

RESOURCE ARTICLE

Comparison of materials for rapid passive collection of environmental DNA

Cindy Bessey^{1,2,3}  | Yuan Gao⁴ | Yen Bach Truong⁴  | Haylea Miller²  |
Simon Neil Jarman^{3,5}  | Oliver Berry² 

¹Commonwealth Scientific and Industrial Research Organisation, Indian Oceans Marine Research Centre, Oceans and Atmosphere, Crawley, Western Australia, Australia

²Commonwealth Scientific and Industrial Research Organization, Indian Oceans Marine Research Centre, Environmental Future Science Platform, Crawley, Western Australia, Australia

³University of Western Australia, UWA Oceans Institute, Crawley, Western Australia, Australia

⁴Commonwealth Scientific and Industrial Research Organization, Manufacturing, Clayton, Victoria, Australia

⁵School of Biological Sciences and the UWA Oceans Institute, University of Western Australia, Crawley, Western Australia, Australia

Correspondence

Cindy Bessey, Commonwealth Scientific and Industrial Research Organisation, Indian Oceans Marine Research Centre, Oceans and Atmosphere, Crawley, WA, Australia.

Email: cindy.bessey@csiro.au

Funding information

Commonwealth Scientific and Industrial Research Organisation, Grant/Award Number: Environmental Future Science Platform

Handling Editor: Carla Lopes

Abstract

Passive collection is an emerging sampling method for environmental DNA (eDNA) in aquatic systems. Passive eDNA collection is inexpensive and efficient, and requires minimal equipment, making it suited to high-density sampling and remote deployment. Here, we compare the effectiveness of nine membrane materials for passively collecting fish eDNA from a 3-million-litre marine mesocosm. We submerged materials (cellulose, cellulose with 1% and 3% chitosan, cellulose overlaid with electrospun nanofibres and 1% chitosan, cotton fibres, hemp fibres, and sponge with either zeolite or active carbon) for intervals between 5 and 1080 min. We show that for most materials, with as little as 5 min of submersion, mitochondrial fish eDNA measured with qPCR, and fish species richness measured with metabarcoding, was comparable to that collected by conventional filtering. Furthermore, PCR template DNA concentrations and species richness were generally not improved significantly by longer submersion. Species richness detected for all materials ranged between 11 and 37 species, with a median of 27, which was comparable to the range for filtered eDNA (19–32). Using scanning electron microscopy, we visualized biological matter adhering to the surface of materials, rather than entrapped, with images also revealing a diversity in size and structure of putative eDNA particles. eDNA can be collected rapidly from seawater with a passive approach and using a variety of materials. This will suit cost- and time-sensitive biological surveys, and where access to equipment is limited.

KEYWORDS

biotechnology, DNA barcoding, environmental DNA, fish

1 | INTRODUCTION

Environmental DNA (eDNA) metabarcoding is a sensitive, non-invasive and broadly applicable tool for species detection (Deiner et al., 2017; Taberlet et al., 2018). It has been used for a range of

applications, including biodiversity measurement and biosecurity surveillance (Taberlet et al., 2018). Organisms shed their DNA into the air, soil and water, which can be sampled by collecting, extracting, amplifying and sequencing, and ultimately identified by comparing against a reference database of known DNA sequences (Taberlet

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Molecular Ecology Resources* published by John Wiley & Sons Ltd.

et al., 2018; Thomsen & Willerslev, 2015). Diverse applications have been developed for eDNA metabarcoding and the field has grown rapidly in recent years (Jarman et al., 2018; Koziol et al., 2019).

Insufficient replication of biological sampling in eDNA studies has been identified as a consistent deficiency (Prosser, 2010; Zinger et al., 2019). For example, if the objective is to identify as much biodiversity as possible, then low replication will under-represent true diversity through false negatives (Burian et al., 2021). Both small sampling volumes and few replicates at a site can lead to false negatives if the concentration of target DNA is low. If comparisons of alpha diversity are being made, then sampling designs must consider the appropriate scale required because sampling a few sites can result in false negatives arising from small-scale heterogeneity in the distribution of the target species. Appropriate sampling can be very difficult to achieve with traditional eDNA filtering from water in aquatic systems because of the time and expense involved in sufficient water sampling. The potential for sampling effects to impact interpretation of eDNA surveys has been highlighted in recent studies which emphasize that the distribution of eDNA can be highly heterogeneous (Buxton et al., 2021; Derocles et al., 2018; Dickie et al., 2018; Zinger et al., 2019). If small numbers of samples are taken, then large volumes of water are needed to recover maximum biodiversity in eDNA analysis (Bessey et al., 2020).

Passive eDNA collection methods (Bessey et al., 2021; Kirtane et al., 2020), which involve direct submersion of materials into a water body, facilitate increased replication because they are cheaper, simpler and faster to apply than active filtration. For example, both commercially available membranes and sachets of granular materials have all been left in water bodies to effectively capture eDNA (Bessey et al., 2021; Kirtane et al., 2020). The ease of sample replication enables analyses that are generally not practical with time-consuming water filtering methods. Frequency of occurrence methods, which rely on replicate sampling to statistically evaluate detection probabilities (Strickland & Roberts, 2019; MacKenzie et al., 2002), are becoming more feasible, as well as mapping residence of species of interest, because a large number of samples can be easily collected from an area. For studies investigating diversity, greater biological replication improves the reliability of both alpha and beta diversity estimates (Zinger et al., 2019; Prosser, 2010). Furthermore, because passive eDNA requires minimal or no supporting technology (no vacuum or peristaltic pumps are required), it suits deployment to remote environments, and by nonexperts.

Few studies have investigated the mechanisms or optimal material properties needed for passive eDNA collection. Kirtane et al. (2020) used adsorbent-filled sachets of montmorillonite clay and granular activated carbon to passively capture eDNA in freshwater laboratory, microcosm and field experiments. In the laboratory, they found that extracellular DNA adsorbed to these materials at different rates, depending on the water matrix. In their field experiments, granular activated carbon sachets captured significantly more eDNA than clay and detected the same concentration of fish eDNA as a 1-L conventional grab sample. These materials were chosen for their high adsorption capacity to trap DNA but also for their low adsorption affinity to allow high yield during extraction. They

suggest that adsorption mechanisms for granular activated carbon are dependent on the water matrix, whereas that of clay is more dependent on adsorption kinetics and capacity. Bessey et al. (2021) compared the effectiveness of positively charged nylon and non-charged cellulose ester membrane materials for passive collection of fish eDNA at both a species-rich tropical and species-poor temperate marine site. They found that both materials detected fish as effectively as conventional active eDNA filtration methods and provided similar estimates of total fish biodiversity but only in temperate, not tropical, waters. Their materials were chosen to investigate the possible role of electrostatic attraction and because both are commonly used in conventional aquatic eDNA studies using filtration methods. The observations that significant material effects exist, and may be system-specific, indicates there is potential for improvements to passive eDNA collection through material selection that could create greater efficiencies for users.

The optimal submersion time for efficient passive eDNA collection is also unclear. Kirtane et al. (2020) found that, regardless of material used (clay or granular carbon) or water matrix (molecular-grade water, microcosm tank water or natural creek water), an equilibrium concentration of eDNA was absorbed in <24 h. In field trials, they also found that fish species detection did not significantly increase with longer submersion duration (7 compared to 21 days). In both tropical and marine waters, Bessey et al. (2021) likewise found that increased submersion time did not increase species richness (comparing 4, 8, 12 and 24 h of submersion). Combined, these studies indicate that long-duration submersion (days or hours) may not be necessary and therefore investigations into minimal submersion times are another potential avenue to increase passive eDNA collection efficiency.

Using a DNA metabarcoding approach, here we evaluate the effect of materials and submersion time on the efficiency with which fish eDNA could be collected passively from a large marine mesocosm. We chose or manufactured a range of materials for their structural complexity, availability, physical robustness to sustain marine deployment, or for enhanced DNA anionic binding efficiency. We also used scanning electron microscopy to visualize eDNA capture by each material to gain a qualitative understanding of the mechanism of eDNA adherence, hypothesizing that structurally complex materials would increase eDNA capture through entanglement or that DNA binding efficiency would be increased by using a polycation polymer. We show that, for most materials, passively collected eDNA consistently performs similarly to conventionally filtered eDNA samples, and that high collection efficiency can be achieved in as little as 5 min.

2 | MATERIALS AND METHODS

2.1 | Study site and design

Sampling was conducted in the main tank at The Aquarium of Western Australia (AQWA; aqwa.com.au), which offered a relatively controlled system containing 50 known fish species in 3 million litres of seawater. This system draws incoming seawater from 0.5 m below the seabed (natural sand filter) of the nearshore ocean waters. It is

then filtered (pressure glass media filter) before entering the AQWA facility where the water supplies several display tanks before entering the main tank of the mesocosm. The main tank has its own gravity filter system (volume of filter tank is 2 million litres) that uses a 50-cm sand bed with 2 mm (± 0.5 mm) size particles, over 50 cm of 6 mm (± 3 mm) gravel which we hypothesized would minimize DNA shed from external sources and adjacent display tanks. The turnover rate between the gravity filter and main tank is 5 million litres every 2 hr. Passive eDNA sampling was conducted between 8 a.m. and 4 p.m. on January 21 and 22, 2021, by submerging nine different membrane materials just below the surface in the mesh pockets of a pearl oyster aquaculture frame (Figure 1, see Bessey et al., 2021). Each of the nine membrane materials was deployed in quadruplicate for specified time intervals (5, 10, 30, 60 min and overnight for 18 h; except electrospun nanofibres which were deployed for only 5, 10 and 30 min due to limited production) to examine whether increased submersion time led to increased eDNA collection. Of the quadruplicate samples, three were used for eDNA extractions while the other was used for scanning electron microscopy to visualize how eDNA collected on the different membrane surfaces.

2.2 | Membrane materials

We trialled nine different membrane materials based on properties including structural complexity, availability and physical robustness to sustain marine deployment, or for enhanced DNA anionic binding efficiency (Table 1). The first was a cellulose ester membrane (0.45 μ m Pall GN-6 Metrice) commonly used in eDNA studies (Tsuji et al., 2019). To investigate whether chitosan coating would increase eDNA capture, the cellulose membranes were impregnated with either 1% or 3% (w/w) chitosan, which was then crosslinked under glutaraldehyde vapour to confer stability. Loadings of chitosan on the membranes were confirmed by Fourier-transform infrared spectroscopy (FT-IR) as well as by staining with the anionic dye Eosin Y. Chitosan is a polycation polymer that efficiently binds anionic DNA under acidic conditions and has been used for DNA enrichment and purification (Pandit et al., 2015). Chitosan is derived from chitin in crustacean shells and is readily available, inexpensive and biocompatible. To investigate if eDNA would

become entrapped in highly complex materials, we trialled overlaying the cellulose esters with electrospun nanofibres, while also trialling a combination of electrospun nanofibres that were subsequently covered in 1% (w/w) chitosan. Electrospinning is a technique for producing fibres from submicrometre down to nanometre in diameter with high surface area (Bhardwaj & Kundu, 2010). We used solution electrospinning, where the polymer(s) and other additive materials are first dissolved in a suitable solvent at an optimized concentration before electrospinning. A high-voltage electric field is applied to the droplet of fluid coming out of the tip of a die or spinneret, which acts as one of the electrodes. When the electric field supply is strong enough, it will lead to droplet formation and finally to ejection of a charged jet from the tip of the cone accelerating toward the counter collector electrode, leading to the formation of a nanofibrous membrane. These nanofibrous membranes have found applications in many areas, including biomedical areas (e.g., scaffolds for tissue engineering, drug delivery, wound dressing and medical implants), filtration, protective textiles and battery cells (Gao et al., 2014). Our electrospinning was carried out using polyether-based thermoplastic polyurethane (TPU) grade (RE-FLEX 585A; Townsend Chemicals) with a 10% (w/v) solution in dimethyl formamide solvent (DMF) using a 23G needle spinneret, with an applied voltage of 20 kV at 15 cm from the collecting drum. To ensure sufficient physical robustness for use in the marine environment, a composite was prepared using a thermal bonding (Protechnic 114P [13 gsm]) net material to bond the electrospun membrane attached through thermal adhesive. This backing plate was needed to prevent the nanofibre cellulose membranes from curling, and therefore we also trialled these backing plates separately in the downstream processing to determine their effect on eDNA capture. We also trialled natural fibres, cotton and hemp, which were contained in a nylon bag for practical deployment purposes so they would remain anchored within the mesh of the pearl frame. The cotton and hemp materials were processed independently of their nylon encasings, although a subset of these encasings was retained for downstream processing in the same fashion as the trialled materials. These cotton and hemp fibres were 5 mm in diameter and cut into 40-mm lengths so they could fit in a 2-ml Eppendorf tube for DNA extraction. Finally, we trialled two sponge materials that would be highly robust in aquatic settings: one was a tightly woven filter pad with 100% active carbon embedded into the fibres (Aqua One),

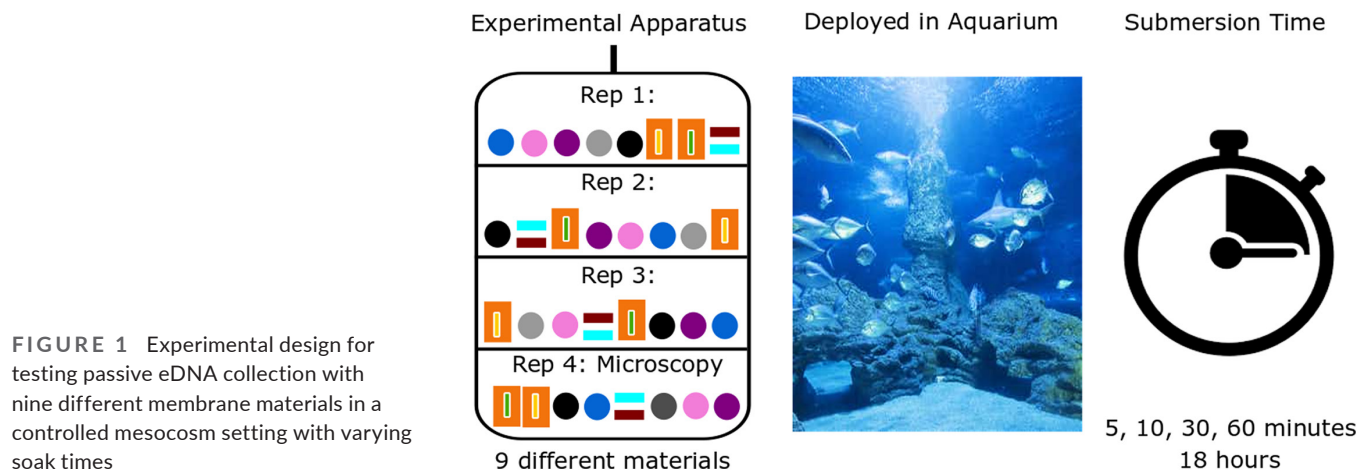


FIGURE 1 Experimental design for testing passive eDNA collection with nine different membrane materials in a controlled mesocosm setting with varying soak times

while the other was a tightly woven filter pad with zeolite embedded into the fibres (Aqua One). The sponge was cut into 40-mm rectangular lengths and had a 5-mm width and depth. All materials were placed under ultraviolet sterilizing light for a minimum of 30 min, except for the cellulose membranes which were certified sterile upon purchase.

2.3 | Scanning electron microscopy

We used scanning electron microscopy (SEM) to qualitatively investigate how biological matter attaches to each of the different materials. SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. These signals are converted into two-dimensional high-resolution images and reveal information about the external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. A subsection of each membrane material was dissected using sterile surgical scissors and mounted onto 10-mm-diameter SEM stubs with an adhesive carbon tab to prevent charge build-up. The stubs were then air dried at ambient temperature in a fume hood while partially covered to prevent dust or debris on the samples. Once completely dry, samples were coated with a thin layer of platinum (sputter coated with 3 nm of platinum using a Leica EM MED020; Leica Microsystems), a fine conducting material for high-resolution electron imaging. The samples were visualized and imaged on a Zeiss 1555 VP-FESEM with SmartSEM software (Zeiss) at the Centre for Microscopy, Characterization and Analysis (CMCA), University of Western Australia, Perth, Western Australia. We provide example SEM images of materials at 10,000 \times (cellulose) and 100 \times (cotton, hemp, sponge-active carbon and sponge-nitrate) magnification prior to deployment (Table 1) and provide an example of all deployed materials with biological matter attached. Due to limited supplies of chitosan and electrospun nanofibre-covered cellulose membranes, none was available for SEM imaging prior to deployment.

2.4 | Active eDNA collection

We collected water for active eDNA filtration to compare with the results of passive eDNA collection. Five 1-L surface water samples were collected in sterile 1-L containers at five different times over the day and filtered with cellulose ester membranes (47 mm diameter, 0.45 μ m pore size) using a peristaltic Sentino Microbiology Pump on a clean benchtop at the aquarium facility. All water samples were taken on the first day.

2.5 | Contamination control

A sterile technique was used throughout the experiment and consisted of wearing gloves and using sterile tweezers to handle all materials. All materials were preserved by freezing after collection and stored at -20°C until further processing in the laboratory. All

collection and deployment apparatus was sterilized by soaking in 10% bleach solution for at least 15 min and rinsed in deionized water.

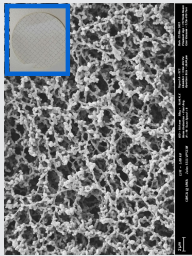
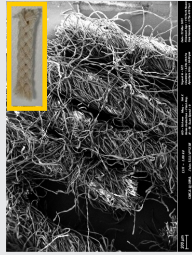
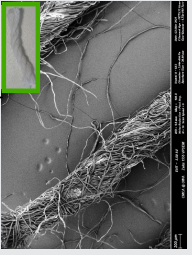
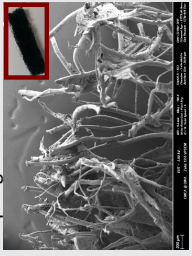
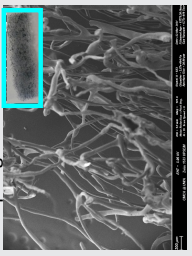
2.6 | eDNA extraction from passive and active collection materials

All cellulose ester materials, as well as the nylon bags, were cut or flash frozen (-80°C) and crushed into small pieces that were placed in a 2-ml Eppendorf tube in preparation for extraction. All other materials were placed directly into a 2-ml Eppendorf tube as for extraction. Total nucleic acid was extracted from all materials in the same fashion using a DNeasy Blood and Tissue Kit (Qiagen), with an additional 40 μ l of Proteinase K used during a 3-h digestion period at 56°C on rotation (300 rpm). DNA was eluted into 200 μ l of AE buffer. All extractions took place in a dedicated DNA extraction laboratory using a QIAcube (Qiagen), where benches and equipment were routinely bleached and cleaned.

2.7 | DNA metabarcode amplification for fish detection

We followed the same procedures used by Bessey et al. (2021). One-step qPCRs were performed in duplicate for each sample using 2 μ l of extracted DNA and a mitochondrial DNA 16S rDNA universal primer set targeting fish taxa (16SF/D 5' GACCCTATGGAGCTTTAGAC 3' and 16S2R-degenerate 5' CGCTGTTATCCCTADRGTAAC 3' with a 178–228 bp amplicon size; Berry et al., 2017; Deagle et al., 2007), with the addition of fusion tag primers unique to each sample that included Illumina P5 and P7 adaptors. qPCR was performed in a dedicated PCR laboratory. qPCR reagents were combined in a dedicated clean room and included 5 μ l AllTaq PCR Buffer (Qiagen), 0.5 μ l AllTaq DNA Polymerase, 0.5 μ l dNTPs (10 mM), 1.0 μ l Ultra BSA (500 $\mu\text{g}\mu\text{l}^{-1}$), SYBR Green I (10 units μl^{-1}), 0.5 μ l forward primer (20 μM) and 5.0 μ l reverse primer (20 μM), 2 μ l of DNA and Ultrapure Distilled Water (Life Technologies) made up to a total volume of 25 μ l. Mastermix was dispensed manually, and qPCR was performed on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of 30 s at 95°C , 30 s at the primer annealing temperature of 54°C , and 45 s at 72°C , with a final extension for 10 min at 72°C . All duplicate qPCR products from the same subsample were combined prior to library pooling. The mean quantification cycle (C_q) from qPCR duplicates was used as an indication of initial DNA copy number. The C_q (or C_t) value is the cycle in which fluorescence exceeds a specified threshold above background noise. Lower C_q values indicate a higher initial copy number of the target sequence. A sequencing library was made by pooling amplicons into equimolar ratios based on qPCR C_t values and sequenced on an Illumina MiSeq platform (Illumina). The libraries were size selected using a Pippin Prep (Sage Science) and purified using the Qiaquick PCR Purification Kit (Qiagen). The volume of purified library added to the sequencing

TABLE 1 Materials trialled for passive eDNA collection accompanied by scanning electron micrographs of each material at 10,000X (cellulose) and 100X (all other materials) magnification, inlaid with images of the materials prior to deployment

Material	Name	Supplier	Description
Cellulose membrane	Cellulose	Pall	GN-6 Metricel 0.45 µm 47 mm S-Pack white gridded
Cellulose + 1% chitosan	Chitosan - 1%	Pall + CSIRO	Cellulose membrane covered with 1% chitosan solution
Cellulose + 3% chitosan	Chitosan - 3%	Pall + CSIRO	Cellulose membrane covered with 3% chitosan solution
Cellulose + electrospun nanofibres	Espun	Pall + CSIRO	Cellulose membrane covered with electrospun nanofibres
Cellulose + electrospun nanofibres + 1% chitosan	Espun w/ chitosan	Pall + CSIRO	Cellulose membrane covered with electrospun nanofibres and 1% chitosan
Cotton fibres in nylon bag	Cotton	Arbee	100% cotton secured in place using a nylon bag (Ribtex)
Hemp fibres in nylon bag	Hemp	Shamrockcraft	Twisted hemp cord secured in place using a nylon bag (Ribtex)
Activated carbon sponge	Sponge - active carbon	Aqua One	Tightly woven filter pad with 100% active carbon
Zeolite sponge	Sponge - zeolite	Aqua One	Tightly woven filter pad with enzyme bacteria and zeolite
Cellulose			
Cotton			
Hemp			
Sponge - Active Carbon			
Sponge - Zeolite			

run was determined by quantifying the concentration (Murray et al., 2015) using a Qubit 4 fluorometer (ThermoFisher Scientific). The library was unidirectionally sequenced using a 300-cycle MiSeq V2 Reagent Kit and standard flow cell.

PCR plates included blank laboratory extraction controls (extraction reagents used with no DNA template), PCR-negative controls (2 μ l of distilled water used rather than DNA template) and positive controls (dhufish, *Glaucosoma hebraicum*; and swordfish, *Xiphias gladius*). Dhufish inhabit the mesocosm, whereas swordfish do not, so the latter was a more appropriate positive control. No negative control (extraction or PCR) contained more than 17 reads, with the maximum number of reads per fish species being four (see Table S1 for number of sequence variants and reads per sample prior to data processing; see Table S2 for sample number treatment details). Therefore, we used a detection rate of greater than five sequences to classify something as a positive detection. All positive controls amplified multiple reads identifying dhufish and swordfish with 100% identity. Swordfish was not detected in any sample except for our positive PCR control.

2.8 | DNA sequence data processing

Our DNA sequence data processing is detailed in Bessey et al. (2021); it directly follows the procedure described at <https://pythonhosted.org/OBITools/wolves.html>, and we briefly outline those procedures here again. Data generated by Illumina sequencing were processed using the OBITOOLS (<https://pythonhosted.org/OBITools/>) command “ngsfilter” to assign each sequence record to the corresponding sample based on tag and primer. Then “obiuniq” was used to dereplicate reads into unique sequences. Reads <190bp and with counts <10 were discarded. Denoising was performed using “obiclean” to retain only head sequences, which are sequences with no variants containing a count >5% of the head sequence. Sequences were assigned to taxa using “ecotag” and a result table was generated using “obiannotate.” Our reference database was built in silico using our universal fish primer assay on Augusts 3, 2021. Only fish species with identities $\geq 90\%$ and whose sequence variants could be assigned to at least family (and lower) were included. All variants were assigned a single name (e.g., to family, genus or species) and directly compared to the known species in the mesocosm (Table 2). For example, an assignment to genus could be compared to the species of that genus that are known to inhabit the mesocosm. If multiple species were a 100% match to a single variant, then the variant was assigned to genus.

2.9 | Statistics

A Box–Cox transformation was used to normalize all Cq data (Shapiro–Wilks test), which allowed for the use of parametric statistics, where mean Cq was the predictor variable. We used an analysis of variance on the linear model fit of mean Cq value by material,

followed by a Tukey honest significant difference test to compare materials. We also used an analysis of variance on the linear model fit between mean Cq value and submersion duration for each material. A linear model fit of mean Cq values by material and submersion duration, and their interactions, produced the same results. These statistics were likewise used to determine differences in the number of species detected between materials and submersion intervals. We fitted a smoothing spline to the interval data for a visual estimation of how mean Cq values and species detections varied with time. All statistics and graphics were produced using R (version 2.14.0; R Core Team, 2021; library MASS was used for Box–Cox transformations) and graphics were edited in INKSCAPE (<https://inkscape.org/>).

3 | RESULTS

3.1 | Multiple materials enable passive eDNA collection

All nine membrane materials collected detectable fish eDNA (Figure 2). Although significant differences in mean Cq values existed between materials ($F = 21.69$, $df = 11$; $p < .001$), with cotton and hemp fibres exhibiting higher mean Cq values (indicating lower initial copy number of the target sequence), all other materials were similar to each other, including those obtained from conventionally filtering five 1-L samples. Both cotton and hemp were deployed within nylon bags, and the mean Cq values for the nylon bags (22.3, 23.1, 23.7; min., mean, max.) were not significantly different from those of the filtered 1-L samples (21.1, 23.5, 28.5), nor most other materials. Additionally, the mean Cq values of the nylon bags were lower than that of the zeolite sponge ($p = .01$). The backing plate attached to the electrospun nanofibre-covered cellulose membranes did not inhibit eDNA collection, as evidenced by their comparable mean Cq values.

3.2 | Increased submersion time did not increase eDNA collection

No significant differences in mean Cq values were detected over time for any of the nine trialled materials (Figure 3; $F = 1.28$ for submersion time \times material, $df = 9$, $p = .25$). Smoothing splines were fitted to the time interval data for each material, and a trend downward would be indicative that the material collected more eDNA over time. Only hemp and both sponge materials showed a decline in trendline over time.

3.3 | The majority of fish species were detected with all materials

For all materials, we assigned 8,822,884 sequence reads to 71 fish taxa (Table 2). Of the 50 species known to inhabit the mesocosm, 37 (74%) were detected through passive eDNA. The additional 34

TABLE 2 Fish species inhabiting the main tank compared to those detected by passive eDNA collection, the materials upon which each species were detected (C = cellulose, C1 = chitosan - 1%, C3 = chitosan - 3%, E = espun, ET = espun w/chitosan, cot = cotton, H = hemp, S-AC = sponge-active carbon, S-Z = sponge-zeolite), the number of reads and associated comments per species (Y-g = yes to genus)

Family	Species	AQWA	eDNA	Materials	Reads	Comments
Aracanidae	<i>Anoplocapros</i>		Y	C, C1, C3, E, ET, H, S-AC, S-Z	394	Found in tanks running to filter tank
Arripidae	<i>Arripis georgianus</i>	Y	Y	All	806,729	
	<i>Arripis trutta</i>	Y	Y	C1, C3, H	893	
Aulopidae	<i>Latropiscis purpurissatus</i>	Y	Y	C3, ET	20	
Belonidae	<i>Tylosurus crocodilus</i>		Y	H	17	Found in local intake waters
Carangidae	Carangidae unknown		Y	All	3884	
	<i>Pseudocaranx georgianus</i>	Y	Y-g	All	289,581	
	<i>Seriola hippos</i>	Y	Y	All	29,516	Detected as <i>Seriola lalandi</i>
	<i>Trachurus declivis</i>	Y	Y-g	All	76,500	
	<i>Carcharias taurus</i>	Y	Y	C, C1, C3, Cot, H, S-AC, S-Z	195	
Carcharhinidae	<i>Carcharhinus cautus</i>	Y	Y-g	All	1160	
	<i>Carcharhinus plumbeus</i>	Y				
Chaetodontidae	<i>Chelmonops curiosus</i>	Y				
	<i>Hemiochus diphreutes</i>	Y	Y	All	2340	
Clupeidae	<i>Hyperlophus vittatus</i>		Y	C, C1, C3, ET, H, S-Z	220	Feed for mesocosm inhabitants
	<i>Sardinella</i>		Y	All	655,224	Feed for mesocosm inhabitants
	<i>Sardinops</i>		Y	C, C1, C3, Cot, ET, E, S-AC, S-Z	487	Feed for mesocosm inhabitants
	<i>Spratelloides delicatulus</i>		Y	All	40,336	Feed for mesocosm inhabitants
	<i>Bathytoshia brevicaudata</i>	Y	Y	All	1,184,978	
Dasyatidae	<i>Diodon nictemerus</i>		Y	C, C1, C3	33	Found in tanks running to filter tank
Emmelichthyidae	<i>Emmelichthys nitidus</i>		Y	Cot, H	108	Feed for mesocosm inhabitants
	<i>Engraulis</i>		Y	All	4346	Feed for mesocosm inhabitants
Enoplosidae	<i>Enoplosus armatus</i>	Y				
Ephippidae	<i>Platax orbicularis</i>		Y	S-Z	9	Found in tanks running to filter tank
Gadidae	<i>Gadidae unknown</i>		Y	C, C3, S-Z	41	
	<i>Girella tephraeops</i>	Y	Y-g	All	2718	
Glucosomatidae	<i>Glucosoma hebraicum</i>	Y	Y	All	55,325	Found in tank and also used as a positive control for two PCR plates
Haemulidae	<i>Plectorhinchus flavomaculatus</i>	Y	Y	All	6890	
	<i>Plectorhinchus polytaenia</i>	Y	Y	C, C1, C3, ET, E, H, S-AC, S-Z	1384	
Hemiscylliidae	<i>Chiloscyllium punctatum</i>	Y				

(Continues)

TABLE 2 (Continued)

Family	Species	AQWA	eDNA	Materials	Reads	Comments
Kyphosidae	<i>Kyphosus sydneyanus</i>	Y	Y-g	All	1335	
	<i>Microcanthus strigatus</i>	Y	Y	C, C1, C3, E, ET, H, S-AC, S-Z	357	
	<i>Scorpius georgiana</i>	Y	Y-g	All	5059	
	<i>Tilodon sexfasciatus</i>	Y	Y	C, C3, Cot, ET, E, H, S-AC, S-Z	380	
Labridae	<i>Bodianus frenchii</i>	Y	Y-g	All	826	
	<i>Choerodon rubescens</i>	Y	Y	All	13,180	
	<i>Coris auricularis</i>	Y	Y-g	C1, C3, ET, E, H, S-Z	185	
	<i>Labroides dimidiatus</i>	Y	Y	C3, Cot, H, S-AC	34	
	<i>Notolabrus parilus</i>	Y	Y	All	4403	
	<i>Pseudolabrus biserialis</i>	Y				
Latidae	<i>Goniistius gibbosus</i>	Y	Y-g	All	1160	
	<i>Goniistius rubrolabiatus</i>	Y	Y-g	All	3053	
	<i>Psammoperca datnioides</i>	Y	Y	C1, C3	32	
	<i>Lethrinus nebulosus</i>	Y	Y	All	877	
Lutjanidae	<i>Lutjanus</i>		Y	C1, H	106	
Monacanthidae	<i>Paramonacanthus choirocephalus</i>		Y	C, S-Z	21	Found in tanks running to filter tank
Monodactylidae	<i>Schuettea woodwardi</i>	Y				
Mugilidae	<i>Mugil cephalus</i>		Y	All	1137	Feed for mesocosm inhabitants
	<i>Parupeneus</i>		Y	C1	9	Found in tanks at aquarium
Mullidae	<i>Upeneus tragula</i>		Y	C, C3, Cot, H	106	Feed for mesocosm inhabitants
	<i>Gymnothorax woodwardi</i>		Y	C1	15	Detected as <i>Gymnothorax pseudothyrsoides</i>
Myliobatidae	<i>Aetobatus narinari</i>		Y	C3, E, H, S-Z	598	
	<i>Myliobatis tenuicaudatus</i>	Y	Y	All	14,066	
Nemipteridae	<i>Pentapodus vitta</i>	Y				
Osmeriidae	<i>Hypomesus</i>		Y	All	7693	Feed for mesocosm inhabitants
	<i>Ostracion</i>		Y	S-AC	8	Found in tanks at aquarium
Pemperphidae	<i>Parapriacanthus elongatus</i>		Y	C, C1, C3, ET, E, S-AC, S-Z	77	Found in tanks running to filter tank
	<i>Pempheris klunzingeri</i>	Y	Y	All	1363	Detected as <i>Pempheris</i> cf. <i>Multiradiata</i>
Platycephalidae	<i>Inegocia japonica</i>		Y	Cot	19	Found in tanks running to filter tank
Plesiopidae	<i>Paraplesiops meleagris</i>	Y				
Pomacentridae	<i>Chromis</i>		Y	C, C1, C3, E, ET, S-AC, S-Z	226	Found in tanks running to filter tank

TABLE 2 (Continued)

Family	Species	AQWA	eDNA	Materials	Reads	Comments
	<i>Parma mccullochi</i>	Y	Y	C3, ET, E, S-Z	197	Detected as <i>Parma microlepis</i>
	<i>Pomatopus saltatrix</i>	Y	Y	All	19,246	
Sciaenidae	<i>Argyrosomus japonicus</i>	Y	Y	All	616,576	
Scombridae	<i>Scombridae unknown</i>		Y	All	7411	Feed for aquarium inhabitants
Serranidae	<i>Epinephelides armatus</i>	Y				
	<i>Epinephelus malabaricus</i>	Y	Y-g	All	4516	
	<i>Ephinephilus rivulatus</i>	Y				
	<i>Othos dentex</i>	Y				
	<i>Pseudanthias dispar</i>		Y	C3, ET, E, H, S-Z	92	Found in tanks running to filter tank
	<i>Variola louti</i>		Y	ET	10	
Siganidae	<i>Siganus doliatus</i>		Y	C3, Cot	48	Found in tanks running to filter tank
Sillaginidae	<i>Sillago ingenuua</i>		Y	All	702,881	Feed for mesocosm inhabitants
Sparidae	<i>Acanthopagrus butcheri</i>	Y	Y-g	All	1,374,144	
	<i>Chrysophrys auratus</i>	Y	Y	All	2,577,546	
	<i>Rhabdosargus sarba</i>	Y	Y	All	5012	
Synodontidae	<i>Synodontidae unknown</i>		Y	C1, C3, Cot	57	Feed for mesocosm inhabitants
Terapontidae	<i>Helotes octolineatus</i>		Y	C, C1, C3, Cot, H, S-AC, S-Z	716	Feed for mesocosm inhabitants
	<i>Torquigener pleurogramma</i>		Y	All	1413	Feed for mesocosm inhabitants
Toxotidae	<i>Toxotidae unknown</i>		Y	C1, C3	20	Found in tanks running to filter tank
Triakidae	<i>Triakidae unknown</i>		Y	E	42	Found in tanks running to filter tank
Trygonorrhinidae	<i>Aptychotrema vincentiana</i>	Y				
	<i>Trygonorrhina fasciata</i>	Y	Y	S-Z	9	Detected as <i>Trygonorrhina guaneri</i>

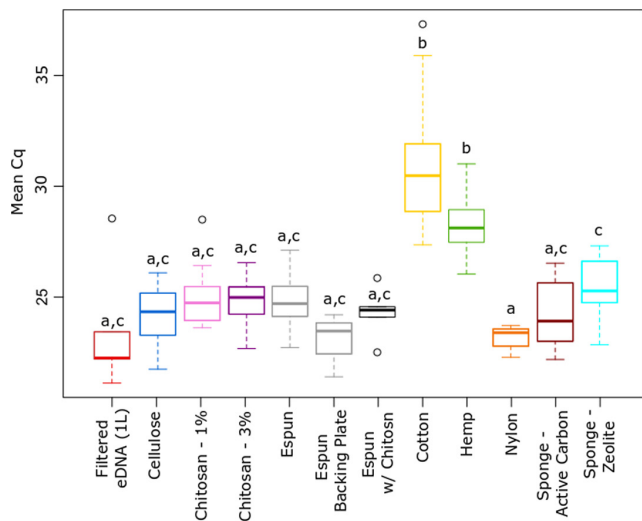


FIGURE 2 Mean Cq values from quantitative PCR by membrane material compared to conventional filtration of 1-L eDNA samples. Note that both cotton and hemp were placed inside nylon bags. Lower Cq values indicate higher DNA yield and different letters indicate statistical significance ($\alpha = .05$)

species detected include known feed taxa, fish found in local intake waters, and fish occupying tanks within the same facility and within the same water system. The number of species detected differed between materials (Figure 4; $F_{21.69, df = 11, p < .01}$), with cotton, hemp and nylon detecting the fewest number of fish species on average. However, all materials detected a comparable number of species to the conventionally filtered eDNA samples.

The median number of fish species detected by material was 27 (filtered eDNA; range = 19–32), 29 (cellulose; 19–33), 31 (chitosan – 1%; 17–36), 31 (chitosan – 3%; 23–37), 29 (espun; 22–31), 26.5 (espun with chitosan; 21–36), 19.5 (cotton; 11–28), 24 (hemp; 14–30), 24 (nylon bags; 18–29), 27 (sponge – active carbon; 13–37) and 29 (sponge – nitrate; 21–34). The number of species detected did not differ with submersion time for any of the nine trialled materials (Figure 5; $F = 0.68$ for submersion time \times material, $df = 9$, $p = .72$).

3.4 | SEM of eDNA collection materials

The SEM images showed how biological matter adhered to the surface of all material (Figure 6) and did not appear entrapped or bound in a consistent manner. These images also revealed the diversity in size and structure of biological matter found on the materials. For example, a “slick” of biological material can be seen on some materials (see Figure 6, cellulose and chitosan – 3%) while others contain small, rounded particles of biological material (see Figure 6, espun, cotton and hemp) or larger particles with irregular shapes that have a crystalline appearance or extremely smooth surface, consistent with inorganic materials and debris such as salt crystals and sediment (see Figure 6, sponge – active carbon).

4 | DISCUSSION

We provide the first comprehensive evaluation of the capacity of a variety of porous materials to passively collect eDNA from a marine environment. Further, we also test the importance of submersion time. We reveal that numerous inexpensive materials are highly effective for the passive collection of eDNA. Remarkably, we also show that passive eDNA collection can be as effective as conventional water filtering and achieved quickly, in as little as 5 min.

4.1 | Materials

We identify multiple materials that can be used for passive eDNA collection. Material choice can influence capture efficiency, and ideally, the selected material will maximize eDNA capture without interfering or complicating the extraction process. We investigated the use of materials which varied in structural complexity and robustness, and found no significant difference in capture efficiency, apart from the reduced capacity of hemp and cotton fibres that were contained in nylon bags. The nylon bag had similar mean Cq values to all other materials, which was presumably capturing eDNA that may have been captured by the hemp and cotton fibres had they not been encased. Kirtane et al. (2020) likewise found that the pore size used to encase an adsorbent had a significant impact on DNA adsorption. They suggest that restricted flow over the adsorbent was associated with smaller pore sizes, and that increasing encasing pore size increases capture. These results highlight the importance of membrane encasing and suggest the maximum surface area of a material should come into direct contact with the water.

There was also some indication that the addition of chitosan to a material could increase capture efficiency, since both chitosan treatments detected the highest median and maximum fish species richness. Although this was not statistically significant, our experiment was conducted in a low-diversity, temperate mesocosm. Since water characteristics, such as pH and temperature, can influence DNA adsorption to different materials (Lorenz & Wackernagel, 1987), it is possible the addition of chitosan could result in increased capture efficiencies in some environments. For example, Bessey et al. (2021) found that nylon membranes performed as well as conventional filtering for fish species detection in low-diversity temperate waters but not in high-diversity tropical waters, presenting a situation where the addition of chitosan could potentially increase the effectiveness of nylon materials. Further investigation into material optimization will be particularly important for high-diversity systems.

Practical considerations for material choice will play an important role because our results reveal many materials are effective for passive eDNA collection. The cost of material, availability, robustness, ease of deployment and downstream processing may all influence material choice. For example, nanomaterials are more time-consuming and costly to produce than readily available cellulose ester membranes and aquarium-grade sponges, which are

FIGURE 3 Mean Cq values from quantitative PCR by submersion time for each membrane material. Open circles represent data for nylon bags (used with both cotton and hemp) which were sampled only at the end of the experiment. A smoothing spline (dashed line) is used to visualize possible time trends and could not be fitted for electrospun nanofibres due to the small sample size

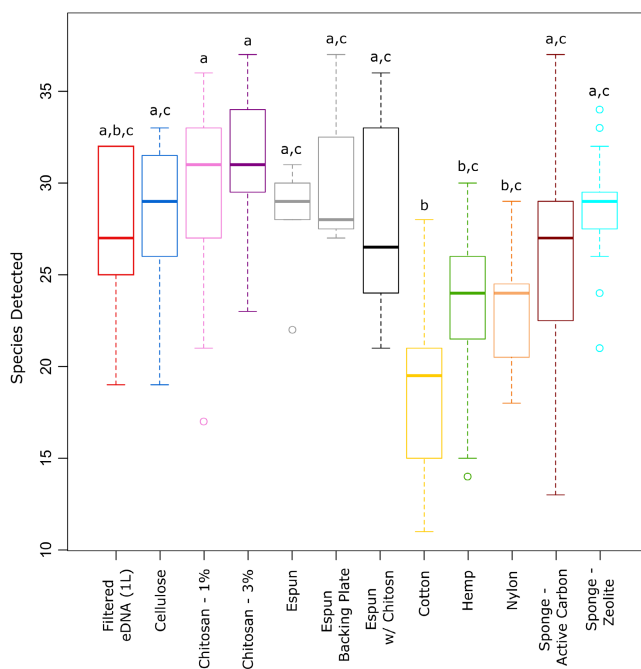
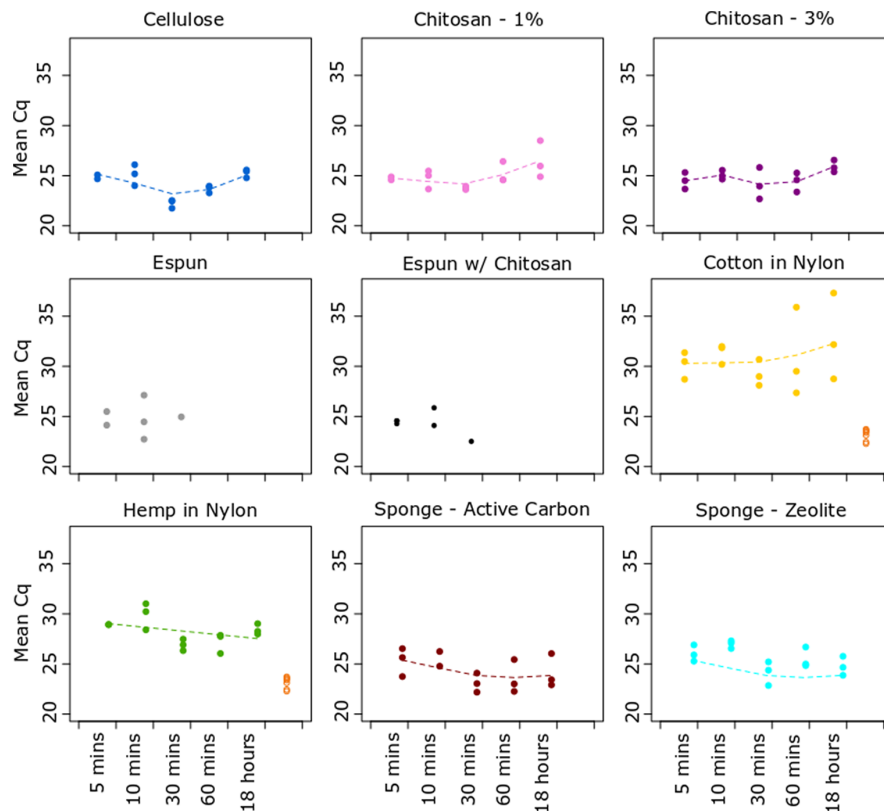


FIGURE 4 The number of fish species detected by membrane material compared to conventional filtration of 1-L eDNA samples. Note that both cotton and hemp were placed inside nylon bags. Different letters indicate statistical significance ($\alpha = .05$)

commercially available (Liu, 2012). Cellulose ester membranes require less handling time during downstream processing than granular materials, which require weighting, or sponge and nylon materials that require cutting prior to DNA extraction (Bessey

et al., 2021). However, in a turbulent, high-flow water environment, a more robust material, such as sponge, may be desirable over the more fragile cellulose membranes. A challenge for employing passive eDNA collection will be finding a standard that can be consistently used so that time series and spatial comparisons are meaningful within and between studies. This challenge similarly exists for conventional eDNA studies (Trujillo-Gonzalez et al., 2021).

4.2 | Time

We determined that long submersion times are not necessary for passive eDNA collection and effective sampling can be achieved in as little as 5 min. Conventional eDNA filtration methods are time-consuming, especially when considering the amount of water needed to effectively filter an area for accurate biodiversity estimates (Bessey et al., 2020; Koziol et al., 2019). A quick eDNA collection method would have considerable benefit for end users and increase sampling capacity. Increased sampling capacity enables a broader range of ecological question to be addressed through comparative frequency analysis (Strickland & Roberts, 2019), which are statistically more powerful with larger sample sizes. Therefore, exploring the minimum amount of time required for passive eDNA collection membranes to saturate would be a worthwhile endeavour to maximize efficiency. Laboratory experiments revealed no difference in adsorbed extracellular DNA concentrations over a time gradient between 1 min to 2 h for granular active carbon in tank water but did find a difference in creek waters (Kirtane et al., 2020). These

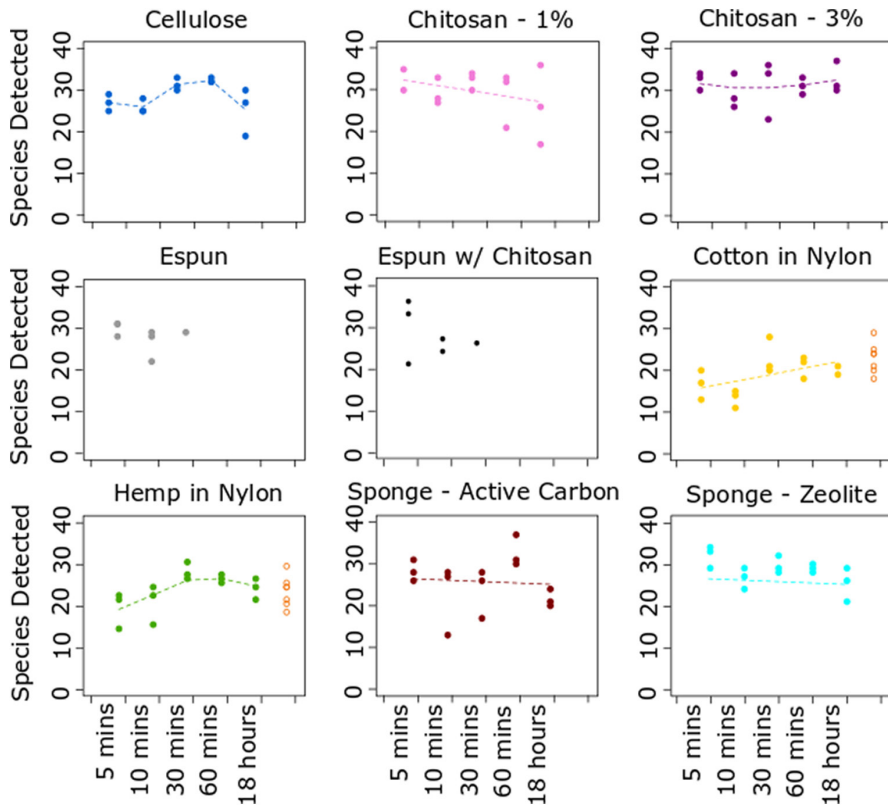


FIGURE 5 The number of fish species detected by submersion time and membrane material. Open circles represent data for nylon bags (used with both cotton and hemp) which were sampled only at the end of the experiment. A smoothing spline (dashed line) is used to visualize possible time trends and could not be fitted for electrospun nanofibres due to the small sample size

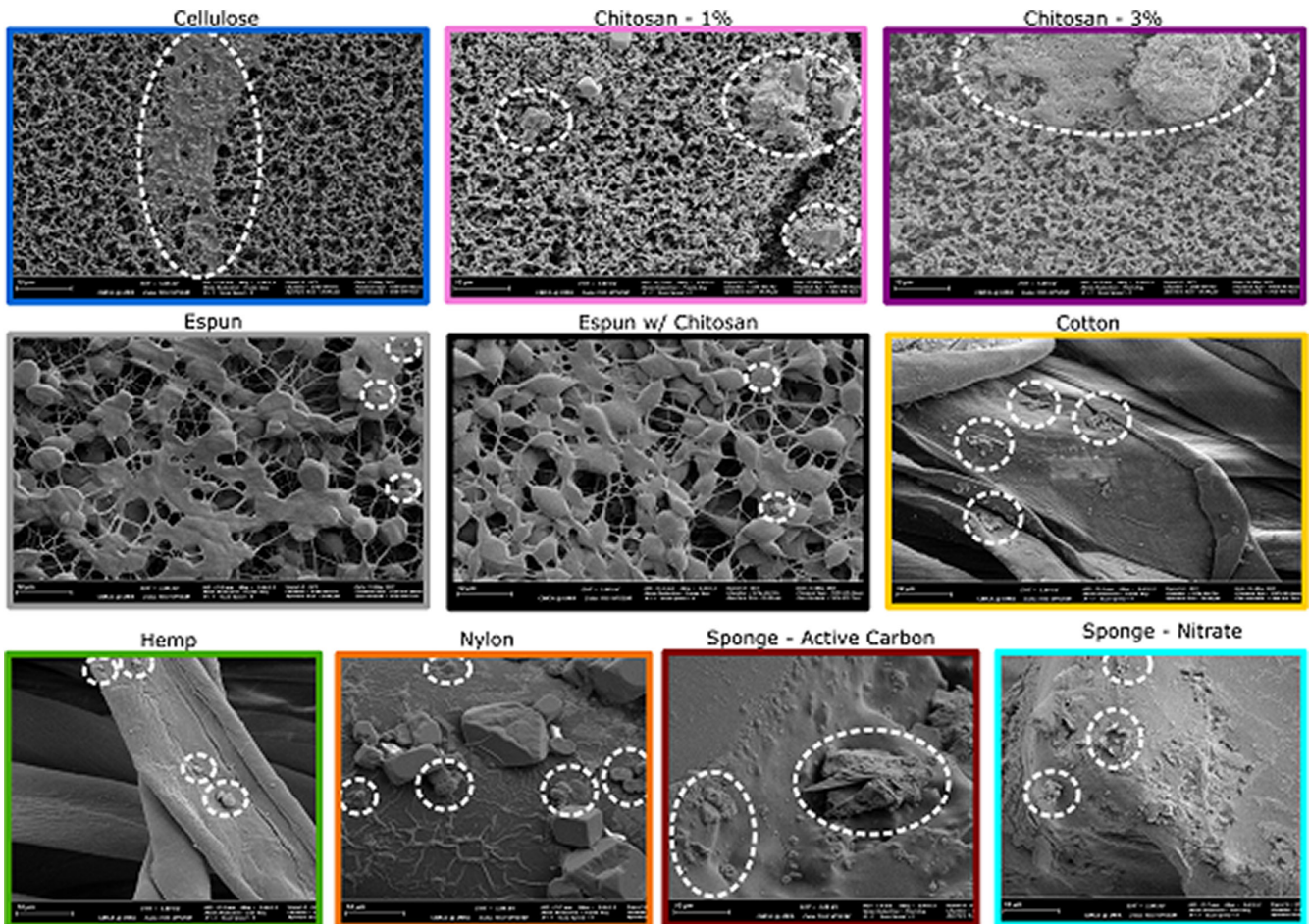


FIGURE 6 Scanning electron micrographs of materials at 5000x magnification after submersion in tank water where dashed circles identify biological matter. All scale bars are 10 µm

previous studies indicate that site-specific water chemistry affects the effectiveness of passive eDNA collection. Therefore, a better understanding of the mechanism of eDNA adherence to materials could help optimize passive eDNA collection methods.

4.3 | Mechanism of eDNA adherence to materials

The mechanism by which eDNA adheres to materials in natural aquatic systems remains unclear. We used SEM to gain insight into the mechanism of attachment but found no consistent patterns. Despite trialling materials with a range of surface complexities, we found no supporting evidence that eDNA was entrapped within the interstitial spaces of the materials. Rather, biological matter appeared to adhere randomly to any available surface and showed great diversity in size and shape. For example, morphologically distinct single-cell eukaryotes and bacteria could be seen on the surfaces of the membrane materials, many embedded in larger bodies of apparent biological material, probably biofilms. An important component of biofilm development is extracellular polymeric substances (Hancock, 2001; Vilain et al., 2009) which are mainly composed of polysaccharides, proteins, metabolites and extracellular DNA (Das et al., 2013). These extracellular polymeric substances occur in a range of molecular sizes, conformations and physical/chemical properties, although little is known about the physical ultrastructure of how they interact (Decho & Gutierrez, 2017). They are known to adhere to both natural and engineered surfaces (Das et al., 2013). The diversity of biological compounds and structures that eDNA might be associated with in aquatic systems, including “sticky” transparent exopolymer particles, is huge (Passow, 2002). Dissolved organic matter (DOM) may contain more than 20,000 compounds in a single seawater sample (Mentges et al., 2017). Particulate organic matter (POM) as seen in Figure 6, contains equal or greater diversity as well as structural complexity because much of it is derived from dead organisms (Kharbush et al., 2020). A deeper understanding of the adhesive properties of different fractions of the POM pool and biofilms associated with passive eDNA collection materials may provide deeper insights into eDNA binding to collection materials.

4.4 | Future research

We contribute to building evidence that passive eDNA collection is effective and offers important advantages over conventional water filtration methods. Our study identified fish species in a relatively low-diversity temperate mesocosm, but future studies should evaluate the effectiveness of materials and submersion time in warmer, high-diversity systems since we know environmental conditions influence eDNA particle size distribution (Barnes et al., 2021; Turner et al., 2014). Even in situations where passive eDNA collection may not perform as optimally as conventional filtering methods, the time and cost efficiencies may still warrant its use, making cost-benefit analysis of which method to use a worthwhile consideration. Future

studies focused on a mechanistic understanding of not only how eDNA adheres to materials, but investigating the physical/chemical properties of eDNA, could lead to the greatest advances in passive eDNA collection methods and optimization of materials.

AUTHOR CONTRIBUTIONS

C.B., Y.G., Y.T., H.M., S.J., O.B. contributed intellectual direction. C.B. designed the study; organized/participated in mesocosm sampling; performed molecular research; processed and analysed all data; conducted statistical analysis, produced graphics and Tables. Y.G., Y.T. designed membrane materials. H.M. conducted SEM imaging and interpretation. C.B., Y.G., Y.T., H.M., S.J., O.B. assisted with manuscript writing. All authors contributed to manuscript revisions.

ACKNOWLEDGEMENTS

This project was funded by the CSIRO Environomics Future Science Platform. We thank Matt O'Malley and AQWA for allowing our experiments to be conducted at the facility. We acknowledge the facilities, and the scientific and technical assistance of the Australian Microscopy, Characterization & Analysis, the University of Western Australia, a facility funded by the University, State and Commonwealth Governments. We also thank Sylvia Osterrieder and Zoe Slatter for their invaluable help during the mesocosm experiment and in the laboratory.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Raw sequences, bioinformatic script, reference database, and the final datasets are available on the CSIRO Data Access Portal at TBD on acceptance (raw sequence and final dataset) and <https://data.csiro.au/collections/collection/Clcisiro:46025v1> (bioinformatic script and reference database).

ORCID

Cindy Bessey  <https://orcid.org/0000-0002-4081-0214>

Yen Bach Truong  <https://orcid.org/0000-0003-2210-2414>

Haylea Miller  <https://orcid.org/0000-0003-3312-7107>

Simon Neil Jarman  <https://orcid.org/0000-0002-0792-9686>

Oliver Berry  <https://orcid.org/0000-0001-7545-5083>

REFERENCES

- Barnes, M. A., Chadderton, W. L., Jerde, C. L., Mahon, A. R., Turner, C. R., & Lodge, D. M. (2021). Environmental conditions influence eDNA particle size distribution in aquatic systems. *Environmental DNA*, 3(3), 643–653.
- Berry, T. E., Osterrieder, S. K., Murray, D. C., Coghlan, M. L., Richardson, A. J., Grealy, A. K., Stat, M., Bejder, L., & Bunce, M. (2017). DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecology and Evolution*, 7, 5435–5453.
- Bessey, C., Jarman, S. N., Simpson, T., Miller, H., Stewart, T., Keesing, J. K., & Berry, O. (2021). Passive eDNA collection enhances aquatic biodiversity analysis. *Communications Biology*, 4, 236. <https://doi.org/10.1038/s42003-021-01760-8>

- Bessey, C., Jarman, S. N., Berry, O., Olsen, Y. S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G. J., & Keesing, J. (2020). Maximizing fish detection with eDNA metabarcoding. *Environmental DNA*, 2(4), 493–504.
- Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances*, 28, 325–347.
- Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., & Sweet, M. (2021). Improving the reliability of eDNA data interpretation. *Molecular Ecology Resources*, 21(5), 1422–1433.
- Buxton, A., Matechou, E., Griffin, J., Diana, A., & Griffiths, R. A. (2021). Optimizing sampling and analysis protocols in environmental DNA studies. *Scientific Reports*, 11, 637.
- Das, T., Sehar, S., & Manefield, M. (2013). The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. *Environmental Microbiology Reports*, 5, 778–786.
- Deagle, B. E., Gales, N. J., Evans, K., Jarman, S. N., Robinson, S., Trebilco, R., & Hindell, M. A. (2007). Studying seabird diet through genetic analysis of faeces: A case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS ONE*, 2(9), e831.
- Decho, A. W., & Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSs) in ocean systems. *Frontiers in Microbiology*, 8, 922.
- Deiner, K., Bik, H. M., Machler, E., Seymour, M., Lacoursiere-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology*, 26, 5872–5895.
- Derocles, S. A. P., Bohan, D. A., Dumbrell, A. J., Kitson, J. J. N., Massol, F., Pauvert, C., Plantegenest, M., Vacher, C., & Evans, D. M. (2018). Biomonitoring for the 21st century: Integrating next-generation sequencing into ecological network analysis. *Advances in Ecological Research*, 58, 1–62.
- Dickie, I. A., Boyer, S., Buckley, H. L., Duncan, R. P., Gardner, P. P., Hogg, I. D., Holdaway, R. J., Lear, G., Makiola, A., Morales, S. E., Powell, J. R., & Weaver, L. (2018). Towards robust and repeatable sampling methods in eDNA-based studies. *Molecular Ecology Resources*, 18, 940–952.
- Gao, Y., Truong, Y. B., Zhu, Y., & Kyrtzsis, L. I. (2014). Electrospun antibacterial nanofibers: Production, activity, and in vivo applications. *Journal of Applied Polymer Science*, 15, 131.
- Hancock, R. E. W. (2001). A brief on bacterial biofilms. *Nature Genetics*, 29, 360.
- Jarman, S. N., Berry, O., & Bunce, M. (2018). The value of environmental DNA biobanking for long-term biomonitoring. *Nature Ecology & Evolution*, 2, 1192–1193.
- Kharbush, J. J., Close, H. G., Van Mooy, B. A. S., Arnosti, C., Smittenberg, R. H., Le Moigne, F. A. C., Mollenhauer, G., Scholz-Bottcher, B., Obrecht, I., Koch, B. P., Becker, K. W., Iversen, M. H., & Mohr, W. (2020). Particulate organic carbon deconstructed: Molecular and chemical composition of particulate organic carbon in the ocean. *Frontiers in Marine Science*, 7, 818.
- Kirtane, A., Atkinson, J. D., & Sassoubre, L. (2020). Design and validation of passive environmental DNA samplers using granular activated carbon and montmorillonite clay. *Environmental Science and Technology*, 54, 11961–11970. <https://doi.org/10.1021/acs.est.0c01863>
- Kozioł, A., Stat, M., Simpson, T., Jarman, S., DiBattista, J. D., Harvey, E. S., Marnane, M., McDonald, J., & Bunce, M. (2019). Environmental DNA metabarcoding studies are critically affected by substrate selection. *Molecular Ecology Resources*, 19(2), 366–376.
- Liu, J. W. (2012). Adsorption of DNA onto gold nanoparticle and graphene oxide: Surface science and applications. *Physical Chemistry Chemical Physics*, 14, 10485–10496.
- Lorenz, M. G., & Wackernagel, W. (1987). Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. *Applied and Environmental Microbiology*, 53, 2948–2952.
- MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A., & Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, 83(8), 2248–2255.
- Mentges, A., Feenders, C., Seibt, M., Blasius, B., & Dittmar, T. (2017). Functional molecular diversity of marine dissolved organic matter is reduced during degradation. *Frontiers in Marine Science*, 4, 194.
- Murray, D. C., Coghlan, M. L., & Bunce, M. (2015). From benchtop to desktop: Important considerations when designing amplicon sequencing workflows. *PLoS One*, 10, e0124671.
- Passow, U. (2002). Transparent exopolymer particles (TEP) in aquatic environments. *Progress in Oceanography*, 55, 287–333.
- Pandit, K. R., Nanayakkara, I. A., Cao, W., Raghavan, S. R., & White, I. M. (2015). Capture and direct amplification of DNA on chitosan micro-particles in a single PCR-optimal solution. *Analytical Chemistry*, 87, 11022–11029.
- Prosser, J. I. (2010). Replicate or lie. *Environmental Microbiology*, 12(7), 1806–1810.
- R Core Team (2021). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna. <https://www.R-project.org/>
- Strickland, G. J., & Roberts, J. H. (2019). Utility of eDNA and occupancy models for monitoring an endangered fish across diverse riverine habitats. *Hydrobiologia*, 826, 129–144.
- Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA, for biodiversity research and monitoring*. Oxford University Press.
- Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183, 4–18.
- Trujillo-Gonzalez, A., Villacorta-Rath, C., White, N. E., Furlan, E. M., Sykes, M., Gossel, G., Divi, U. K., & Gleeson, D. (2021). Considerations for future environmental DNA accreditation and proficiency testing schemes. *Environmental DNA*, 1049–1058. <https://doi.org/10.1002/edn3.243>
- Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods in Ecology and Evolution*, 5(7), 676–684.
- Tsuji, S., Takahara, T., Doi, H., Shibata, N., & Yamanaka, H. (2019). The detection of aquatic macroorganisms using environmental DNA analysis – A review of methods for collection, extraction, and detection. *Environmental DNA*, 1, 99–108.
- Vilain, S., Pretorius, J. M., Theron, J., & Brozel, V. S. (2009). DNA as an adhesion: *Bacillus cereus* requires extracellular DNA to form biofilms. *Applied Environmental Microbiology*, 75, 2861–2868.
- Zinger, L., Bonin, A., Alsos, I. G., Balint, M., Bik, H., Boyer, F., Chariton, A. A., Creer, S., Coissac, E., Deagle, B. E., De Barba, M., Dickie, I. A., Dumbrell, A. J., Ficetola, G. F., Fierer, N., Fumagalli, L., Gilbert, M. T. P., Jarman, S., Jumpponen, A., ... Taberlet, P. (2019). DNA metabarcoding – Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology*, 28, 1857–1862.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Bessey, C., Gao, Y., Truong, Y. B., Miller, H., Jarman, S. N., & Berry, O. (2022). Comparison of materials for rapid passive collection of environmental DNA. *Molecular Ecology Resources*, 22, 2559–2572. <https://doi.org/10.1111/1755-0998.13640>