Received: 26 June 2023 | Revised: 5 October 2023 | Accepted: 10 October 2023

DOI: 10.1002/edn3.485

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences
WILEY

ORIGINAL ARTICLE

Seas the DNA? Limited detection of cetaceans by low-volume environmental DNA transect surveys

Chloe V. Robinson | Amy Migneault | Karina Dracott | Robin D. Glover

Whales Initiative, Ocean Wise, Vancouver, British Columbia, Canada

Correspondence
Chloe V. Robinson, Whales Initiative, Ocean Wise, Vancouver, BC, Canada.
Email: chloe.robinson@ocean.org

Funding information
Fisheries and Oceans Canada, Grant/Award Number: 2022-083-Pac-PR

Abstract

Environmental DNA (eDNA) has begun to show promise as a robust and reproducible tool for monitoring cetaceans in coastal and offshore waters. Some limiting factors preventing the wider application of eDNA for cetacean monitoring includes lack of species-specific qPCR assays and limited in situ validation. In this study, we determined 15 monitoring stations within cetacean hotspots in Chatham Sound (British Columbia, Canada), from which we collected a combination of visual and acoustic data, and low-volume eDNA samples (equivalent to ~250 mL seawater). We designed novel eDNA assays for gray whale and Dall's porpoise and validated existing assays for harbor porpoise, killer whale, and humpback whale. Overall, we collected a total of 120 paired eDNA samples across four sampling intervals, 60 preserved with absolute ethanol and 60 preserved with propylene glycol antifreeze. Positive rates for visual (18%) and acoustic (4%) detections were higher than the eDNA detection rate (<3%), with only one sample (antifreeze-preserved) producing a positive detection for humpback whales at one of the stations. We discuss factors which could have influenced the lack of detections and highlight the need for higher sample volumes and species-specific sample approaches to improve detection success and confidence in eDNA applicability for cetacean monitoring.

KEYWORDS
environmental DNA, *Eschrichtius robustus*, marine mammal monitoring, *Megaptera novaeangliae*, *Orcinus orca*, *Phocoena phocoena*, *Phocoenoides dalli*, qPCR

1 | INTRODUCTION

Environmental DNA (eDNA) is a proven successful approach for capturing species diversity metrics in aquatic systems (Belle et al., 2019; Jerde et al., 2011; Lodge et al., 2012; Thomsen & Willerslev, 2015). More recently, there has been an increased uptake of utilizing eDNA as a tool for detecting and monitoring marine biodiversity (Dalongeville et al., 2022; Gilbey et al., 2021; Kopp et al., 2023; Miya, 2022). Despite this, there remains limited application of eDNA for marine mammal monitoring (Suarez-Bregua et al., 2022). Though scarce, previous cetacean (whales, dolphins, and porpoise) studies have primarily focused on detecting single species through either targeted 'flukeprint' sampling (Alter et al., 2022; Baker et al., 2018; Parsons et al., 2018; Robinson et al., unpublished data; Székely et al., 2021), or indirect sampling via transects (or similar; Juhel et al., 2021; Ma et al., 2016; Székely et al., 2021; Zhang et al., 2023). A portion of these studies have employed DNA metabarcoding via Next-Generation Sequencing (NGS) to detect target taxa

(Alter et al., 2022; Juhel et al., 2021; Valsecchi et al., 2021; Zhang et al., 2023), with a majority of these studies employing species-specific detection approaches (Baker et al., 2018; Foote et al., 2012; Ma et al., 2016; Parsons et al., 2018; Qu & Stewart, 2019; Robinson et al., unpublished data; Székely et al., 2021). To date, species-specific assays currently exist for only five out of the global 94 species of *Cetacea*, including killer whales (*Orcinus orca*; Baker et al., 2018), harbor porpoises (*Phocoena phocoena*; Foote et al., 2012; Parsons et al., 2018), bowhead whales (*Balaena mysticetus*; Székely et al., 2021), Yangtze finless porpoise (*Neophocaena asiaeorientalis*; Ma et al., 2016; Qu & Stewart, 2019), and humpback whales (*Megaptera novaeangliae*; Robinson et al., unpublished data).

Similar to the scarcity of cetacean-based studies, reported detection rates of cetacean eDNA in situ are equally as limited (Suarez-Bregua et al., 2022). A combination of volume sampled, environmental conditions at the time of DNA deposition and collection, technology and approaches used for DNA detection (i.e., quantitative PCR (qPCR) vs. droplet digital PCR), and proximity to target species are factors that determine both DNA degradation rates and/or likelihood of detecting target species (Barnes et al., 2014; Cao et al., 2012; Padilla et al., 2015; Pinfield et al., 2019; Schabacker et al., 2020; Sepulveda et al., 2019; Strickler et al., 2015; Suarez-Bregua et al., 2022; Xiong et al., 2016). Lack of knowledge surrounding the role of environmental factors on the temporal and spatial persistence of cetacean eDNA has been identified as a major limitation for the application of eDNA for cetacean monitoring (Suarez-Bregua et al., 2022).

More common methods for monitoring cetacean presence mostly consist of a combination of visual and acoustic techniques (Cartagena-Matos et al., 2021; Dalpaz et al., 2021; Liu et al., 2022), including aerial and satellite surveillance (Boulent et al., 2023; Charry et al., 2021). Each of these approaches includes inherent biases to data collection; acoustic monitoring can be spatially limited and focused toward only a portion of species depending on the device used (Barkley et al., 2021; Liu et al., 2022; Rice et al., 2021), whereas visual boat-based surveys that cover a wider spatial area than acoustic monitoring, however, are limited to when animals surface to breathe, time of day and year, visibility, weather, sea state, observer bias, and vessel avoidance bias (Dalpaz et al., 2021; Forney et al., 1991; Marsh & Sinclair, 1989; Oliveira-Rodrigues et al., 2022). In addition to these methods, citizen science, or community-based monitoring initiatives, worldwide also contribute important data toward understanding cetacean distribution and abundance (Cheeseman et al., 2023; Gutiérrez et al., 2021; Mancini & Elsadek, 2019; Mwango'mbe et al., 2021; Pirotta et al., 2020; Rodriguez et al., 2021).

Combining citizen science (or community-based monitoring) with eDNA tools has been shown to be a powerful approach for monitoring aquatic species (Feng & Lougheed, 2023; Miya et al., 2022; Valsecchi et al., 2023). There is the potential for community-based eDNA monitoring initiatives to be pivotal for monitoring cetaceans. However, there are factors which need to first be addressed to ensure quality and consistency of data collected. For cetacean eDNA approaches, there is a lack of standardization concerning water volumes collected, preservative used, and time taken between collection and processing of samples (Alter et al., 2022; Baker et al., 2018; Foote et al., 2012; Juhel et al., 2021; Ma et al., 2016; Parsons et al., 2018; Pinfield et al., 2019; Qu & Stewart, 2019; Suarez-Bregua et al., 2022). In previous studies, volumes of water collected vary between 50 mL and 30 L (Foote et al., 2012; Juhel et al., 2021) have been low at ~1 L (Alter et al., 2022; Ma et al., 2016; Pinfield et al., 2019; Székely et al., 2021), and preservatives vary from conservation buffer (Juhel et al., 2021), to Longmire's buffer (Parsons et al., 2018; Pinfield et al., 2019), DMSO (Székely et al., 2021), and ethanol (Ma et al., 2016; Robinson et al., unpublished data). Propylene glycol-based antifreeze is effective as a safe, cost-friendly, and easy preservative that has been incorporated into freshwater-based community-based monitoring initiatives (Robinson et al., 2021); however, its utility has not yet been tested on seawater samples. qPCR detection of eDNA offers higher sensitivity compered to metabarcoding approaches (McColl-Gausden et al., 2023; Yu et al., 2022), making it a good candidate for cetacean studies. Employing eDNA monitoring programs for cetaceans in conjunction with existing techniques, such as community-based monitoring, has the potential to improve upon species detections, particularly within cetacean hotspots and for elusive species, while also increasing public engagement with conservation (Alter et al., 2022; Miya et al., 2022).

Within the northeast Pacific Ocean, Chatham Sound and surrounding waters in British Columbia (BC, Canada), provides an important habitat for several species of cetacean listed on the Canadian *Species at Risk Act* (SARA), including humpback whales, gray whales (*Eschrichtius robustus*), killer whales, and harbor porpoises (Dracott et al., 2022; Frouin-Mouy et al., 2022; Wright et al., 2021), and non-SARA-listed species including Dall's porpoises (*Phocoenoides dalli*). This cetacean hotspot can be challenging to monitor particularly during winter months due to the frequency of storms caused by a combination of wind, large tides, shoals, and shallows. From the northeast, katabatic winds are generated by cold air funneling down from the mountains through the many region's fjords and often oppose Arctic low-pressure systems born over Alaska. These severe weather systems are a large part as to why temporal and spatial patterns in cetacean habitat use remains relatively understudied along this part of the coast (Frouin-Mouy et al., 2022). In addition, Chatham Sound is an area undergoing rapid anthropogenic development, with shipping traffic due to increase substantially because of projects such as the DP World (Dubai Ports World) Fairview Terminal expansion (Prince Rupert Port Authority, 2022). Considering this pending expansion and subsequent increase in human activity, employing robust monitoring methods to simultaneously close cetacean distribution data gaps and monitor potential effects of increased anthropogenic activity is of great importance.

In this study, we aimed to (1) develop specific eDNA high-resolution melt (HRM) assays for gray whales and Dall's porpoise; (2) compare low-volume eDNA detection with visual and acoustic monitoring methods for five target cetacean species – humpback whales,

Dall's porpoise, harbor porpoise, killer whales, and gray whales within Chatham Sound, and (3) explore utility of non-toxic propylene glycol-based antifreeze as an ethanol preservative alternative for cetacean eDNA.

2 | MATERIALS AND METHODS

2.1 | Data collection

Total of 15 stations were identified as locations of cetacean hotspots around Chatham Sound near Prince Rupert (BC, Canada), determined through examination of historical Ocean Wise Sightings Network cetacean sightings reports and local knowledge (Figure 1; Table S1). At each station, a dedicated 10 min 'Stop, Look, Listen' protocol was used onboard the research vessel – 'Tsitika', a 6 m purpose-designed research vessel, where one team member conducted a 360° visual scan of the surrounding water using binoculars and recorded any cetaceans in the area including species, best count estimate, behavior, and distance. Simultaneously, a second team member deployed an Ocean Sonics icListen hydrophone at a depth range of 15–30 m (depending on the depth of the station location), to record the presence of any audible cetacean vocalizations. For collecting low-volume eDNA samples, a third team member (wearing sterile nitrile gloves) collected two 500 mL water samples using sterile 500 mL Nalgene bottles attached to a 2 m long sampling pole submerged ~1 m into the water column. These samples were immediately filtered in situ onboard the research vessel using 0.45 µm Polyethersulfone self-preserving filter units (Smith-Root, USA; Thomas et al., 2019) and the eDNA Citizen Scientist Sampler (Smith-Root). Filter units were re-placed into individual packets stored at room temperature (20–22°C) until further processing. Each station was sampled four times within the months of June–October 2022 for a total of 60 'Stop, Look, Listen' surveys (Table 1).

2.2 | eDNA processing

After storage at room temperature for between 0 and 3 days following sample collection, filters from the eDNA self-preserving filter units collected during each 'Stop, Look, Listen' survey were carefully removed and folded into a cone shape using sterile forceps in a space that was decontaminated using a 30% bleach solution. In order to test the two preservatives on these desiccated filters, one filter was placed in a 1.5 mL Eppendorf tube containing 90% EtOH and the other in an Eppendorf tube containing Absolute Zëro™ RV Waterline Antifreeze. New nitrile gloves were worn for processing each filter. eDNA samples in this study were stored at 4°C for ~20 days before being shipped to the Pacific Science Enterprise Center (PSEC) in Vancouver (BC, Canada), following all Transportation of Dangerous Goods (TDG) regulations, for subsequent DNA extraction and qPCR screening.

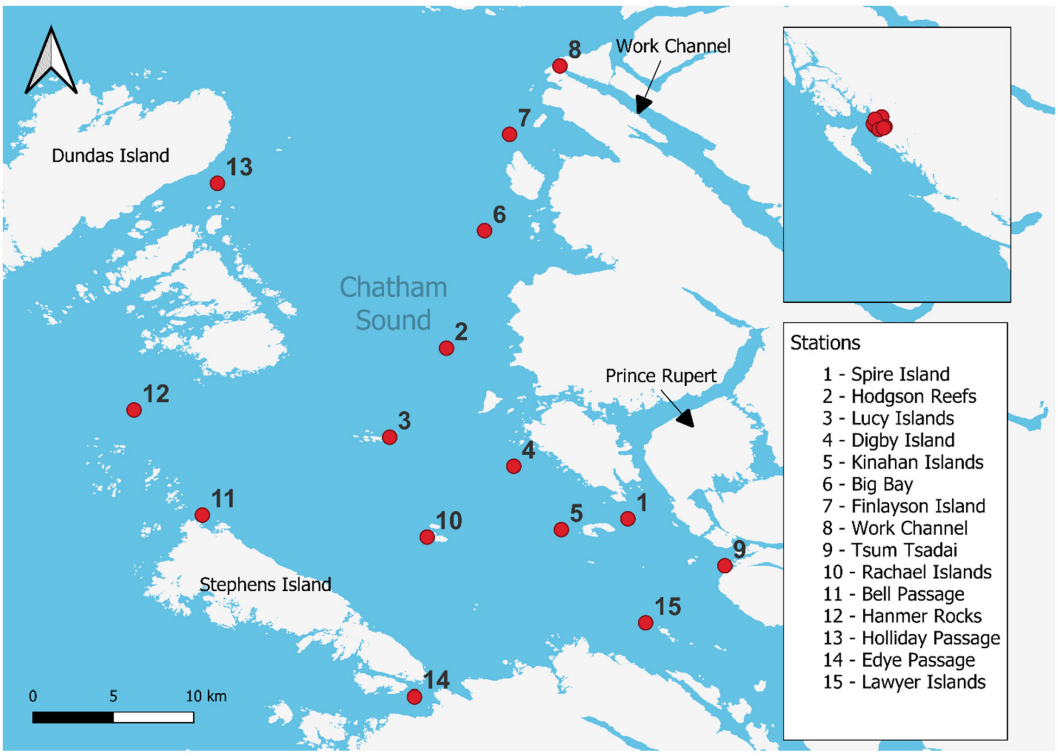

FIGURE 1 Map depicting the 15 'Stop, Look, Listen' stations within Chatham Sound and surrounding waters near Prince Rupert (British Columbia, Canada). Inset map (top right) shows study area in relation to wider central and northern British Columbia.

TABLE 1 Summary table of each sampling period (1–4) across the 15 monitoring stations related to the number of visual, acoustic, and environmental DNA (eDNA) detections.

Sample period	Sample period start (2022)	Sample period end (2022)	Sample numbers	Number of visual detections (species)	Number of acoustic detections (species)	Number of eDNA detections (species)
1	June 22	July 7	1–30	3 (HW ×2, HP)		
2	July 20	August 5	31–60	3 (HW ×3)	1 (BKW)	1 (HW)
3	August 24	September 8	61–90	3 (HW ×2, HP)		
4	September 20	October 20	91–120	2 (HW ×2, HP)	1 (HW)	

Abbreviations: BKW, Bigg's (Transient) killer whale; HP, harbor porpoise; HW, humpback whale.

2.3 | qPCR assay design and validation

Species-specific qPCR primers were designed for gray whales and Dall's porpoises using PrimerBlast (NCBI, http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Parameters were set to produce a DNA product between 50 and 180 bp in length (all other parameters remained at default). Resulting primers EscRobu_COI (forward: 5′-CGTGCTAGTAACAGCCCAC-3′; reverse: 5′- GTGCTCCG ATCATTAGGGGG-3′) for gray whales and PhoDall_COI (forward: 5′- ATTGGAGCCCCTGATATGGC-3′; reverse: 5′-CATGTGCTAGG TTCCCTGC-3′) for Dall's porpoises were designed to amplify the cytochrome oxidase I (COI) gene on mitochondrial DNA (mtDNA) to produce products of 105 bp (gray whale) and 157 bp (Dall's porpoise). Specificity checks were conducted for both primer sets, using BioEdit (Hall, 1999) to align COI sequences to the designed primers to check for number of mismatches from commonly occurring species including killer whale, Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), minke whale (*Balaenoptera acutorostrata*), humpback whale, and harbor porpoise; Figures S1 and S2). For species which had three or fewer mismatches with both forward and reverse primers, we conducted further specificity testing by using extracted DNA from tissue samples (CITES permit no. 22CA02349) to test the assays for non-specific amplification.

We performed in silico testing of both gray whale and Dall's porpoise primers on an Applied Biosystems StepOne™ qPCR cycler to determine the amplification efficiency of each assay. Standard curves were run to calculate the limit of detection (LOD) via a 10-fold dilution series, ranging from 5 ng/μL to 5×10^{-5} ng/μL (quantified by a NanoDrop™ 2000 spectrophotometer), using extracted DNA from gray whale and Dall's porpoise tissue respectively. The annealing temperatures for each primer set were optimized at 58°C ($R^2=0.99$; gray whale) and 56°C ($R^2=0.98$; Dall's porpoise; Table 2).

For killer whales and harbor porpoises, we used previously designed and published eDNA primers (Baker et al., 2018; Parsons et al., 2018). Assays Oordlp6.5F/dlp8G (for killer whales) and Ppho_Cytb F/R (for harbor porpoises; Table 2) were also tested in vitro to determine efficiency for both species ($R^2=0.99$ and $R^2=0.98$, respectively). Lastly, qPCR primers (MegNova_COI) previously designed and validated for humpback whales were used in this study (see Appendix S1 for details); however, no in silico tests were required as these primers have already undergone in silico validation in our previous flukeprint study (Robinson et al., unpublished data).

Assay optimizations for killer whale, harbor porpoise, gray whale, and Dall's porpoise primer assays were undertaken in a total reaction volume of 10 μL, with 7.5 μL PowerUp™ SYBR™ Green Master Mix, 0.5 μL of each forward and reverse primer, 4.5 μL molecular-grade water, and 2 μL of template DNA (at ~2 ng/μL), using the following cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 56°C for 20 s and 72°C for 30 s. We applied an additional HRM step ranging from 65 to 95°C in 0.1°C increments to the end of qPCR protocol, to assess the consistency of amplicon melt temperature (T_m) for each species.

2.4 | DNA extraction and qPCR screening

DNA extractions were completed in a dedicated section of the genomics laboratory. Movements between this space and the main laboratory were restricted to a one-way flow, to prevent the contamination of eDNA samples by PCR products.

DNA extractions were performed using the Qiagen DNeasy Blood & Tissue Kit. The extraction protocol was based on the Qiagen DNeasy Blood & Tissue Kit instructions with some modifications. Half of each filter membrane was cut into small pieces (estimated 3 mm in diameter) using sterile razor blade and forceps and then placed into a 1.5 mL microcentrifuge tube. The remaining half of each filter membrane was placed back into its original sample tube and kept as an archive. Next, 300 μL of Buffer ATL, 340 μL of Phosphate-buffered saline, and a single sterile metal bead was added to each 1.5 mL tube containing a filter membrane. The tubes were shaken in a tissue lyser for 45 s before 30 μL of Proteinase K was added to each tube. The tubes were then mixed by vortexing and then incubated at 56°C in a tube rotator overnight. The remainder of the extraction protocol followed manufacturer recommendations for the Qiagen DNeasy Blood & Tissue Kit, except for warming the elution buffer to 55°C prior to adding to the column membrane in two 25 μL quantities instead of a single 100 μL step to increase DNA yield.

After DNA extraction, eDNA samples were quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific™) before being run with each of the five primer assays in duplicate on an Applied Biosystems StepOne™ qPCR cycler. Reaction and cycling conditions used to optimize the assays were the same as the conditions used to amplify eDNA station samples. On each plate, a positive control (DNA from respective target species tissue) and

TABLE 2 List of the five quantitative PCR (qPCR) assays used in this study, including primer sequences, product length, annealing temperature of assay (°C), limit of detection (LOD; ng/μL), assay efficiency (R^2), and melt temperature (°C), of DNA product.

Species common name	Assay name	Forward primer (3′ to 5′)	Reverse primer (3′ to 5′)	Product length (bp)	Annealing temperature (°C)	LOD (ng/μL) (R^2)	Product melt temperature (°C ± 0.2)
Harbor porpoise	Ppho_Cytb F/R[a]	GTTCTTCATTTGTCTTTATATCCATATTG	GCACCTCAAAATGATATTTGTCCT	160	55	0.05 (0.98)	78.5
Dall's porpoise	PhoDall_COI[b]	ATTGGAGCCCCTGATATGGC	CATGTGCTAGGTTCCCTGC	157	56	0.05 (0.98)	81.9
Killer whale	Oordlp6.5F/dlp8G[c]	AGCTTGCACCGACTCAGCTAT	GGAGTACTATGTCCTGTAACCA	139	58	0.05 (0.99)	79.6[d]
Humpback whale	MegNova_COI[b]	GACCCAATATCAGACCCCTC	AACGGGTAATGATAGTAGGAGTAAT	76	56	0.05 (0.98)	75.1
Gray whale	EscRobu_COI[b]	ACGTGCTAGTAACAGCCCAC	GTGCTCCGATCATTAGGGGG	105	58	0.0005 (0.99)	77.9

[a]Parsons et al. (2018).
[b]Assay developed for this study.
[c]Baker et al. (2018).
[d]Offshore killer whale product melt temperature = 80.2°C.

negative controls (molecular grade water in place of eDNA) were run to test for false negatives and contamination. An eDNA sample was considered a positive if one out of two replicates amplified, resulting in a product with a melt peak specific for killer whale DNA (all ecotypes other than offshores: 79.6 ± 0.2°C; offshore killer whales 80.2 ± 0.2°C), harbor porpoise DNA (78.5 ± 0.2°C), gray whale DNA (77.9 ± 0.2°C), Dall's porpoise DNA (81.9 ± 0.2°C), and humpback whale DNA (75.0 ± 0.2°C; Table 2; Figure 2).

2.5 | Statistical analyses

All statistical analyses were carried out in R Studio version 2023.03.1 (RStudio, 2023) using R version 4.3.0 (R Core Team, 2023). To determine if there was any significant difference between the average total eDNA concentrations of samples between the two preservatives, we first conducted a Shapiro–Wilk normality test to assess normality of the data (Shapiro & Wilk, 1965). Following this, we conducted a Kruskal–Wallis test (Kruskal & Wallis, 1952). We used package *ggplot2* (Wickham et al., 2022) to plot DNA concentrations for both each station and preservative.

3 | RESULTS

3.1 | qPCR primer optimization and specificity

Assays for both gray whale and Dall's porpoise assays were found to be specific for each target species. There was no evidence of non-specific amplification with minke whale or humpback whale DNA for the gray whale assay and (Table S2), and no evidence of non-specific amplification of killer whale or harbor porpoise DNA for the Dall's porpoise assay (Table S2). The LOD for each assay ranged between 0.05 and 0.005 ng/μL, efficiency (R^2) ranged from 0.98 to 0.99, and species-specific product melt temperatures were consistent for each species within ±0.2°C (Table 2; Table S3). Previous assay tests for humpback whale primers concluded that MegNova_COI primers were specific for the target species, with no non-specific cross amplification (Robinson et al., unpublished data).

3.2 | Cetacean visual and acoustic detection

Cetaceans were visually observed at 11 separate station visits throughout the four sampling periods. Humpback whales were observed at station two and station eight across three sampling periods, station 11 across two sampling periods, and station 14 for one sampling period (Table S4; Table 1). Harbor porpoises were observed at station one, station 10, and station 11 for a single sampling period (Table S4; Table 1). For both species, visual detections ranged from an estimated 60 m to 8 km. Acoustically, Bigg's (Transient) killer whales and humpback whales were detected at station 12 within two separate sampling periods (Table S4; Table 1). There was no

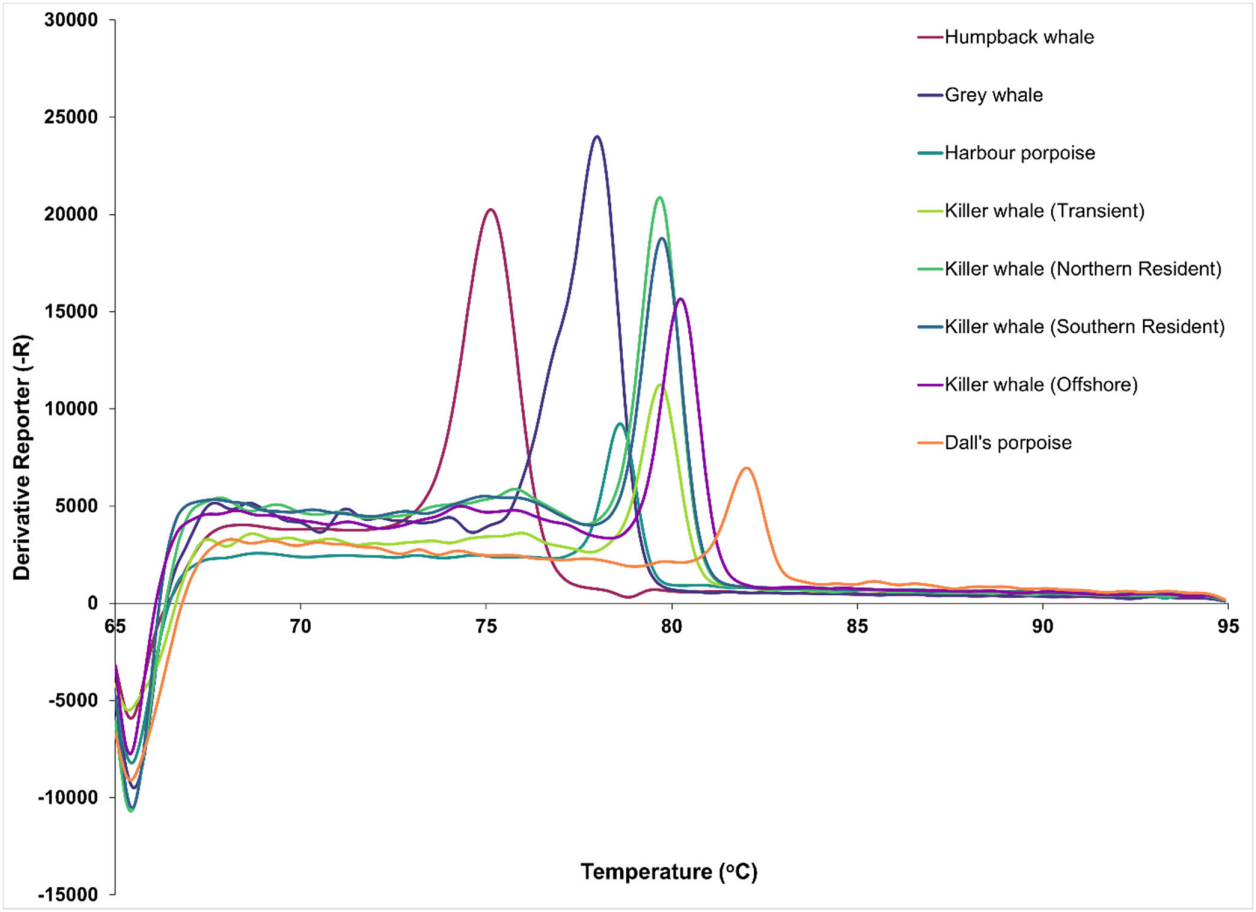

FIGURE 2 Optimized DNA product melt curves for humpback whale (*Megaptera novaeangliae*; 75.0 ± 0.2°C), gray whale (*Eschrichtius robustus*; 77.9 ± 0.2°C), harbor porpoise (*Phocoena phocoena*; 78.5 ± 0.2°C), killer whale (*Orcinus orca*; Transient, Northern Resident, and Southern Resident: 79.6 ± 0.2°C; Offshore: 80.2 ± 0.2°C), and Dall's porpoise (*Phocoenoides dalli*; 81.9 ± 0.2°C). Note: DNA concentrations for each species were between 5 and 20 ng/μL.

overlap of both positive visual and acoustic detections for the same time point at any one station for any of the five target species.

3.3 | Effect of preservatives

A total of 120 eDNA samples were collected throughout the study period. Total DNA concentrations for these samples ranged from 0.5 to 66.7 ng/μL (Table S4). Mean DNA concentration from samples was 9.6 ng/μL, with the highest concentration of DNA originating from station 14 (Edye Pass; Figure S3). The mean DNA concentration was higher for ethanol (12.7 ng/μL) compared to antifreeze (6.6 ng/μL) across all samples. Overall, there was a significant difference in DNA concentration and preservative used ($W = 0.69$, $p < 0.001$; Figure 3), with ethanol-preserved samples producing higher DNA yields (Figure S3, Figure 3).

3.4 | Cetacean eDNA detection

Despite positively detecting cetaceans by visual or acoustic observation at 21% of the total number of station visits, only one species of cetacean was detected at one station via eDNA analyzed in ~250 mL water (humpback whale at station eight; Table S4; Table 1). At this station, 6–7 humpback whales were observed at the time of collection at an approximate distance of 300 m from the research vessel (Table S4; Figure S4). For this eDNA sample, humpback whale DNA was detected in both replicates of the antifreeze-preserved sample (DNA concentration of 1.5 ng/μL), with no positive detection from the corresponding ethanol-preserved sample (DNA concentration of 17.4 ng/μL) from the same station. All negative controls in qPCR reactions were negative.

4 | DISCUSSION

Laboratory testing revealed that the four qPCR assays used provided sensitive detection limits for the five target species. Robust in vitro testing resulted in successful amplification of serial dilution series for each species down to 0.05 ng/μL for killer whale, Dall's porpoise, humpback whale, and harbor porpoise positive controls, and 0.005 ng/μL for gray whale positive controls, suggesting that any cetacean eDNA in the environment was below this threshold

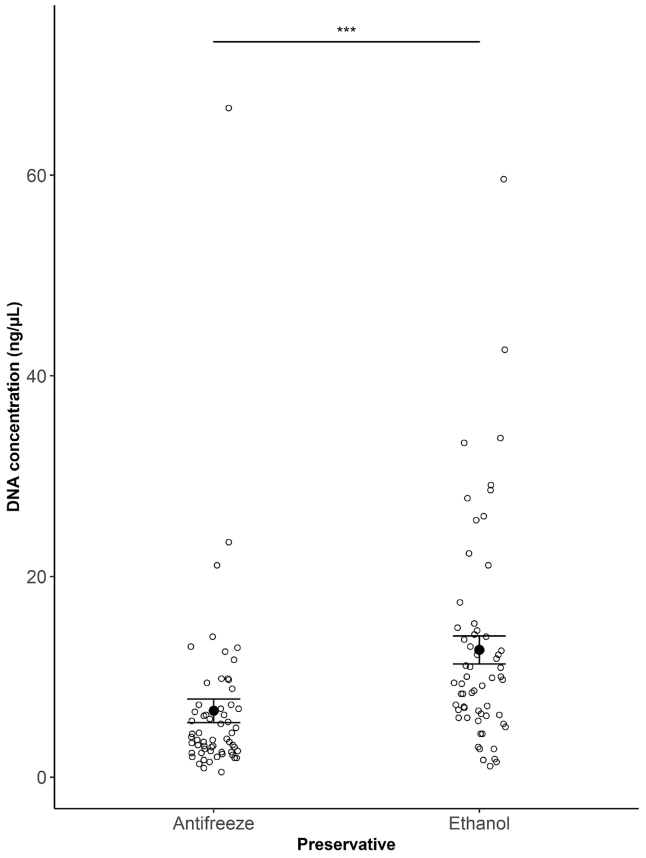

FIGURE 3 Total DNA concentration (ng/µL) of 120 environmental DNA (eDNA) samples (open circles) collected from 15 stations over four sample periods, plotted based on preservative (ethanol or antifreeze) used. Significance level: $p < 0.001$.

and therefore undetectable. Additionally, acoustic detections were also low, with a 15% detection rate for visual detections and 3% detection rate for acoustic detections. Nanodrop and qPCR control results ruled out gross failures of sample processing and positive detection of humpback whale DNA at station eight reinforces that eDNA processing, extraction, and amplification protocols were not the cause for lack of detections.

For visual detections, the individuals were on average between ~200 m (for harbor porpoises) and ~1400 m (for humpback whales) away from the station being sampled at time of observation. Previous studies have demonstrated that distance from cetaceans can have variable results in terms of eDNA detection. Some earlier studies with captive harbor porpoises reported a lack of DNA detections >10 m from the animal (Foote et al., 2012), and a more recent study highlighted the lack of killer whale DNA detections despite collecting samples within 20 m of killer whales (Pinfield et al., 2019). Contrary to this, Székely et al. (2021) reported positive target species detection despite no bowhead whale individuals being observed at time of collection. Studies from non-captive environments have reported positive DNA detections of between 10 min and 2 h for targeted flukeprint studies (Baker et al., 2018; Székely et al., 2021). Success rates of detecting cetacean DNA from non-target samples have shown to vary, from 15% transect success rate for Dwarf sperm whales (*Kogia sima*) to 81% success rate for bowhead whale transects (Juhel et al., 2021; Székely et al., 2021). Considering the relatively low number of visual sightings recorded during this study (11 sightings in total), and the average sighting being 1 km away from the point of collection, a 9.1% successful eDNA detection rate of the sampling events coinciding with visual detections (1 out of 11) is not surprising. We used low-sample volumes in this study, which is the most likely cause of the limited positive detections observed (Govindarajan et al., 2022; Mächler et al., 2016; Schabacker et al., 2020; Sepulveda et al., 2019). Our positive DNA detection of humpback whales was at a station where six to seven humpback whales were observed feeding ~300 m from the point of sample collection for a minimum of 1 h in the same location. This particular location exhibits a large tidal exchange and the combination of a greater number of individuals shedding DNA and the duration of time the individuals were in the area prior to sample collection would have influenced this detection.

Additional factors which could have influenced the lack of detection in our study includes shedding rates of target species, depth of sample collection, and influence of environmental factors such as current direction and strength (Baker et al., 2018; Foote et al., 2012; Govindarajan et al., 2022; Mächler et al., 2016; Mathieu et al., 2020; Pinfield et al., 2019; Schabacker et al., 2020; Sepulveda et al., 2019; Suarez-Bregua et al., 2022). Based on a combination of physiological (i.e., body size) and behavioral (i.e., breaching, feeding, socializing, or diving) factors, it is expected that different cetacean species shed DNA into their environment at different rates (Alter et al., 2022; Barnes et al., 2014; Parsons et al., 2018). Our limited success in this study is mostly likely driven by the low-volume approach used. We have demonstrated that ~250 mL was not optimal for detecting smaller species such as Dall's and harbor porpoises in a non-captive environment (Parsons et al., 2018) and it is clear that low-volume approaches are not appropriate for rare and dilute molecular targets. This reinforces that species-specific sampling approaches should be developed to improve chances of detecting DNA, with special emphasis given to ensuring sufficient water volumes are collected. Lastly, recent studies have indicated that eDNA dispersal in nearshore and open water environments is relatively localized both horizontally and vertically in the water column (Kelly et al., 2018; Monuki et al., 2021; O'Donnell et al., 2017). Considering this, the current and/or tides at the time of collection could have made detection less likely, especially if sample collection was conducted 'up flow' of observed individuals.

Although we had limited success with cetacean DNA detections, the positive sample was one that was preserved in antifreeze. Propylene glycol antifreeze has been used to preserve invertebrate tissue samples (Ferro & Park, 2013; Patrick et al., 2016; Steininger et al., 2015; Weigand et al., 2021) and river sediment samples (Robinson et al., 2021) with noted success. Antifreeze is a beneficial preservative to use instead of the more commonly used absolute ethanol, due to the fact it is easily accessible, non-toxic and non-regulated, and does not inhibit the DNA extraction process (Demeke & Jenkins, 2010; Robinson et al., 2021; Sales et al., 2019). This means

that propylene glycol antifreeze could be used to preserve marine eDNA samples in remote areas without the issues of sample storage and transit (i.e., no storage requirements and can be shipped by air), which are often encountered when using absolute ethanol to preserve samples (Robinson et al., 2021). Unfortunately, due to the fact there was only a single positive sample in this study, it is not possible to draw any definitive conclusions regarding the application of antifreeze for preserving marine eDNA samples. There is the need to validate antifreeze for this utility under controlled conditions (e.g., mesocosm studies) to fully establish its effectiveness.

5 | CONCLUSION

While eDNA provides a novel opportunity to monitor the distribution of cetaceans, methods require stringent optimization and in situ validation before this approach can be fully applied as a realized monitoring tool. It is clear from this study that low-volume sampling is not appropriate for cetacean eDNA monitoring and that minimum sample volumes for detection need to be established to prevent future application failures. We also need to improve our understanding of species-specific DNA shedding rates, and how this influences the sampling approach taken, to maximize success of monitoring efforts. As with most eDNA studies, there is a trade-off between the volume of water collected in terms of financial, temporal costs, and PCR inhibition (Sepulveda et al., 2019) and the likelihood of detection, especially when considering the potential for combining cetacean eDNA tools with community-based monitoring approaches. Despite some successful studies regarding non-targeted monitoring of cetaceans via eDNA, there is a large literature gap concerning less successful studies, as previously highlighted by Pinfield et al. (2019). Our study, while largely unsuccessful for detecting cetacean eDNA, did produce and optimize species-specific primer assays for an additional two cetacean species. Overall, 'one size fits all' is not the best avenue for cetacean eDNA monitoring, and further research should focus on pilot studies to optimize species-specific cetacean methods, including sample techniques, assay design, and analysis techniques (Altermatt et al., 2023). Additionally, as previously suggested by Suarez-Bregua et al. (2022), more research on the fate of eDNA in the dynamic marine environment is required, which can then be combined with species-specific approaches to improve confidence in eDNA outcomes and ultimately move eDNA tools into the next era of biodiversity monitoring.

AUTHOR CONTRIBUTIONS

CR and KD conceived the study, KD and AM collected samples with support from volunteers and other Ocean Wise staff, CVR and AM completed primer design, CVR and RG conducted eDNA analysis, CVR wrote the manuscript with support from all co-authors.

ACKNOWLEDGMENTS

We wish to acknowledge that this research took place on the traditional territory of the Nine Allied Tribes of Metlakatla and Lax Kw'alaams who have been stewards of these lands and waters since time immemorial. This project was supported partially by a financial contribution from Fisheries and Oceans Canada/Ce projet fut partiellement appuyé par une contribution financière de Pêches et océans Canada. Thank you to Ashley Bachert and Sally Best for support with sample collection.

FUNDING INFORMATION

This project was supported partially by a financial contribution from Fisheries and Oceans Canada/Ce projet fut partiellement appuyé par une contribution financière de Pêches et océans Canada.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

DATA AVAILABILITY STATEMENT

Data is available via GitHub https://github.com/chloerobinson23/Robinson-et-al-2023.

ORCID

Chloe V. Robinson https://orcid.org/0000-0003-3776-9166
Karina Dracott https://orcid.org/0000-0001-9225-1816

REFERENCES

Alter, S. E., King, C. D., Chou, E., Chin, S. C., Rekdahl, M., & Rosenbaum, H. C. (2022). Using environmental DNA to detect whales and dolphins in the New York Bight. *Frontiers in Conservation Science*, *3*, 820377. https://doi.org/10.3389/fcosc.2022.820377

Altermatt, F., Carraro, L., Antonetti, M., Albouy, C., Zhang, Y., Lyet, A., Zhang, X., & Pellissier, L. (2023). Quantifying biodiversity using eDNA from water bodies: General principles and recommendations for sampling designs. *Environmental DNA*, *5*(4), 671–682. https://doi.org/10.1002/edn3.430

Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. *Frontiers in Marine Science*, *5*, 133. https://doi.org/10.3389/fmars.2018.00133

Barkley, Y. M., Nosal, E.-M., & Oleson, E. M. (2021). Model-based localization of deep-diving cetaceans using towed line array acoustic data. *The Journal of the Acoustical Society of America*, *150*(2), 1120–1132. https://doi.org/10.1121/10.0005847

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827. https://doi.org/10.1021/es404734p

Belle, C. C., Stoeckle, B. C., & Geist, J. (2019). Taxonomic and geographical representation of freshwater environmental DNA research in aquatic conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(11), 1996–2009. https://doi.org/10.1002/aqc.3208

Boulent, J., Charry, B., Kennedy, M. M., Tissier, E., Fan, R., Marcoux, M., Watt, C. A., & Gagné-Turcotte, A. (2023). Scaling whale monitoring using deep learning: A human-in-the-loop solution for analyzing aerial datasets. *Frontiers in Marine Science*, *10*, 1099479. https://doi.org/10.3389/fmars.2023.1099479

Cao, Y., Griffith, J. F., Dorevitch, S., & Weisberg, S. B. (2012). Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing the impact of inhibition while measuring enterococcus in environmental waters. *Journal of Applied Microbiology*, *113*(1), 66–75. https://doi.org/10.1111/j.1365-2672.2012.05305.x

Cartagena-Matos, B., Lugué, K., Fonseca, P., Marques, T. A., Prieto, R., & Alves, F. (2021). Trends in cetacean research in the Eastern North Atlantic. *Mammal Review*, *51*(3), 436–453. https://doi.org/10.1111/mam.12238

Charry, B., Tissier, E., Iacozza, J., Marcoux, M., & Watt, C. A. (2021). Mapping Arctic cetaceans from space: A case study for beluga and narwhal. *PLoS One*, *16*(8), e0254380. https://doi.org/10.1371/journal.pone.0254380

Cheeseman, T., Johnson, T., Southerland, K., & Muldavin, N. (2023). Happywhale: Globalizing marine mammal photo identification via a citizen science web platform. *International Whaling Commission*, *7*, 1–7.

Dalongeville, A., Boulanger, E., Marques, V., Charbonnel, E., Hartmann, V., Santoni, M. C., Deter, J., Valentini, A., Lenfant, P., Boissery, P., Dejean, T., Velez, L., Pichot, F., Sanchez, L., Arnal, V., Bockel, T., Delaruelle, G., Holon, F., Milhau, T., … Mouillot, D. (2022). Benchmarking eleven biodiversity indicators based on environmental DNA surveys: More diverse functional traits and evolutionary lineages inside marine reserves. *Journal of Applied Ecology*, *59*(11), 2803–2813. https://doi.org/10.1111/1365-2664.14276

Dalpaz, L., Paro, A. D., Daura-Jorge, F. G., Rossi-Santos, M., Norris, T. F., Ingram, S. N., & Wedekin, L. L. (2021). Better together: Analysis of integrated acoustic and visual methods when surveying a cetacean community. *Marine Ecology Progress Series*, *678*, 197–209. https://doi.org/10.3354/meps13898

Demeke, T., & Jenkins, G. R. (2010). Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and Bioanalytical Chemistry*, *396*(6), 1977–1990. https://doi.org/10.1007/s00216-009-3150-9

Dracott, K., Robinson, C. V., Brown-Dussault, A., Birdsall, C., & Barrett-Lennard, L. (2022). Porpoises after dark: Seasonal and diel patterns in Pacific harbour porpoise (*Phocoena phocoena vomerina*) aggregations at one of North America's fastest growing ports. *Frontiers in Marine Science*, *9*, 1010095. https://doi.org/10.3389/fmars.2022.1010095

Feng, W., & Lougheed, S. C. (2023). Integrating eDNA and citizen science observations to model distribution of a temperate freshwater turtle near its northern range limit. *PeerJ*, *11*, e15120. https://doi.org/10.7717/peerj.15120

Ferro, M. L., & Park, J.-S. (2013). Effect of propylene glycol concentration on mid-term DNA preservation of coleoptera. *The Coleopterists Bulletin*, *67*(4), 581–586. https://doi.org/10.1649/0010-065X-67.4.581

Foote, A. D., Thomsen, P. F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L. A., Salling, A. B., Galatius, A., Orlando, L., & Gilbert, M. T. P. (2012). Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS One*, *7*(8), e41781. https://doi.org/10.1371/journal.pone.0041781

Forney, K. A., Hanan, D. A., & Barlow, J. (1991). Detecting trends in harbor porpoise abundance from aerial surveys using analysis of covariance. *Fishery Bulletin*, *89*, 367–377.

Frouin-Mouy, H., Mouy, X., Pilkington, J., Küsel, E., Nichol, L., Doniol-Valcroze, T., & Lee, L. (2022). Acoustic and visual cetacean surveys reveal year-round spatial and temporal distributions for multiple species in northern British Columbia, Canada. *Scientific Reports*, *12*(1), 19272. https://doi.org/10.1038/s41598-022-22069-4

Gilbey, J., Carvalho, G., Castilho, R., Coscia, I., Coulson, M. W., Dahle, G., Derycke, S., Francisco, S. M., Helyar, S. J., Johansen, T., Junge, C., Layton, K. K. S., Martinsohn, J., Matejusova, I., Robalo, J. I., Rodríguez-Ezpeleta, N., Silva, G., Strammer, I., Vasemägi, A., & Volckaert, F. A. M. (2021). Life in a drop: Sampling environmental DNA for marine fishery management and ecosystem monitoring. *Marine Policy*, *124*, 104331. https://doi.org/10.1016/j.marpol.2020.104331

Govindarajan, A. F., McCartin, L., Adams, A., Allan, E., Belani, A., Francolini, R., Fujii, J., Gomez-Ibañez, D., Kukulya, A., Marin, F., Tradd, K., Yoerger, D. R., McDermott, J. M., & Herrera, S. (2022). Improved biodiversity detection using a large-volume environmental DNA sampler with in situ filtration and implications for marine eDNA sampling strategies. *Deep Sea Research Part I: Oceanographic Research Papers*, *189*, 103871. https://doi.org/10.1016/j.dsr.2022.103871

Gutiérrez, P., Walters, A., Dolman, S., & Pierce, G. (2021). Patterns and trends in cetacean occurrence revealed by Shorewatch, a land-based citizen science program in Scotland (United Kingdom). *Frontiers in Marine Science*, *8*, 642386. https://doi.org/10.3389/fmars.2021.642386

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). 'Sight-unseen' detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Juhel, J., Marques, V., Polanco Fernández, A., Borrero-Pérez, G. H., Mutis Martinezguerra, M., Valentini, A., Dejean, T., Manel, S., Loiseau, N., Velez, L., Hocdé, R., Letessier, T. B., Richards, E., Hadjadj, F., Bessudo, S., Ladino, F., Albouy, C., Mouillot, D., & Pellissier, L. (2021). Detection of the elusive Dwarf sperm whale (*Kogia sima*) using environmental DNA at Malpelo island (Eastern Pacific, Colombia). *Ecology and Evolution*, *11*(7), 2956–2962. https://doi.org/10.1002/ece3.7057

Kelly, R. P., Gallego, R., & Jacobs-Palmer, E. (2018). The effect of tides on nearshore environmental DNA. *PeerJ*, *6*, e4521. https://doi.org/10.7717/peerj.4521

Kopp, D., Faillettaz, R., Le Joncour, A., Simon, J., Morandeau, F., Le Bourdonnec, P., Bouché, L., & Méhault, S. (2023). Assessing without harvesting: Pros and cons of environmental DNA sampling and image analysis for marine biodiversity evaluation. *Marine Environmental Research*, *188*, 106004. https://doi.org/10.1016/j.marenvres.2023.106004

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*(260), 583–621. https://doi.org/10.2307/2280779

Liu, M., Lin, M., Dong, L., Caruso, F., & Li, S. (2022). An integrated strategy for monitoring cetaceans in data-poor regions. *Biological Conservation*, *272*, 109648. https://doi.org/10.1016/j.biocon.2022.109648

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., Mahon, A. R., & Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, *21*(11), 2555–2558. https://doi.org/10.1111/j.1365-294X.2012.05600.x

Ma, H., Stewart, K., Lougheed, S., Zheng, J., Wang, Y., & Zhao, J. (2016). Characterization, optimization, and validation of environmental DNA (eDNA) markers to detect an endangered aquatic mammal. *Conservation Genetics Resources*, *8*(4), 561–568. https://doi.org/10.1007/s12686-016-0597-9

Mächler, E., Deiner, K., Spahn, F., & Altermatt, F. (2016). Fishing in the water: Effect of sampled water volume on environmental DNA-based detection of macroinvertebrates. *Environmental Science & Technology*, *50*(1), 305–312. https://doi.org/10.1021/acs.est.5b04188

Mancini, A., & Elsadek, I. M. (2019). The role of citizen science in monitoring megafauna of the Red Sea. In N. M. A. Rasul & I. C. F. Stewart (Eds.), *Oceanographic and biological aspects of the Red Sea* (pp. 507–519). Springer International Publishing. https://doi.org/10.1007/978-3-319-99417-8_28

Marsh, H., & Sinclair, D. F. (1989). Correcting for visibility bias in strip transect aerial surveys of aquatic Fauna. *The Journal of Wildlife Management*, *53*(4), 1017. https://doi.org/10.2307/3809604

Mathieu, C., Hermans, S. M., Lear, G., Buckley, T. R., Lee, K. C., & Buckley, H. L. (2020). A systematic review of sources of variability and uncertainty in eDNA data for environmental monitoring. *Frontiers in Ecology and Evolution*, *8*, 135. https://doi.org/10.3389/fevo.2020.00135

McColl-Gausden, E. F., Weeks, A. R., Coleman, R., Song, S., & Tingley, R. (2023). Using hierarchical models to compare the sensitivity of metabarcoding and qPCR for eDNA detection. *Ecological Informatics*, *75*, 102072. https://doi.org/10.1016/j.ecoinf.2023.102072

Miya, M. (2022). Environmental DNA metabarcoding: A novel method for biodiversity monitoring of marine fish communities. *Annual Review of Marine Science*, *14*(1), 161–185. https://doi.org/10.1146/annurev-marine-041421-082251

Miya, M., Sado, T., Oka, S., & Fukuchi, T. (2022). The use of citizen science in fish eDNA metabarcoding for evaluating regional biodiversity in a coastal marine region: A pilot study. *Metabarcoding and Metagenomics*, *6*, e80444. https://doi.org/10.3897/mbmg.6.80444

Monuki, K., Barber, P. H., & Gold, Z. (2021). eDNA captures depth partitioning in a kelp forest ecosystem. *PLoS One*, *16*(11), e0253104. https://doi.org/10.1371/journal.pone.0253104

Mwango'mbe, M. G., Spilsbury, J., Trott, S., Nyunja, J., Wambiji, N., Collins, T., Gomes, I., & Pérez-Jorge, S. (2021). Cetacean research and citizen science in Kenya. *Frontiers in Marine Science*, *8*, 642399. https://doi.org/10.3389/fmars.2021.642399

O'Donnell, J. L., Kelly, R. P., Shelton, A. O., Samhouri, J. F., Lowell, N. C., & Williams, G. D. (2017). Spatial distribution of environmental DNA in a nearshore marine habitat. *PeerJ*, *5*, e3044. https://doi.org/10.7717/peerj.3044

Oliveira-Rodrigues, C., Correia, A. M., Valente, R., Gil, Á., Gandra, M., Liberal, M., Rosso, M., Pierce, G., & Sousa-Pinto, I. (2022). Assessing data bias in visual surveys from a cetacean monitoring programme. *Scientific Data*, *9*(1), 682. https://doi.org/10.1038/s41597-022-01803-7

Padilla, C. C., Ganesh, S., Gantt, S., Huhman, A., Parris, D. J., Sarode, N., & Stewart, F. J. (2015). Standard filtration practices may significantly distort planktonic microbial diversity estimates. *Frontiers in Microbiology*, *6*, 547. https://doi.org/10.3389/fmicb.2015.00547

Parsons, K. M., Everett, M., Dahlheim, M., & Park, L. (2018). Water, water everywhere: Environmental DNA can unlock population structure in elusive marine species. *Royal Society Open Science*, *5*(8), 180537. https://doi.org/10.1098/rsos.180537

Patrick, H. J. H., Chomič, A., & Armstrong, K. F. (2016). Cooled propylene glycol as a pragmatic choice for preservation of DNA from remote field-collected diptera for next-generation sequence analysis. *Journal of Economic Entomology*, *109*(3), 1469–1473. https://doi.org/10.1093/jee/tow047

Pinfield, R., Dillane, E., Runge, A. K. W., Evans, A., Mirimin, L., Niemann, J., Reed, T. E., Reid, D. G., Rogan, E., Samarra, F. I. P., Sigsgaard, E. E., & Foote, A. D. (2019). False-negative detections from environmental DNA collected in the presence of large numbers of killer whales (*Orcinus orca*). *Environmental DNA*, *1*(4), 316–328. https://doi.org/10.1002/edn3.32

Pirotta, V., Reynolds, W., Ross, G., Jonsen, I., Grech, A., Slip, D., & Harcourt, R. (2020). A citizen science approach to long-term monitoring of humpback whales (*Megaptera novaeangliae*) off Sydney, Australia. *Marine Mammal Science*, *36*(2), 472–485. https://doi.org/10.1111/mms.12651

Prince Rupert Port Authority. (2022). Terminal Expansion to 1.8M TEU Capacity (2 Stages). https://www.rupertport.com/active_project/fairview-container-terminal/

Qu, C., & Stewart, K. A. (2019). Evaluating monitoring options for conservation: Comparing traditional and environmental DNA tools for a critically endangered mammal. *The Science of Nature*, *106*(3), 9. https://doi.org/10.1007/s00114-019-1605-1

R Core Team. (2023). *R: A language and environment for statistical computing* [Computer software]. https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006

Rice, A., Debich, A. J., Širović, A., Oleson, E. M., Trickey, J. S., Varga, L. M., Wiggins, S. M., Hildebrand, J. A., & Baumann-Pickering, S. (2021). Cetacean occurrence offshore of Washington from long-term passive acoustic monitoring. *Marine Biology*, *168*(8), 136. https://doi.org/10.1007/s00227-021-03941-9

Robinson, C. V., Porter, T. M., Wright, M. T. G., & Hajibabaei, M. (2021). Propylene glycol-based antifreeze is an effective preservative for DNA metabarcoding of benthic arthropods. *Freshwater Science*, *40*(1), 77–87. https://doi.org/10.1086/712232

Rodriguez, L. K., Fandel, A. D., Colbert, B. R., Testa, J. C., & Bailey, H. (2021). Spatial and temporal variation in the occurrence of bottlenose dolphins in the Chesapeake Bay, USA, using citizen science sighting data. *PLoS One*, *16*(5), e0251637. https://doi.org/10.1371/journal.pone.0251637

RStudio. (2023). *RStudio: Integrated Development Environment for R* [Computer software]. http://www.rstudio.com/

Sales, N. G., Wangensteen, O. S., Carvalho, D. C., & Mariani, S. (2019). Influence of preservation methods, sample medium and sampling time on eDNA recovery in a neotropical river. *Environmental DNA*, *1*(2), 119–130. https://doi.org/10.1002/edn3.14

Schabacker, J. C., Amish, S. J., Ellis, B. K., Gardner, B., Miller, D. L., Rutledge, E. A., Sepulveda, A. J., & Luikart, G. (2020). Increased eDNA detection sensitivity using a novel high-volume water sampling method. *Environmental DNA*, *2*(2), 244–251. https://doi.org/10.1002/edn3.63

Sepulveda, A. J., Schabacker, J., Smith, S., Al-Chokhachy, R., Luikart, G., & Amish, S. J. (2019). Improved detection of rare, endangered and invasive trout in using a new large-volume sampling method for eDNA capture. *Environmental DNA*, *1*(3), 227–237. https://doi.org/10.1002/edn3.23

Shapiro, S., & Wilk, M. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*, 591–611.

Steininger, S., Storer, C., Hulcr, J., Lucky, A., Steininger, S., Storer, C., Hulcr, J., & Lucky, A. (2015). Alternative preservatives of insect DNA for citizen science and other low-cost applications. *Invertebrate Systematics*, *29*(5), 468–472. https://doi.org/10.1071/IS15003

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038

Suarez-Bregua, P., Álvarez-González, M., Parsons, K. M., Rotllant, J., Pierce, G. J., & Saavedra, C. (2022). Environmental DNA (eDNA) for monitoring marine mammals: Challenges and opportunities. *Frontiers in Marine Science*, *9*, 987774. https://doi.org/10.3389/fmars.2022.987774

Székely, D., Corfixen, N. L., Mørch, L. L., Knudsen, S. W., McCarthy, M. L., Teilmann, J., Heide-Jørgensen, M. P., & Olsen, M. T. (2021). Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. *Environmental DNA*, *3*(1), 248–260. https://doi.org/10.1002/edn3.176

Thomas, A. C., Nguyen, P. L., Howard, J., & Goldberg, C. S. (2019). A self-preserving, partially biodegradable eDNA filter. *Methods in Ecology and Evolution*, *10*(8), 1136–1141. https://doi.org/10.1111/2041-210X.13212

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Valsecchi, E., Arcangeli, A., Lombardi, R., Boyse, E., Carr, I. M., Galli, P., & Goodman, S. J. (2021). Ferries and environmental DNA: Underway

sampling from commercial vessels provides new opportunities for systematic genetic surveys of marine biodiversity. *Frontiers in Marine Science*, *8*, 704786. https://doi.org/10.3389/fmars.2021.704786

Valsecchi, E., Tavecchia, G., Boldrocchi, G., Coppola, E., Ramella, D., Conte, L., Blasi, M., Bruno, A., & Galli, P. (2023). Playing 'hide and seek' with the Mediterranean monk seal: A citizen science dataset reveals its distribution from molecular traces (eDNA). *Scientific Reports*, *13*(1), 2610. https://doi.org/10.1038/s41598-023-27835-6

Weigand, A. M., Desquiotz, N., Weigand, H., & Szucsich, N. (2021). Application of propylene glycol in DNA-based studies of invertebrates. *Metabarcoding and Metagenomics*, *5*, e57278. https://doi.org/10.3897/mbmg.5.57278

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., & RStudio. (2022). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics (3.3.6)*. [Computer software]. https://CRAN.R-project.org/package=ggplot2

Wright, B. M., Nichol, L. M., & Doniol-Valcroze, T. (2021). *Spatial density models of cetaceans in the Canadian Pacific estimated from 2018 ship-based surveys* (2021/049; DFO Canadian Science Advisory Secretariat, pp. 1–46). Pacific Biological Station. https://publications.gc.ca/collections/collection_2021/mpo-dfo/fs70-5/Fs70-5-2021-049-eng.pdf

Xiong, W., Li, H., & Zhan, A. (2016). Early detection of invasive species in marine ecosystems using high-throughput sequencing: Technical challenges and possible solutions. *Marine Biology*, *163*(6), 139. https://doi.org/10.1007/s00227-016-2911-1

Yu, Z., Ito, S., Wong, M. K.-S., Yoshizawa, S., Inoue, J., Itoh, S., Yukami, R., Ishikawa, K., Guo, C., Ijichi, M., & Hyodo, S. (2022). Comparison of species-specific qPCR and metabarcoding methods to detect small pelagic fish distribution from open ocean environmental DNA. *PLoS One*, *17*(9), e0273670. https://doi.org/10.1371/journal.pone.0273670

Zhang, S., Cao, Y., Chen, B., Jiang, P., Fang, L., Li, H., Chen, Z., Xu, S., & Li, M. (2023). Assessing the potential use of environmental DNA for multifaceted genetic monitoring of cetaceans: Example of a wandering whale in a highly disturbed bay area. *Ecological Indicators*, *148*, 110125. https://doi.org/10.1016/j.ecolind.2023.110125

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Robinson, C. V., Migneault, A., Dracott, K., & Glover, R. D. (2023). Seas the DNA? Limited detection of cetaceans by low-volume environmental DNA transect surveys. *Environmental DNA*, *00*, 1–11. https://doi.org/10.1002/edn3.485