and then the *tabasco* function in 'vegan' v2.5.7 (Oksanen et al., 2020) to show a compact community figure (Fig. S3), and the *protest* function in 'vegan' to test the contribution of the turnover and nestedness components to the overall β-diversity (comparing the matrices generated by metaMDS). To determine the key species associated with sponges, we used the package 'indicspecies' v1.7.12 (De Cáceres et al., 2010) to calculate the difference between nsDNA and water eDNA. All results were visualised using the R package 'ggplot2' v3.3.5.

Acoustic tracking data were provided by the Ocean Tracking Network, with the permission of the data owners. Data were available throughout 2021 across the Halifax Line, allowing refinement to a five-day interval (two days before and after sponge collection), making species detection directly comparable with nsDNA/water eDNA.

DFO (Fisheries and Ocean Canada) Maritime research vessel survey data (the annual groundfish biodiversity and stock assessment) was downloaded from GBIF (Global Biodiversity Information Facility). These data include species observed by trawling on the Scotian Shelf. The groundfish survey is conducted annually by DFO, who visit different locations in Nova Scotia each year. Due to the fact that there is no temporal overlap and mostly disjunct spatial locations of the groundfish survey data with our study, we chose the closest data from the DFO trawl surveys, namely the survey period from 5 to 29 July 2020. In these dates, the survey covered an area close to our sampling site, so we restricted the spatial source of the data to the vicinity of the sponge sampling sites, as shown in Fig. 1, but it should be stressed that the nsDNA data are not directly comparable with the DFO groundfish surveys data.

3. Results

Sequencing produced 9,682,568 raw reads (6,106,864 for the sponge library, 3,575,704 for the seawater library); however, after all the quality control steps above, we were left with a total of 4,124,898 reads for the Tele02 primer (including 51 sponge samples, 63 water samples and 21 controls) and 2,034,788 reads for the Elas02 primer (including 50 sponge samples, 86 water samples and 21 controls). After quality control, the negative and positive controls showed no evidence of contamination.

Comparisons of species richness and species composition were carried out by comparing nsDNA with water eDNA at each mooring location. Our results show that compared to either surface or bottom water

eDNA, nsDNA captured greater species richness (Fig. 2A). nsDNA detected all species found in bottom water eDNA and had higher detection frequency across samples for most of these overlapping species. We also found eight species (35 %) from nsDNA that bottom water eDNA did not detect (Fig. 2B). Although nsDNA yielded slightly higher overall estimates of species richness than surface water eDNA, these two data sets showed less overlap in detected species. Pelagic predators such as harbour porpoise (*Phocoena phocoena*), white-sided dolphin (*Lagenorhynchus acutus*) and porbeagle shark (*Lamna nasus*) were only detected in surface water samples in this study (light blue in Fig. 2B). In contrast, small pelagic species such as Atlantic mackerel (*Scomber scombrus*) and Atlantic saury (*Scomberesox saurus*) were detected more frequently in sponges than in surface water, and the long-finned pilot whale (*Globicephala melas*) was only found in nsDNA (Fig. 2B). Furthermore, when bottom and surface water eDNA samples were pooled, the species richness was similar to that detected by nsDNA (Fig. S1). At species level, we found that eight of the species detected were statistically more likely to be sampled with nsDNA compared to water eDNA, such as *Pollachius virens* (saithe), *Sebastes fasciatus* (Acadian redfish) and *Citharichthys arctifrons* (Gulf Stream flounder) (Table S1), all of which are key fish species of the *V. pourtalesii* habitat (Fuller, 2011; Ward-Paige and Bundy, 2016; Hawkes et al., 2019).

Subsequently, we investigated the spatial and temporal changes in community composition. Although the study area could be grouped into four oceanographically distinguishable areas, based on a set of 48 environmental variables (see Fig. S2), no significant differences in alpha or beta diversity could be detected among the areas (Tables S2, mvabund analysis, $P = 0.083$). Instead, nsDNA detections suggested that community change was mainly driven by seasonality (Fig. 3B), with more similar assemblages found in October and August, and a more divergent community composition in April, these patterns were evident in both water eDNA and nsDNA data sets (Fig. 3).

Lastly, we compared the nsDNA and water eDNA species inventories with two established biodiversity assessment data sources available for the study area. OTN's moored receivers (acoustic tracking data) detect tagged fish within approximately 500 m, providing an independent source of data on tagged species, which are predominantly large (usually longer than 1 m) and pelagic. Data were available across the Halifax Line throughout 2021, which also allowed refining data to a five-day interval (two days before and after sponge collection), to make species detection directly comparable to water eDNA/nsDNA samples. The groundfish survey is conducted annually by DFO, visiting different Nova Scotian locations each year. In 2020, the survey covered an area close to our sampling site (Fig. 1), providing a referenced list of primarily demersal fish species in this area and their relative abundance, with more pelagic species, such as mackerel (*Scomber scombrus*) and herring (*Clupea harengus*) known to be sampled less efficiently (DFO, 2003).

The acoustic recordings showed that within five days around the sampling events, no faunal detections were recorded by the acoustic receivers. But throughout 2021, the acoustic array detected 10 tagged migratory species present at different times of the year (Fig. 4), namely: 86 detections of great white shark (*Carcharodon carcharias*), 62 of Atlantic salmon (*Salmo salar*), 45 of Atlantic bluefin tuna (*Thunnus thynnus*), eight of Atlantic halibut (*Hippoglossus hippoglossus*), three of short-fin mako shark (*Isurus oxyrinchus*), and two for both swordfish (*Xiphias gladius*) and Atlantic sturgeon (*Acipenser oxyrinchus*). However,

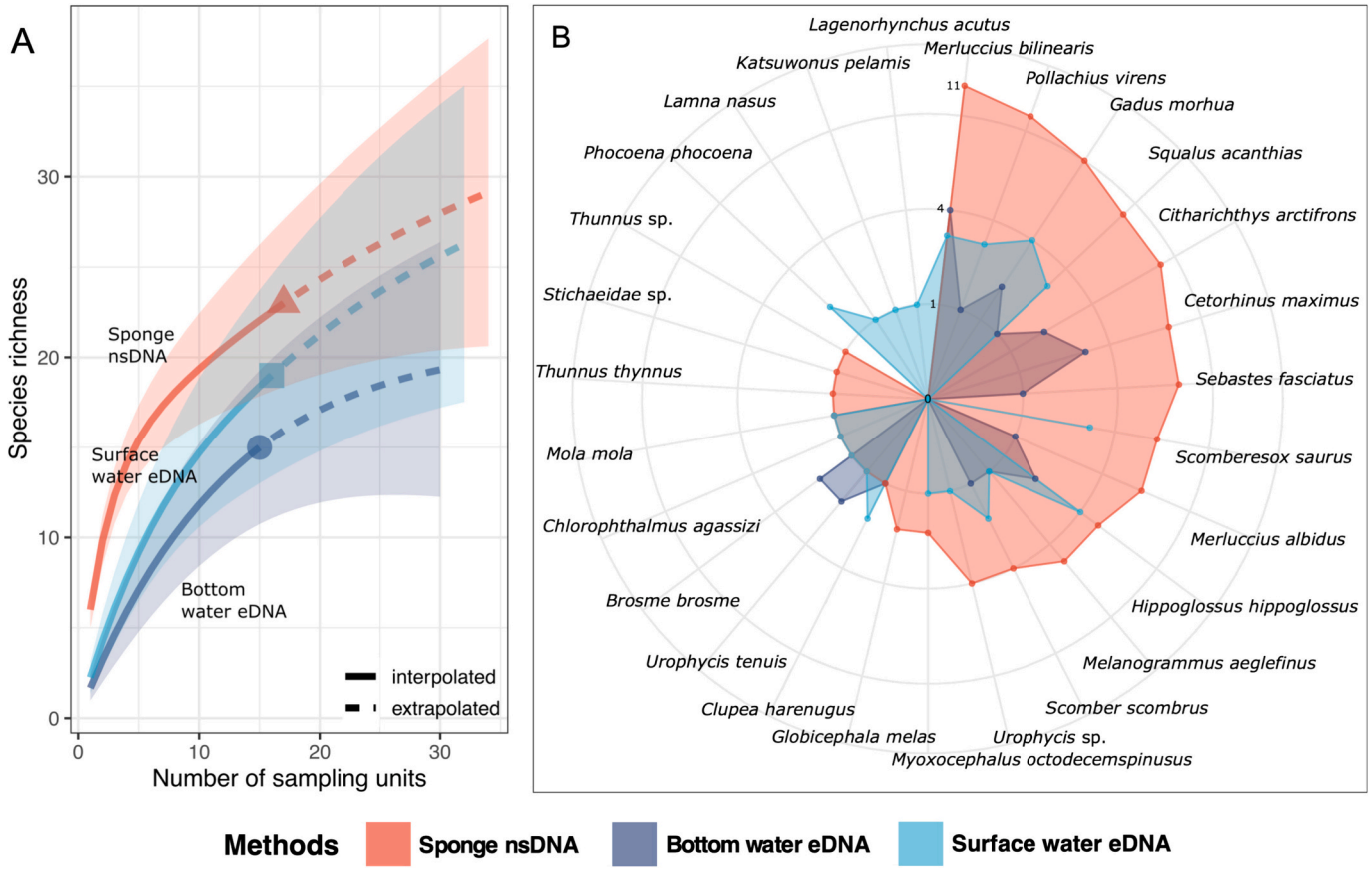

Fig. 2. Comparison between sponge nsDNA and water eDNA. Data from nsDNA in red, and data from water eDNA in shades of blue. A. Alpha diversity analysis using iNEXT (v2.0.12). Sample size-based rarefaction (solid lines) and extrapolation (dashed lines) sampling curves for three measures of species richness in surface water, bottom water and nsDNA. Shaded areas represent 95 % confidence intervals. Symbols (triangle, square, circle) indicate the total sample sites per sampling method, dotted lines indicate the predicted species richness. Where confidence intervals overlap, there is no difference in alpha diversity between sampling methods, whereas non-overlap indicates a significant difference. B. Numbers indicate the total number of detections for each fish species by each method.

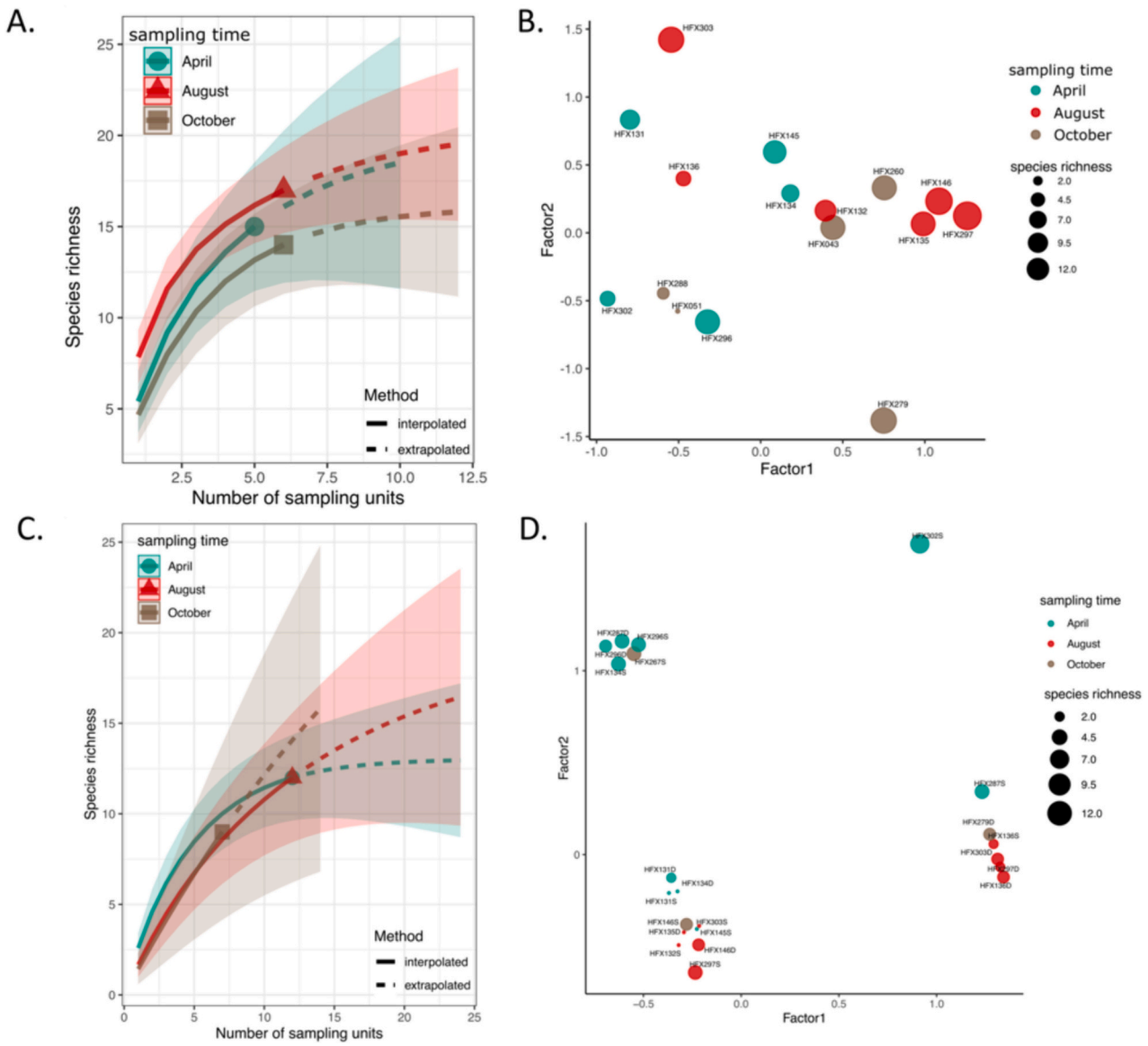

Fig. 3. Seasonal changes in the fish community recorded by sponge nsDNA (panels A and B) and water eDNA (panels C and D). A and C show sample size-based rarefaction (solid lines and symbols) and extrapolation (dashed lines) sampling curves for species richness. Shaded areas represent 95 % confidence intervals. Overlapping confidence intervals indicate no substantial difference between the sampling periods. B and D show the community heterogeneity of the samples displayed in the first two axes of a model based latent factor ordination analyses. Size of dots is proportional to species richness recorded for each sample.

nsDNA (and water eDNA) detected a strong basking shark (*Cetorhinus maximus*) signal, particularly in August and October, at 7 of the 17 sites, as well as ocean sunfish (*Mola mola*) in August. For the Atlantic halibut, although six receivers picked up eight acoustic signals from September to December, nsDNA detected this species in April, August and October. Atlantic bluefin tuna was also detected by nsDNA in August.

The nsDNA approach generated a picture of the demersal assemblage of the outer Bay of Fundy that is broadly consistent with the information gathered via bottom trawling. There are 43 % of the trawl caught species (13 out of 28 species) that overlapped with the nsDNA detection. These 13 overlapping species dominated 88 % of the trawl counts, indicating that the most abundant species recorded by trawl were also detected by nsDNA (Fig. 4). For example, silver hake (*Merluccius bilinearis*) was the most abundant species caught by trawl (green in Fig. 4, representing 66 % of the counts) and was also the most abundant species (53 % of Hellinger-transformed read counts) detected by sponges (33 % in water eDNA). The remaining 12 % of the counts in the trawl catch belonged to 15 species that nsDNA did not detect, which were strictly benthic as would be expected from bottom trawling. However, nsDNA also detected nine additional species that are typically not caught by the trawl survey, such as sharks, tuna, sunfish and other large, fast-moving predators, including marine mammals. In this analysis, the probability of detection of each species for nsDNA/water eDNA is calculated by the proportion of the Hellinger-transformed read counts, while the number of sites where the species is present in all sites for nsDNA/water eDNA is linearly correlated with the proportion of Hellinger-transformed read counts of all nsDNA/water eDNA ($P < 0.001$, $R^2 = 0.79$, $P < 0.001$ for nsDNA, $R^2 = 0.50$ for water eDNA).

4. Discussion

In this study, we provide a first practical demonstration of the potential of biofouling organisms to serve as eDNA collectors to characterize marine assemblages from areas where biological surveys are often difficult and expensive. We generated species inventories from eDNA trapped in samples of the glass sponge *V. pourtalesii* growing on the

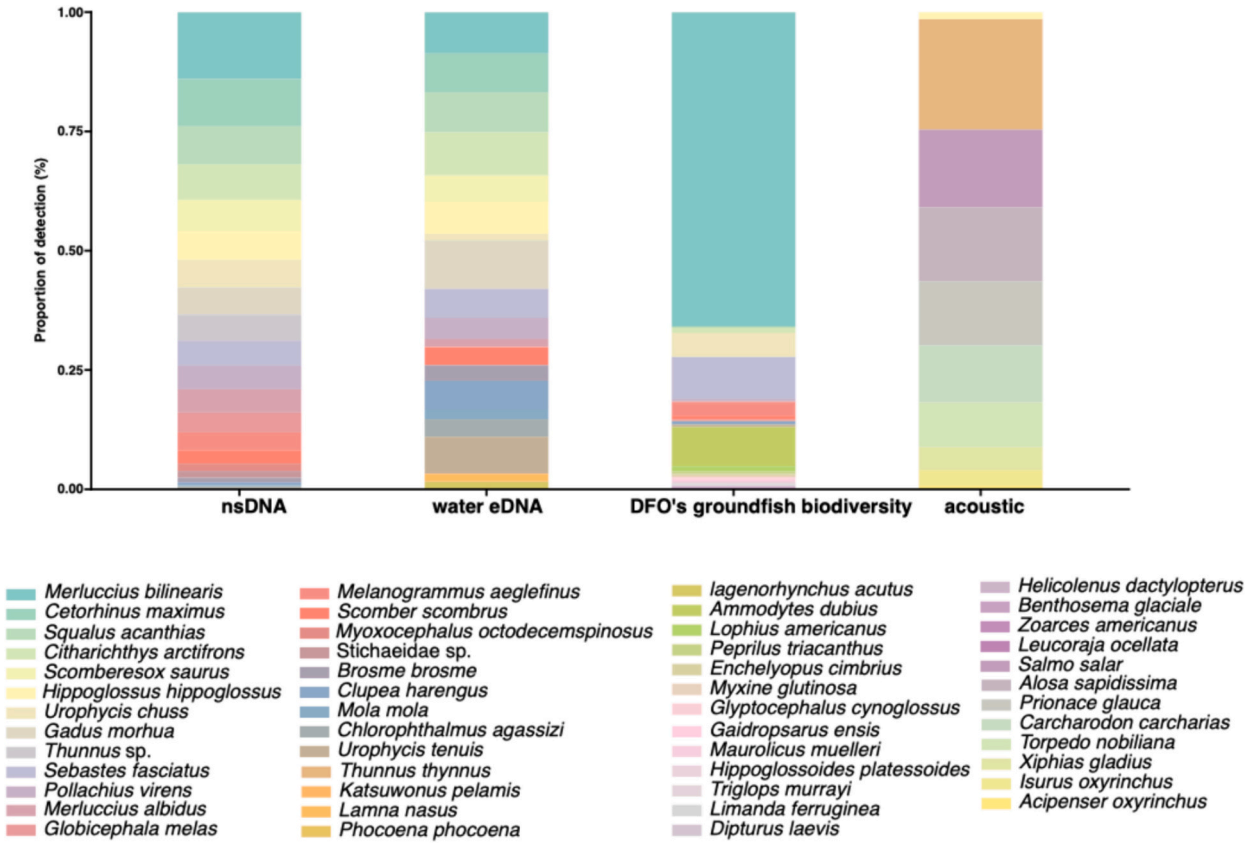

Fig. 4. Summary of relative species abundance recorded by different data sources. Bars represent the relative abundance of species measured by different methods, and colours represent species. The percentages of species detected by sponge nsDNA and water eDNA (including surface and bottom water) are calculated by the proportion of the Hellinger-transformed read counts, the percentages by groundfish survey are calculated as a proportion of total catch abundance, and the percentages by acoustics are calculated as a proportion of the total number of signals received across all species. nsDNA and water eDNA data come from one-day collections made in August and October 2021 and April 2022, the DFO groundfish biodiversity data set is based on a five-day survey conducted in July 2020, and the acoustic data records all species signals returned in the year 2021.

moorings of a long-term acoustic telemetry array and compared them with both surface (5 m) and bottom (~150 m) standard water eDNA samples collected from Niskin bottles. The results indicate that i) biofouling-derived assemblage reconstruction is comparable or even more efficient than that obtained through water eDNA samples; ii) both sets of water eDNA and nsDNA data portrayed the Scotian shelf assemblages accurately, complementing pelagic (acoustic tagging) and demersal (trawling surveys) faunal information from the study region.

4.1. *Biofouling V. pourtalesii nsDNA efficiency*

The eDNA trapped in the biofouling sponges (nsDNA) proved to be more informative than the eDNA from bottom water samples, and was generally comparable with the information gathered from pooled surface and bottom water eDNA samples (Figs. 2, S1). This suggests that nsDNA metabarcoding could be a better strategy for fish biodiversity surveys than bottom water eDNA metabarcoding, especially in deep-sea surveys where sponges are available for collection, are taken as trawling bycatch (Jeunen et al., 2023b), or have already been archived for other purposes (Neave et al., 2023). Since *V. pourtalesii* is a deep-water species, nsDNA detections in this species are expected to show closer resemblance to the water eDNA samples from the bottom rather than the surface. As expected, nsDNA outperformed surface water eDNA for the detection of mesopelagic and demersal fish. However, it is noteworthy that even at depths between 106 m and 171 m, nsDNA yielded more detections for some pelagic species than surface water eDNA. This may reflect the effectiveness of sponge nsDNA in trapping eDNA fragments sinking from surface layers, drifting through water currents, or actively brought into deeper waters through animal vertical migrations. The performance of biofouling *V. pourtalesii* nsDNA provides an integrated biodiversity assessment from both surface and bottom water eDNA for both species composition and species richness. This opens up an avenue whereby routine biofouling control operations could directly provide sponge nsDNA samples for biodiversity monitoring, without the need to invest in bespoke water-based eDNA surveys. The known biofouling properties of sponges across a wide variety of habitats and regions (Callow and Callow, 2002; Stachowitsch et al., 2002) may make this approach globally relevant. Furthermore, the species that are highly associated with sponge nsDNA (Table S1) are emblematic of the *V. pourtalesii* habitat (Fuller, 2011; Ward-Paige and Bundy, 2016; Hawkes et al., 2019), which strengthens the perception of this sponge species as an accurate recorder for local biodiversity. For instance, saithe and flounder are benthic feeders (Bundy et al., 2017), while redfish find shelters in the bottom during the day (Grinyó et al., 2023). Thus, as we begin to understand the biological and environmental variables that underlie the efficiency of the sponge nsDNA approach (Cai et al., 2022; Harper et al., 2023; Jeunen et al., 2023a), it will soon be possible to devise optimal nsDNA protocols for biofouling-assisted biomonitoring, which consider sample sizes, collection, preservation, extraction methods, marker choice.

Ecologically, the temporal and spatial eDNA examination of ichthyofaunal assemblages confirmed that the biome examined in our study was consistent with the 'outer Bay of Fundy' community, as observed by O'Brien et al. (2022). The nsDNA detections suggested that community change was mainly driven by seasonality (Fig. 3B), with more similar assemblages found in October and August, and a more

divergent community composition in April, suggesting seasonal species turnover (β-diversity partitioning test in Fig. S3). These patterns were evident in both water eDNA and nsDNA data sets (Fig. 3) and highlight an important role that DNA methods can play in filling temporal sampling gaps. The explanation for this pattern may reside in seasonal variations in the Gulf Stream kinetics and eddy formation (Kang et al., 2016) which peaks in summer (Zhai et al., 2008) and brings transient warm water species at various life stages onto the shelf, and/or peaks of eDNA abundance linked to spawning cycles (Collins et al., 2022).

4.2. Biofouling nsDNA as a complement to acoustic and capture-based biodiversity assessment

These results show that nsDNA yielded a portrayal of fish assemblages that were consistent with what is known from demersal surveys (despite the very different sampling techniques and timeframes), and revealed important information about seasonal species distribution changes and the presence of megafauna at a time when acoustic receivers did not record movements (Fig. 4).

Both nsDNA and water eDNA approaches are temporal 'snapshot' sampling due to the nature of DNA degradation. This means that non-resident, migratory species will rarely be detected, especially if they have small population sizes. However, the nsDNA approach can have high sensitivity, and more frequent sponge collections could have revealed the roaming of other individuals of acoustically-tagged species. Some notable examples in our study include: i) nsDNA samples detected Atlantic bluefin tuna in August, during its arrival season in Nova Scotia (DFO, 2018), when the acoustic receivers did not record signals from this species (Fig. 4); ii) Ocean sunfish and basking shark are both seasonal migrants species (Sims et al., 2003; Potter et al., 2011), neither of them have been tagged by OTN researchers; iii) Atlantic halibut, whose migratory patterns are little known in our study area (Le Bris et al., 2018), is detected by nsDNA from April to October at a depth of approximately 150 m, even though acoustic receivers only picked up halibut signals from September to December.

It should be noted that in some special cases, such as in very deep, dark and cold ocean conditions, eDNA may remain as a legacy signal in sponge tissues, making sponges useful for detecting rare species, by preserving eDNA for longer compared to its persistence in water (Cai et al., 2022). Overall, our study demonstrates the potential of sponges to provide important information on the movements of large, elusive migratory species, including those that are difficult to fit with acoustic tags. We highlight the versatility and affordability of opportunistic biofouling as collecting capsules for biodiversity records that span a range of habitats and taxonomic realms – from demersal to pelagic, from cryptic benthic fishes to cetaceans – that are impossible to obtain or replicate with other, more expensive survey methods.

4.3. New field of eDNA applications

In conclusion, the evidence presented here paves the way to a new field of eDNA applications, which harnesses biofouling organisms as precious tools for monitoring biodiversity, especially that found in association with offshore, deep-sea, industrial infrastructure at the frontier of ecosystem changes. eDNA-based biodiversity surveys have the remarkable advantage of being non-invasive, less selective, and more efficient compared to virtually every other sampling method. Parallel to the increase in popularity of nsDNA-based biodiversity surveys, there is a concern that oversampling may negatively impact sponge populations, or those of other candidate "natural sampler" species. This study identifies an avenue where the routine maintenance of artificial structures can elegantly be coupled with cutting-edge marine biomonitoring tools, without harming marine life in its natural habitat. It is therefore possible to envisage a proximate future where biofouling organisms can form a network of natural marine sentinels to enhance marine biodiversity monitoring.

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CRediT authorship contribution statement

Wang Cai: Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis. **Barry MacDonald:** Writing – review & editing, Methodology, Data curation. **Michelle Korabik:** Methodology. **Iago Gradin:** Resources, Methodology. **Erika F. Neave:** Writing – review & editing, Methodology. **Lynsey R. Harper:** Writing – review & editing, Data curation, Conceptualization. **Ellen Kenchington:** Writing – review & editing, Supervision, Funding acquisition. **Ana Riesgo:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Frederick G. Whoriskey:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Stefano Mariani:** Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174148.

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