Received: 18 April 2023 | Revised: 2 August 2023 | Accepted: 7 August 2023

DOI: 10.1002/edn3.468

Environmental DNA
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ORIGINAL ARTICLE

Autonomous eDNA collection using an uncrewed surface vessel over a 4200-km transect of the eastern Pacific Ocean

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Funding information
David and Lucile Packard Foundation; National Oceanographic Partnership Program, Grant/Award Number: NA19OAR0110409

Abstract

The collection of environmental DNA (eDNA) samples is often laborious, costly, and logistically difficult to accomplish at high frequency in remote locations and over large geographic areas. Here, we addressed those challenges by combining two robotic technologies: an uncrewed surface vessel (USV) fitted with an automated eDNA sample collection device to survey surface waters in the eastern North Pacific Ocean from Alameda, CA to Honolulu, HI. USV Surveyor SD 1200 (Saildrone) carrying the Environmental Sample Processor (ESP) collected 2-L water samples by filtration followed by RNAlater preservation at regular intervals over a 4200-km, 29-day transit. Sixty samples (52 field and 8 controls) were acquired and used to estimate the concentration of specific genes and assess eukaryotic diversity via targeted qPCR and metabarcoding of the cytochrome oxidase subunit I (COI) gene, respectively. Comparisons of control samples revealed important considerations for interpreting results. Samples stored at ambient temperatures onboard Surveyor over the length of the voyage had less total recoverable DNA and specific target gene concentrations compared to the same material immediately flash-frozen after collection and stored in a laboratory. In contrast, the biodiversity of the COI genes in those samples was similar regardless of sample age and storage condition. COI genes affiliated with 40 eukaryotic phyla were found in native samples collected during the voyage. The distribution and dominance of those phyla varied across different regions, with some taxa spanning large continuous stretches >2000 km, while others were only detected in a single sample. This work highlights the utility and potential of using USVs fitted with autonomous eDNA sample collection devices to improve ocean exploration and support large, basin-scale, systematic biodiversity surveys. Results of this study also inform future technical considerations for using automated eDNA samplers to acquire material and store it over prolonged periods under prevailing environmental conditions.

KEYWORDS
autonomous vehicles [J01.937.500.300], biodiversity [G16.500.275.157.049], DNA barcoding, taxonomic [E05.393.542.249], DNA environmental [D13.444.308.577], Pacific Ocean [Z01.756.700]

1 | INTRODUCTION

The global ocean is the largest living space on Earth. Its bountiful biota provides an invaluable suite of ecosystem services, including oxygen generation, food production, and atmospheric carbon sequestration. Monitoring biodiversity in the sea is crucial to understanding the changes that human activity is imposing on marine ecosystems (Ibarbalz et al., 2019; Tittensor et al., 2010). Crewed ship-based monitoring is expensive and logistically limited, and not practically scalable over large areas, making routine monitoring of the open sea difficult (Cristini et al., 2016; Global Ocean Science Report: The Current Status of Ocean Science around the World, 2017). New autonomous ocean-going platforms (e.g., uncrewed underwater vehicles, UUVs, and uncrewed surface vessels, USVs) offer a means of addressing these issues by making it possible to conduct forays to remote regions that are not readily visited with crewed ships.

In parallel with rapid advances in marine robotics, molecular analytical techniques are increasingly unveiling an unprecedented view of the distribution and activities of marine organisms across wide swaths of the ocean. In that regard, environmental DNA (eDNA) has emerged as a primary tool for assessing marine biodiversity (for review see Compson et al., 2020; Ruppert et al., 2019). The rapid adoption of eDNA metabarcoding in particular reflects the technique's noninvasive and cost-effective attributes, accessibility, and its effectiveness for surveying entire organismal communities from microbes to whales based on a single sample (de Vargas et al., 2015; Djurhuus et al., 2020; Liu et al., 2020; Miya, 2022; O'Rorke et al., 2022; Suarez-Bregua et al., 2022).

Despite the promise of eDNA, the vastness, and inaccessibility of much of the world's oceans make it difficult to obtain representative samples on meaningful scales (Havermans et al., 2022). Historically, eDNA sampling in the open sea has relied on crewed vessels, which adds considerable cost and logistical challenges for implementing routine, open-ocean eDNA biomonitoring programs (Compson et al., 2020). Satellite and in-water optical sensing techniques, along with bioacoustics measurements, provide valuable proxy variables for tracking plankton and vertebrate assemblages (Aiken et al., 2007; Alcocer et al., 2022). However, those methods lack much of the resolving power that is needed to understand spatial and temporal changes over time at the species level, which, in turn, is required for long-term ecosystem structure and function assessments. Scaling up the use of eDNA analyses offers a tantalizing means for addressing this observational gap, but to do so, our capacity for sample collection must be greatly enhanced relative to what is realistically possible at present.

Recent developments in robotic water samplers offer a glimpse of how the sample collection problem can be overcome (Govindarajan et al., 2022; McQuillan & Robidart, 2017; Ottesen, 2016). Deploying automated samplers on uncrewed vehicles provides the added advantages of mobility and opportunities for event response capability. For example, we have coupled the Environmental Sample Processor (ESP) with a long-range autonomous underwater vehicle (LRAUV) to collect eDNA samples in coastal, open ocean, and freshwater lake environments (Den Uyl et al., 2022; Truelove et al., 2022; Yamahara et al., 2019). Similarly, Govindarajan et al. (2022) have utilized a high-volume eDNA sampler onboard *Mesobot* for eDNA collection during midwater surveys, and Jakuba et al. (2018) have used *Clio* to support high-volume, vertical profile sampling for biogeochemical studies. While these eDNA sample collection demonstrations clearly highlight the utility and potential for combining mobile autonomous platforms with automated samplers, they also demonstrate common challenges associated with UUVs that are capable of carrying a relatively large payload, namely limited onboard power and range, and a need for crewed vessel support during operations.

Uncrewed surface vessels provide an attractive alternative to UUVs for certain used cases and are increasingly being used to enable persistent ocean monitoring (Liu et al., 2016; Meinig et al., 2019; Patterson et al., 2022). These platforms have an inherent advantage over UUVs in that many are powered by wind, wave, and/or solar energy. Thus, USVs hold great promise for carrying multiple classes of sensors on a single vessel to support long endurance, over-the-horizon surveys in areas where crewed ship operations are limited or logistically challenging. Like their UUV counterparts, USVs can be configured to meet multiple mission objectives (e.g., seafloor mapping, water column chemistry, etc). For example, USVs carrying acoustic sensors have been used for seafloor mapping (Francis & Traykovski, 2021; Gentemann et al., 2020), fish stock assessments (Dallolio et al., 2022; De Robertis et al., 2019; Komiyama, 2021; Totland & Johnsen, 2022), and marine mammal surveys (Premus et al., 2022). Given the utility and versatility of the platforms, it is somewhat surprising that greater adoption of incorporating water sampling devices on USVs has not yet occurred (Flanigan et al., 2021). The union of USVs and water sampling devices would provide needed advancements to improve the scalability of eDNA sample acquisition: lower cost to manufacture and operate compared to crewed vessels, access the upper water column over much greater distances by remaining at sea for longer periods of time, flexible scheduling, and operation over a wide range of sea states (Gentemann et al., 2020; Patterson et al., 2022; Ricciardulli et al., 2022).

Saildrone, Inc. (https://www.saildrone.com/) is among the USV pioneers who have devised a unique type of rigid-sail vessel capable of long-distance, over-the-horizon operations for extended periods (mission endurances >180 days). They have recently introduced a 22-m long, Surveyor-class, uncrewed ocean-going surface vessel,

SD 1200 (hearafter referred to as Surveyor) specifically designed for seafloor mapping and with payload bays capable of carrying multiple instrument suites. Surveyor has proven to be an effective platform for remotely collecting seafloor bathymetry along with concurrent environmental data, but prior to this study, it had never been used to collect biological samples.

Here, we combined the autonomous sampling capabilities of the ESP with the mobility and durability of Saildrone's Surveyor to collect surface eDNA samples over a 4200-km transect in the eastern Pacific Ocean (Figure 1). Our objective was to investigate two fundamental questions germane to automating long-endurance, large-scale autonomous eDNA sample collections: (1) Is it feasible to collect, preserve, and store surface seawater samples over extended periods of time at ambient temperatures, and (2) does the eDNA recovered reflect the biodiversity of the biomes the autonomous craft traversed? To answer those questions, we applied both targeted qPCR for specific genetic markers along with metabarcoding of the cytochrome oxidase subunit I (COI) gene to assess the stability of eDNA that is recovered and the biodiversity of eukaryotic phyla detected over the course of the transit, respectively. We show that long-endurance USVs do indeed offer a viable means of scaling up eDNA biodiversity observations over large geographical regions, and highlight a number of technical considerations for future studies of this kind.

2 | METHODS

2.1 | Uncrewed surface vehicle (USV) Surveyor and ESP integration

The Surveyor SD 1200 is the 22-m autonomous sailing vessel used in this study. During light or no winds, an onboard diesel/electric engine can generate speeds of 6–10 knots. Under sail, the Surveyor can attain speeds of five knots. Surveyor carried payloads for eDNA sampling (see below) and seafloor mapping, and a profiling winch system for conducting sound velocity profiles. Other instrumentation onboard the vessel provided environmental data, including a Dual Antenna GNSS-Aided Inertial Navigation System (VectorNav, Dallas TX), wave period and height, and an anemometer (B&G Sailing Electronics) for wind speed and direction. Environmental data are available in real time via satellite telemetry using Saildrone's Mission Portal.

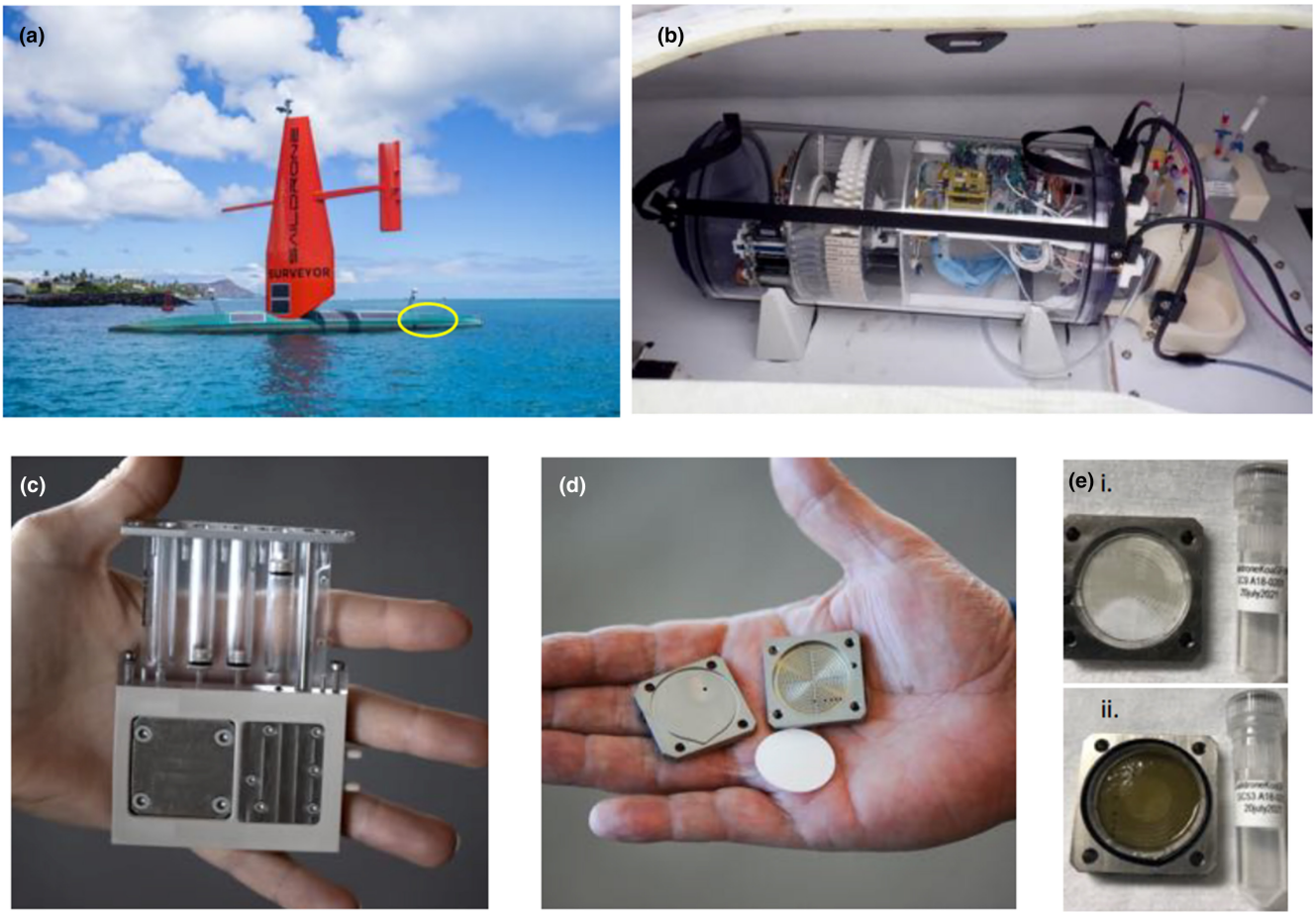

FIGURE 1 USV Surveyor SD 1200 reached Honolulu, HI after a 29-day cruise (a), carrying the Environmental Sample Processor in its aft hold (yellow circle). The ESP mounted in the hold (b). The ESP carried 60 cartridges where filtering and preservation of each eDNA sample took place (c). Each cartridge contained a square "puck" that housed a 25 mm, 0.2 μm filter (d). Opened pucks show biomass collected on filters from two eDNA preserved samples (e) collected off the coastlines of Hawaii (i) and California (ii).

The Environmental Sample Processor (ESP) is a robotic device that automates water sample collection and processing (Scholin et al., 2017). The ESP has been under development since the early 2000's and has been used successfully in a variety of marine and freshwater settings (Den Uyl et al., 2022; Scholin et al., 2017; Sepulveda et al., 2020, 2021; Truelove et al., 2022; Yamahara et al., 2019). The latest iteration of this device, known as the third generation or 3G-ESP (hereafter ESP; Pargett et al., 2016), is configured as a 28-cm diameter, 66-cm long payload for the Monterey Bay Aquarium Research Institute's (MBARI) LRAUV (Hobson et al., 2012). It is capable of collecting and processing up to 60 discrete samples, either preserving the material collected (e.g., Den Uyl et al., 2022; Truelove et al., 2022; Yamahara et al., 2019; Zhang et al., 2021) or homogenizing it for real-time, onboard detection of phycotoxins (W. Ussler, personal communication).

For deployment aboard Surveyor, the ESP was fitted in a waterproof acrylic housing and placed in the aft hold of the vessel (Figure 1). Dampers were added inside the housing and on the support cradle to reduce shock/vibration. Surveyor provided the ESP with a 15-volt power supply and two-way satellite communications, permitting 24-h access to the instrument for reviewing instrument logs, changing sampling parameters, etc., while underway. The sample water intake system consisted of a dedicated tube beginning at a copper-screened intake at ~2-m depth on the ship's rudder's leading edge, running through the interior space of the rudder, and plumbed directly to the ESP (~5.5 m in total length, Figure 2). Water filtered through the ESP was emptied into a scupper within the payload bay. A 2 L reservoir of 0.2% v/v sodium hypochlorite ("dilute bleach;" Sigma-Aldrich) was plumbed to the ESP's sample collection line, which allowed flushing of the intake between collections to reduce biofouling (see Figure 2). A manifold of duckbill check valves (Qosina) external to the ESP on the exhaust line controlled the flow of fluids depending on the direction of the ESP sample pump; sample filtrate generated by the forward direction was discharged as waste into the payload bay, while the reverse direction drew from the bleach container, backflushing the entire sample path through the ESP to the copper screen intake on the ship's rudder.

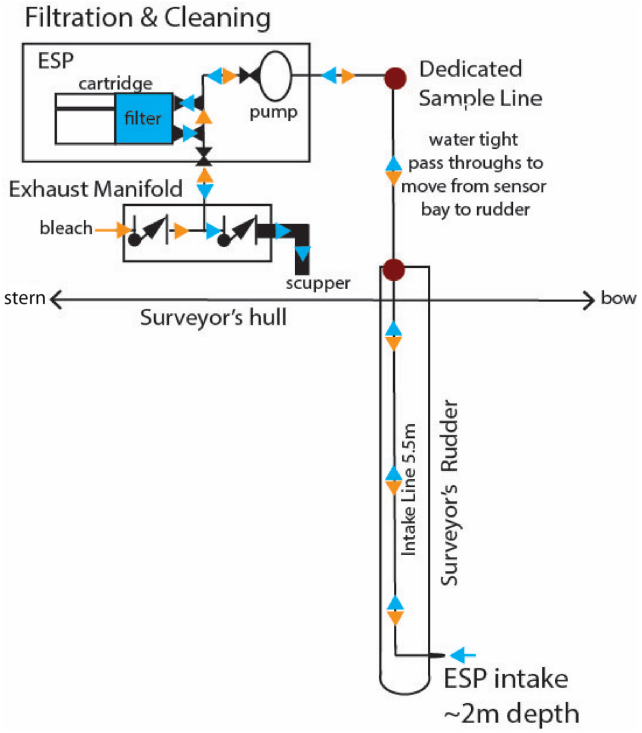

FIGURE 2 Schematic diagram of the sampling path of the ESP. The ESP, located in an aft compartment, was connected to an intake port 2 m deep on the leading edge of the rudder with ~5.5 m of 1.5-mm ID tubing. A bidirectional pump could either draw in seawater for sample collection (blue triangles) or move weak bleach through the entire sampling system (orange triangles) for decontamination. Exterior to the ESP was an exhaust manifold of duck-bill valves that controlled the fluidic path.

2.2 | Surveyor and ESP operations

Surveyor departed Alameda, CA on June 10, 2021, and arrived at Honolulu, HI on July 9, 2021 (Figures 1 and 3). The ESP-collected samples in rapid succession while transiting San Francisco Bay moving across the California current ($n = 6$, June 10–11), and again when approaching the Hawaiian Islands ($n = 6$, June 5–6). From June 12 to July 2, samples ($n = 40$) were collected twice daily at 12 a.m./12 p.m. Pacific Daylight Time (PDT) before crossing 140° W longitude, and then 3 a.m./3 p.m. PDT thereafter. Particulate sample collection and preservation were accomplished as described previously (Den Uyl et al., 2022; Pargett et al., 2016; Truelove et al., 2022; Yamahara et al., 2019). Briefly, at each scheduled sampling event, the ESP first flushed the 35 mL sample loop containing the bleach solution with 200 mL of native seawater. Seawater was then directed through a sample collection cartridge containing a 0.2-μm pore size 25-mm Durapore filter (EMD Millipore). Filtration was terminated when either the target volume of 2 L was reached, or the filtration rate dropped below 0.2 mL per second. In either case, the filtration rate and total volume filtered were recorded by the instrument, and that information was also accessible via satellite communication.

Following sample filtration, 1.6 mL of RNAlater (Invitrogen) was immediately added, displacing residual sample water into the sample cartridge's waste chambers. After a 10-min incubation, the majority of RNAlater was displaced into the cartridge's waste chambers with 1.6 mL of nitrogen gas (Truelove et al., 2022; Yamahara et al., 2019). Following sample preservation, the intake fluidic path was backflushed with the bleach solution. The toxicant remained in the sampling path until displaced with native water at the start of the next sampling event.

Samples collected using the ESP onboard Surveyor were stored at ambient temperatures until retrieval in HI. A Hobo temperature logger (Onset) was placed inside the ESP housing to record temperature data at 5 min intervals, providing a continuous record of the temperatures experienced over the course of the deployment.

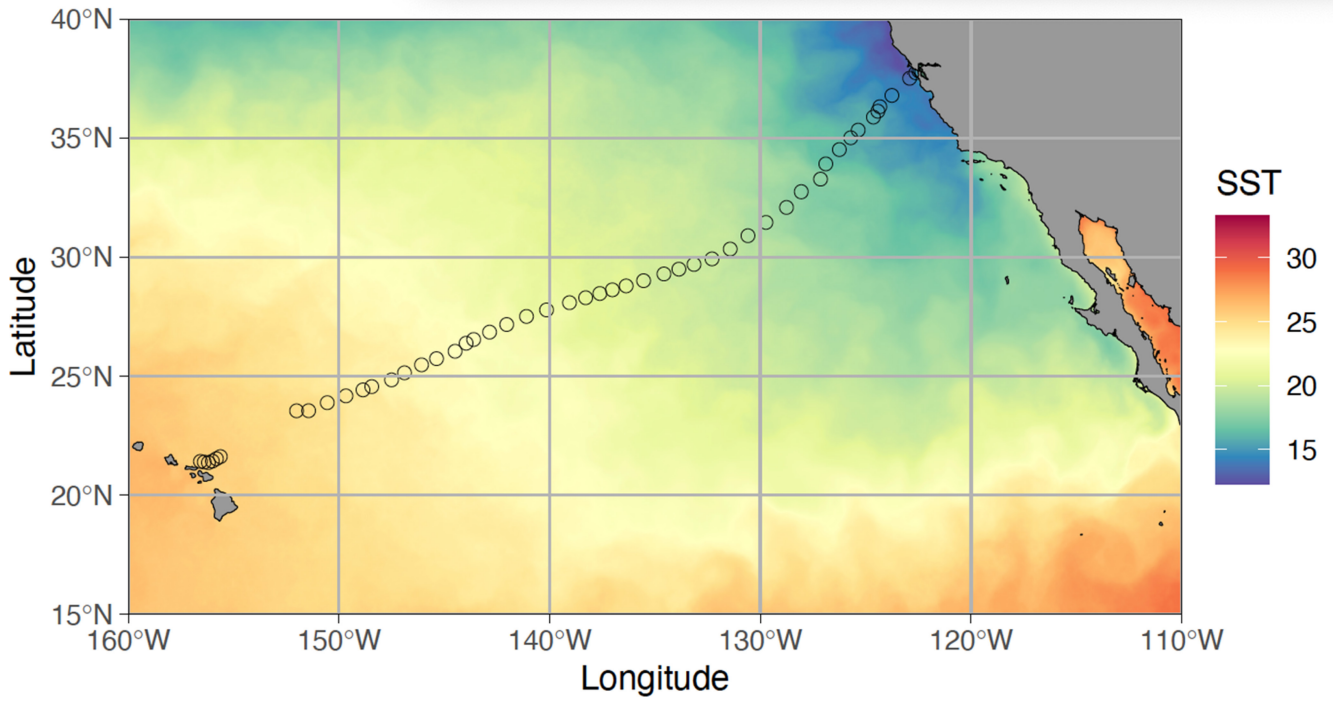

FIGURE 3 Circles denote the location of eDNA samples collected and preserved by the ESP onboard Surveyor during its ~4200-km transit over 29 days (June 10–July 9, 2021) from San Francisco to the Hawaiian Islands. Transit across oceanographic domains, from the California Current upwelling system to the North Pacific Subtropical Gyre, is represented by sea surface temperature (SST) from satellite remote sensing. Shown is the June 2021 average of multiscale ultra-high resolution (MUR) SST Analysis fv04.1, acquired from https://coastwatch.pfeg.noaa.gov/erddap/index.html.

When Surveyor reached HI (July 9th), the ESP, in its acrylic housing, was removed and stored at University of Hawaii Marine Center at room temperature until July 20, after which samples were recovered and frozen on dry ice, shipped to CA, and then stored at −80°C until processed.

Samples collected aboard Surveyor represent an integration of material acquired as the ship moved through the water. Travel distances were determined using filtration start and end times from the ESP logs and the corresponding Surveyor GPS navigational data. The sum of the distance calculated using the Haversine formula (Robusto, 1957) between one-minute latitude and longitude positions provides an estimate of the horizontal distance (km) that the vessel traversed over the course of each collection event.

2.3 | ESP preparation and controls

Environmental Sample Processor cartridge parts and fluidic pathways that contact sample water and reagents were cleaned to reduce nucleic acid and nuclease activity prior to deployment. All parts were cleaned using 1% (v/v) Liquinox (Alconox), followed by 2% (v/v) Citronox (Alconox), and exposed to UV irradiation prior to assembly in a clean hood. The sample path through the ESP was also flushed with a 10% (v/v) bleach solution (Pure Bright Bleach, Ontario, Canada) before loading the instrument with collection cartridges as previously described (Yamahara et al., 2019). The fully assembled ESP was then placed in its waterproof housing and purged with dry nitrogen gas.

In order to test the effects of extended sample storage at ambient temperatures, replicate samples of native seawater (i.e., positive controls) were collected and preserved using the fully assembled ESP instrument prior to its installation in Surveyor and transit to HI. The positive control sample consisted of 20 liters of surface seawater from Monterey Bay, CA, USA (36.807 N, 121.824 W) collected on June 3, 2021, and stored overnight at 10°C in the dark before being processed. Three replicate 400 mL sub-samples were manually processed using vacuum filtration through 0.2-μm pore size 25-mm Durapore filters. After filtration, the filters were aseptically transferred into a 2-mL screw cap microcentrifuge tube (Fisherbrand, Thermo Fisher Scientific) and immediately stored at −80°C. Three additional 400-mL replicates were collected and preserved onboard the ESP; those samples remained onboard the instrument in their collection cartridges for the duration of the transit.

Negative controls were used to access contamination within the ESP's sample fluidic path. Negative controls consist of molecular grade pure water (Sigma-Aldrich) collected using the ESP at the beginning ($n = 1$, 1000 mL) and at the end of the deployment ($n = 1$, 500 mL) as previously described (Truelove et al., 2022; Yamahara et al., 2019). Negative and positive control samples onboard the ESP were recovered and stored with the field samples in Honolulu, HI, as described above.

2.4 | Nucleic acid extraction

Total nucleic acids were extracted from particulates captured on filters using AllPrep PowerViral DNA/RNA kit (Qiagen). Particulate material on sample filters was mechanically homogenized, and a 400-μL aliquot of filtered sample homogenate was then processed for nucleic acid purification according to the manufacturer's instructions as previously described (Preston et al., 2019). Recovered nucleic acids were eluted in 100 μL of RNAase-free water, split into three aliquots, and stored at −80°C in 1.5-mL DNA LoBind tube (Eppendorpf, Hamburg, Germany) until used for targeted qPCR or for metabarcoding analysis. Two extraction blanks were performed during the course of extractions. Extracted DNA concentrations from field samples were measured using Quant-iT™ dsDNA HS Assay Kit (Thermo Fisher Scientific) and ranged from 1.4 to 25.7 ng/mL [average 5.2 ± 1.5 ng/mL (95% CI)].

2.5 | qPCR assays

PCR reagent preparations were performed in a designated DNA-free, UV-PCR hood (UVP) in a separate room from where reactions were thermocycled. Targeted qPCR assays (Table 1) were used to quantify total 16S rRNA genes, total 18S rRNA genes, *Pseudo-nitzschia*-specific 18S rRNA genes, *Pelagibacterales* 16S rRNA genes, and Northern anchovy (*Engraulis mordax*, dLoop) for all ESP and manually collected samples. The anchovy-specific dLoop assay utilized 1X Taqman Environmental Master Mix 2.0 (Life Technologies), SAR11 used Platinum Taq Supermix (Life Technologies), while total 18S rRNA gene, total 16S rRNA gene and *Pseudo-nitzschia*-specific 18S rRNA gene assays used 1X iQ SYBR Green Supermix (BioRad). Triplicate reactions at multiple dilutions for field samples were run on a StepOnePlus real-time PCR system (Applied Biosystems). Thermal conditions for *Pelagibacterales*, *Pseudo-nitzschia* spp., and Northern anchovy were as follows: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at the corresponding annealing temperatures (Table 1). Thermal conditions for the 16S and 18S rRNA genes followed the profiles used for amplicon sequencing (Earth Microbiome Project; Thompson et al., 2017). Upon completion of amplification, a melt curve was performed for 16S rRNA, 18S rRNA, and *Pseudo-nitzschia* spp. 18S rRNA gene assays. The background and cycle thresholds were adjusted manually to compare samples and standards across qPCR runs.

Potential inhibition of qPCR assays was assessed using serial dilutions of the nucleic acid extracts. For the anchovy assay, DNA extracts for qPCR were run undiluted and diluted 5-fold (0.2 dilution). For all other assays, extracts were diluted 5-fold, or 10-fold and 50-fold. A sample less diluted was considered inhibited if the average delta Ct between it and a higher dilution was less than the expected cycle number (e.g., <3.3 for a 10-fold dilution). Of all the eDNA samples collected, only 5, those from San Francisco Bay and nearest the California coastline showed sample inhibition.

gBlocks gene fragments (Integrated DNA Technologies) were synthesized for each target and used in serial dilutions to generate qPCR standard curves for each assay following (Searcy et al., 2022). Triplicate standard curves and no template controls (NTCs) were run on each qPCR plate. All standards for an assay were compiled to generate a master standard curve for sample quantification and used to determine the lower limit of detection (LOD) and lower limit of quantification (LOQ) following (Klymus et al., 2020). Table 1 provides each assay's performance details: master standard curve, PCR efficiency, LOD, and LOQ. All uninhibited reactions for a sample were used to calculate the average copies per mL of seawater filtered. Data analysis comparing eDNA concentrations in ESP-preserved and laboratory-collected samples were performed in R (version 4.1.2; R Core Team, 2021) using Kruskal–Wallis (KW) tests for unpaired data. *P*-values <0.05 were considered statistically significant.

2.6 | Amplicon sequencing and analysis

Recovered nucleic acids were also used to generate amplicon pools for COI Illumina sequencing (Leray et al., 2013) using Fluidigm universal oligomers C1/C2 following published protocols of Truelove et al. (2022). The five samples that exhibited inhibition during qPCR analysis using the 18S or 16S rRNA gene assays were diluted (0.1 or 0.2). Undiluted extracts were used for all other samples. Primary PCR amplifications were performed in triplicate reactions for each sample, then were pooled prior to purification using AMPure XP magnetic beads (Beckman Coulter; after Truelove et al., 2022). Amplified COI genes with Fluidigm oligomers from each sample were visualized on a 2.5% agarose gel and quantified using Quant-iT™ dsDNA HS Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocol.

Primary COI amplicons with Fluidigm oligomers were sent to the RTFS Genomics Core at Michigan State University (East Lansing, MI, USA) to complete library preparation for Illumina sequencing, which included a secondary PCR (11 cycles using 1 μL of the primary amplicon) to add dual indexed Illumina barcodes using primers targeting the Fluidigm CS1/CS2 oligomers. Final products were batch normalized using Invitrogen SequalPrep DNA Normalization plates and pooled. Pools were concentrated using a QIAquick spin column (Qiagen) and cleaned using AMPure XP magnetic beads. The resulting pool was quality controlled with Qubit dsDNA HS and Agilent 4200 TapeStation HS DNA1000 prior to paired-end sequencing (2 × 250 bp) on an Illumina MiSeq platform.

2.7 | COI sequence bioinformatic processing and statistical methods

Resulting COI sequences were processed and taxonomy assigned using a customized shell script adapted from the Banzai pipeline (O'Donnell et al., 2016) as described in the supplementary methods

TABLE 1 Quantitative PCR chemistries and standard curve results for each assay.

Target	Primer/probe ID	Sequences (5′ to 3′)	Conc. (μM)	SYBR/5′ nuclease	Annealing temp. (°C)	Slope (efficiency %)	Intercept	R^2	LOD	LOQ	NTC Ave. C_t[a]	Threshold	References
18S rRNA	Euk_1391F	GTACACACCGCCCGTC	0.2	SYBR	57	−3.42 (99.6)	32.74	0.996	10	340	33.4 (2.4)	1000	Thompson et al. (2017)
	EukBR	TGATCCTTCTGCAGGTTCACCTAC	0.2										
16S rRNA	515F	GTGYCAGCMGCCGCGGTAA	0.2	SYBR	50	−3.41 (99.9)	33.58	0.999	100	100	27.1 (0.2)	1000	Apprill et al. (2015), Parada et al. (2016), Preston et al. (2019)
	806R	GGACTACNNGGGTDTCTAAT	0.5										
Pseudo-nitzchia genus	PnGenusF	CTGTGTAGTGCTTCTTAGAGG	1	SYBR	58	−3.38 (99.9)	31.84	0.999	10	45	NA	1000	Fitzpatrick et al. (2010)
	PnGenusR	AGGTAGAACTCGTTGAATGC	1										
Anchovy (dLoop)	Eng_109F	TTCACTTGGCATTTGACGGG	0.4	5′ Nuclease	60	−3.42 (99.7%)	35.87	0.997	5	145	NA	0.01	Sassoubre et al. (2016)
	Eng_241R	TGCTCCTGAGATCACTTATGC	0.4										
	Eng_152P	FAM-AGGTTGAAC-ZEN-ATTTTCCTTGCTTGCGA-BHQ	0.15										
Pelagibacter (SAR11) 16S rRNA	SAR11-433F	CTCTTTCGTCGGGGAAGAAA	0.5	5′ Nuclease	59	−3.39 (100)	38.62	0.999	5	8	NA	10,000	Suzuki et al. (2001), Varaljay et al. (2015)
	S11V.2-588R	CCACCTACGWACTCTTTAAGC	1.5										
	Univ519bRP	FAM-TTACCGCGG-ZEN-CTGCTGGCAC-BHQ	0.2										

Abbreviation: NA, no amplification.

[a]95% confidence interval in parenthesis.

of Truelove et al. (2022). Briefly, primer sequences were trimmed using the program Atropos (Didion et al., 2017), and low-quality reads within FASTQ files were removed using DADA2 (Callahan et al., 2016) prior to merging the forward and reverse sequences. Lastly, chimeric sequences were removed. Resulting amplicon sequence variants (ASVs) were parsed taxonomically using blastN search and GenBank's nonredundant (nr) database (accessed January 2022; after Sayers et al., 2022) using the following parameters: percent identity = 80, word size = 11, *e* value = 1*e*-5, maximum target matches = 100. BlastN results were filtered using lowest common ancestor (LCA) in MEGAN6 (Bağcı et al., 2019) as previously described (Truelove et al., 2022) using these parameters: hits at ≥80% sequence identity and ≥200 bitscore.

The resulting ASV file, taxonomy file, metafile file from the Banzai pipeline were imported into either R (v.4.1.2; R Core Team, 2021) or Qiime2 (v. 2022.11; Bolyen et al., 2019) for further statistical analysis. In cases where the eukaryal phylum was unknown and a higher taxonomy level was assigned, the phylum was manually updated using taxonomy in the World Register of Marine Species (WoRMs; Costello et al., 2013). Taxa plots were generated in R using the Phyloseq (v.1.38.0; McMurdie & Holmes, 2013) and Phylosmith (v.1.0.6; Smith, 2019) packages.

Alpha diversity analyses were assessed using the Phyloseq package within R. To compare COI sequences recovered from eDNA samples collected, processed, and stored onboard the ESP to those manually collected and immediately frozen, pairwise Wilcox signed-rank tests (Vegan package v. 2.6.2) were performed on each of the alpha diversity tests rarified to 68,000 sequences. Beta diversity analysis using PCA and PERMANOVA with Aitchison distance was performed in Qiime2 using Deicode (Martino et al., 2019). Other beta diversity analyses using Bray–Curtis, weighted unifrac, and unweighted unifrac distances were performed in R using Phyloseq and Adonis2 (Vegan package).

3 | RESULTS

3.1 | Autonomous eDNA sample collection on an uncrewed surface vessel (USV)

The ESP successfully collected and preserved 52 samples over 29 days of the Surveyor's transit (June 10–July 9, 2021) from Alameda, CA to the Hawaiian Islands (Figures 1 and 3, Table S1). As expected, sea surface temperature increased over the ship's westward transit (Figure 3). The ESP initiated eDNA sample collection autonomously at user-specified intervals while Surveyor was simultaneously collecting underway seafloor bathymetry data. The speed and sailing behavior of the vessel in addition to variable filtration rates due to the density of biomass in the water determined the area over which individual sample collections occurred. Overall, ESP samples represented material acquired over transit distances of 0.6–15.1 km (average = 7.6 ± 0.8 km; 95% CI). The first 4 sample collections within San Francisco Bay and nearest the CA coastline did not reach the full target of 2 L due to high particulate/biomass loading. All subsequent collections reached the goal of filtering 2 L. Filtration time ranged from 21 to 49 min within San Francisco Bay, and 59–91 min after reaching the open waters of the Pacific Ocean.

eDNA samples collected over the transit were preserved in RNAlater and stored for 40 (first field sample collected) to 14 days (last field sample collected) before being removed from the ESP and frozen. While stored onboard Surveyor, those samples experienced ambient temperatures from 12.7°C to 38.0°C (mean and standard deviation = 26 ± 4.4°C). From June 10 to July 20th, the maximum daily temperature was above 30°C for 9 days and >35°C for 2 days. The days stored, minimum, maximum, and average temperatures for each eDNA sample acquired are found in Table S1.

3.2 | Stability of eDNA samples

Comparisons of the ESP and manually collected positive control samples collected prior to Surveyor's departure indicated that onboard storage of samples in the ESP for the length of the transit had an effect on the stability of DNA. ESP-processed positive control samples remained onboard the instrument for 46 days (6 days longer than the first sample acquired onboard Surveyor), experiencing a similar range of temperatures (mean = 23.1°C, range 12.7–38.0°C) as field samples collected during the transit. Recovered DNA concentrations were higher in control samples that were processed manually and immediately flash-frozen (average 45.1 ng/μL) versus those filtered, preserved, and stored on the ESP, which were 85% lower (average 6.9 ng/μL, Table S1). Targeted qPCR assays revealed a similar pattern and storage had a significant effect on gene abundance (Figure 4); 91% of 16S rRNA genes were lost (Kruskal–Wallis $H(1) = 12.803$, $p = 0.0003$), 97% of total 18S rRNA genes were lost ($H(1) = 12.816$, $p = 0.0003$), and 98% of *Pelagibacterales* 16S rRNA genes were lost ($H(1) = 12.803$, $p = 0.0003$) in ESP-preserved compared to flash-frozen control samples. Anchovy eDNA was detected below the limit of quantification in all 3 replicate samples collected manually and flash-frozen, as compared to 2 of the 3 samples stored onboard the ESP. *Pseudo-nitzchia* 18S rRNA genes in the control samples were quantifiable for material processed manually but were below the limit of detection for all ESP-stored samples (Figure 4).

In contrast, comparison of eukaryotic COI amplicon sequence data from positive control samples processed manually and immediately frozen, versus those processed and stored aboard the ESP were not significantly different based on alpha and beta diversity analyses (data rarified to 60,000 sequences). Alpha diversity metrics between samples were similar based on Evenness (p-Value = 0.5127, $H = 0.4286$, q-Value = 0.5127), FaithPD (p-Value = 0.5127, $H = 0.4286$, q-Value = 0.5127), and Shannon index (p-value = 0.5127, $H = 0.4286$, q-Value = 0.5127). Beta diversity analysis with PERMANOVA using Aitchison distance (p-value = 0.101, pseudo = 3.7846,

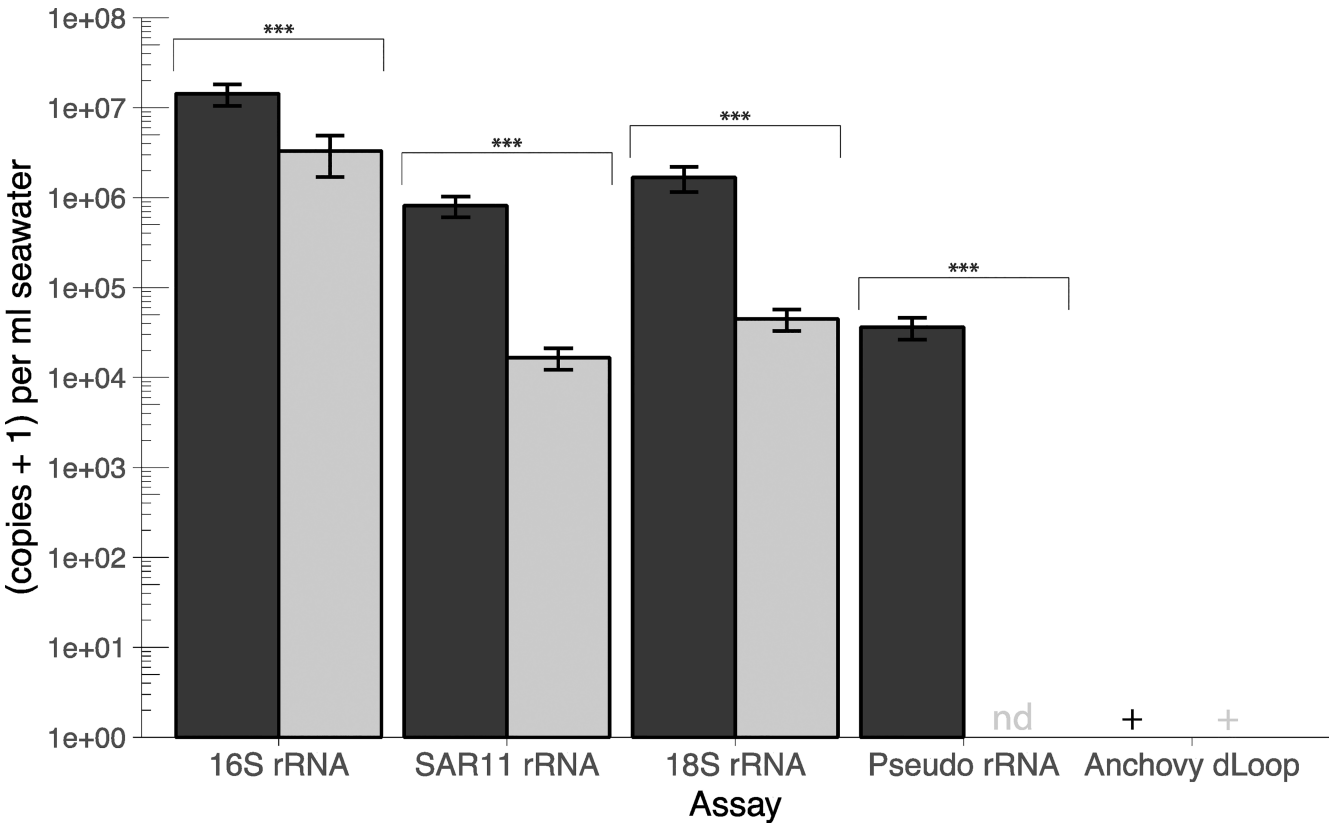

FIGURE 4 Target gene abundance in matched samples filtered and flash-frozen on day 0 (dark bars) and those collected, preserved, stored onboard the ESP, and recovered/frozen on day 46 (gray bars). See Table 1 for qPCR assay details. With the exception of anchovy, significant differences are observed (***Kruskal–Wallis $p < 0.001$) for each assay between day 0 and day 49. The anchovy-specific target was detectable in 3 of 3 replicate samples processed manually and frozen immediately (+), but only in 2 of 3 replicates processed and stored on the ESP (+ gray). All target gene concentrations in low abundance that are detectable but not quantifiable or undetected are shown as + and nd, respectively. Error bars represent the 95% confidence interval.

permutations = 999), Bray–Curtis distance (p-value = 0.289, F statistic = 1.2456, permutations = 999), unweighted unifrac distance (p-value = 0.099, F statistic = 1.376, permutations = 999), and weighted unifrac (p-value = 0.32, F statistic = 1.297, permutations = 999) were insignificant, indicating similar taxonomic diversity of eDNA recovered from manually and ESP-processed samples. Ascomycetes accounted for some of the variation; fungal amplicon sequence variants (ASVs) were at higher relative abundances in 1 of the 3 replicate ESP positive control samples (0.04%, 0.9%, and 75.5%) compared to manually processed samples (<0.1%, Figure S1).

3.3 | Negative controls

To assess contamination in the sample loop at the start and end of the ESP deployment, pure water was filtered and preserved using the ESP before its installation on Surveyor, and immediately prior to sample recovery in HI. DNA recovered from those samples was used to provide a measure of the load and type of contamination present in the instrument's sample path before and after its deployment. Prior to the ESP's installment on Surveyor, the sample path had a very low level of total 16S rRNA gene contamination (Figure 5). Although no 18S rRNA genes were detected in the negative control using qPCR, CO1 amplicon metabarcoding recovered eukarya affiliated with marine and nonmarine taxa. The number of CO1 sequences (8891 sequences) recovered in the predeployment negative control was much lower compared to those from native field samples (average 99,883, range 66,159–165,004). COI sequences (84 ASVs) in the predeployment negative control were affiliated with ascomycetes (73% relative abundance), humans (17.9%), diatoms (5.7%), chlorophytes (0.4%), haptophytes (0.4%) and cnidarians (0.1%). The most abundant ASVs detected in the predeployment negative control were not present in the positive control (ESP-3), which had been collected immediately beforehand (i.e., there was no appreciable sample-to-sample contamination). In addition, no ASV in any of the predeployment positive controls were represented by >25 sequences in the negative control (Figure S2). These results indicate a relatively low level of contamination within the sample pathway of the ESP before the start of the field deployment.

The postdeployment negative control collected 14 days after the last field sample was processed onboard Surveyor showed higher levels of contamination based on total 16S rDNA and total 18S rRNA genes as compared to the predeployment negative control (Figure 5). Nevertheless, total copies of 16S (2.67×10^3/mL) and 18S

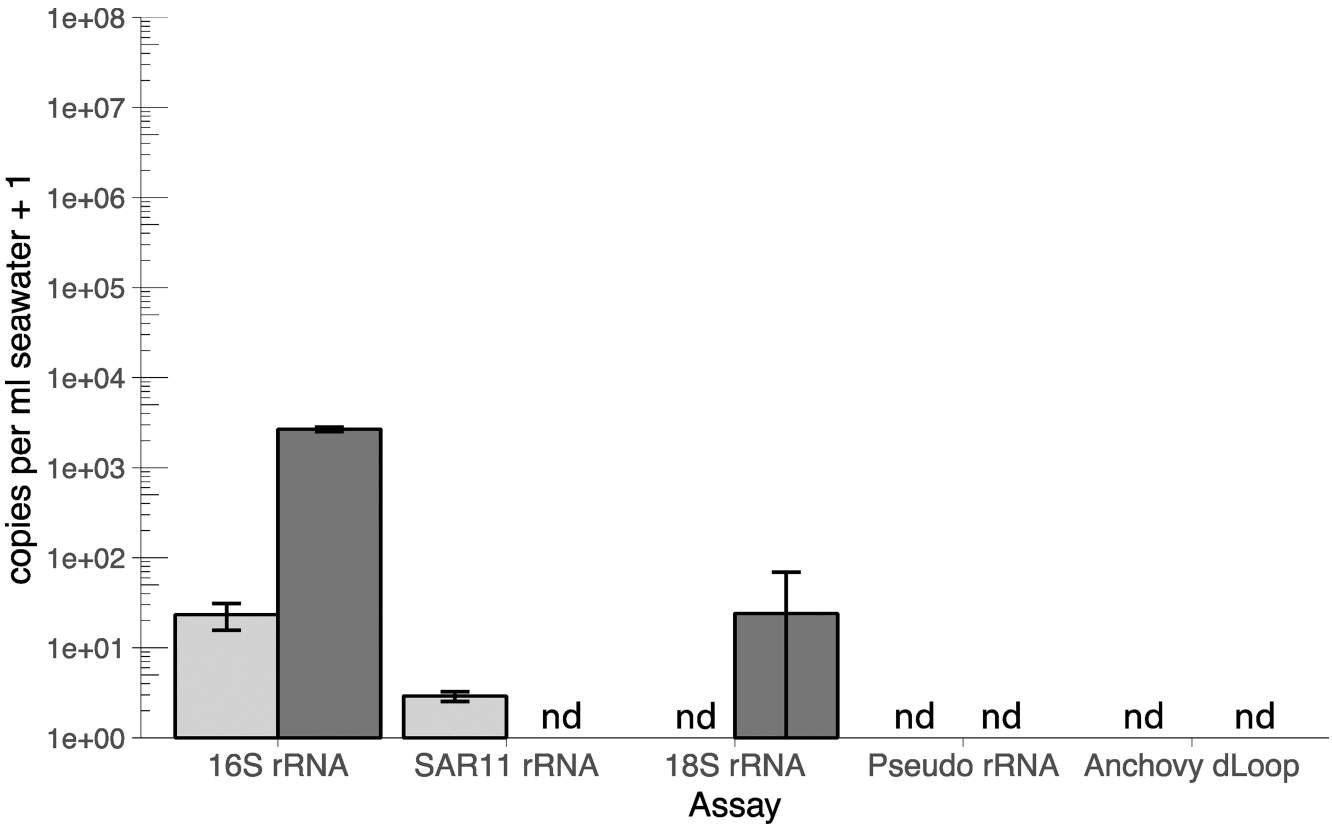

FIGURE 5 Gene concentrations of eDNA in negatives controls collected before integration in Surveyor (light gray) and after the completion of the transit (dark gray). Error bars represent the 95% confidence interval. nd is an eDNA concentration below the limit of detection. See Table 1 for qPCR assay details.

rRNA genes (23/mL) found in the postdeployment negative control were 19.5- and 87-times lower, respectively, than the lowest concentrations quantified from native field samples. In addition, more COI sequences were recovered from the postdeployment negative control (203,507 sequences, 97 ASVs) compared to the predeployment negative control. Nearly all of the postdeployment COI amplicon sequences (99.7%) recovered were affiliated with the ascomycete, *Penicillium sclerotiorum* (203,011 sequences, 23 ASVs). Only two other ASVs were represented by >25 sequences, a marine haptophyte (279 sequences) and an unassigned ASV (38 sequences). The same marine haptophyte ASV was also present in the last field sample collected onboard Surveyor (244 sequences). Previous experience with analogous negative control analyses where no bleach flush was used between sample collections have shown higher levels of contaminating marine taxa fouling accumulation over the course of a deployment (e.g., Truelove et al., 2022). These results indicate that the addition of using a weak bleach solution to flush the sample path between sample collection events combined with an increased volume of flushing native water through the system before initiating sample collection reduced the abundance of contaminating marine taxa in the sample loop.

In addition to controls for the ESP, molecular grade water (no template controls, NTCs) and extraction blanks were analyzed by both qPCR and metabarcoding. Only NTCs for the universal assays (e.g., 16S and 18S rRNA genes) showed a low level of amplification (Table 1). Extraction blanks processed during field sample extraction also indicated a low level of amplification (Table 1) for all qPCR assays except for anchovy. NTCs and the extraction blanks for metabarcoding also showed some amplification of COI genes. From the NTCs ($n=3$), a total of 2204 sequences representing 253 ASVs were found. No ASV was represented by greater than 310 sequences. The top five ASVs were affiliated with marine taxa belonging to Cnideria, Discoea, Haptista, and Arthropoda. The two extraction blanks had different levels of amplification; 53,248 and 76 sequences. Those sequences belonged to 186 ASVs. Of those 11 ASVs were represented by more than 1000 sequences, and 59 ASVs by more than 100 sequences. The top five ASVs were affiliated with Haptista, Ochrophyta, and Bacillariophyta.

3.4 | eDNA analysis of samples collected aboard Surveyor

The overall trend in gene abundances per mL seawater for total 16S and 18S rRNA genes over the course of the transit were largely as expected. Gene abundances for bacteria and archaea (16S rRNA genes) and eukarya (18S rRNA genes) in ESP-collected samples (Figure 6a) were highest inside San Francisco Bay [$n=3$, 16S rRNA

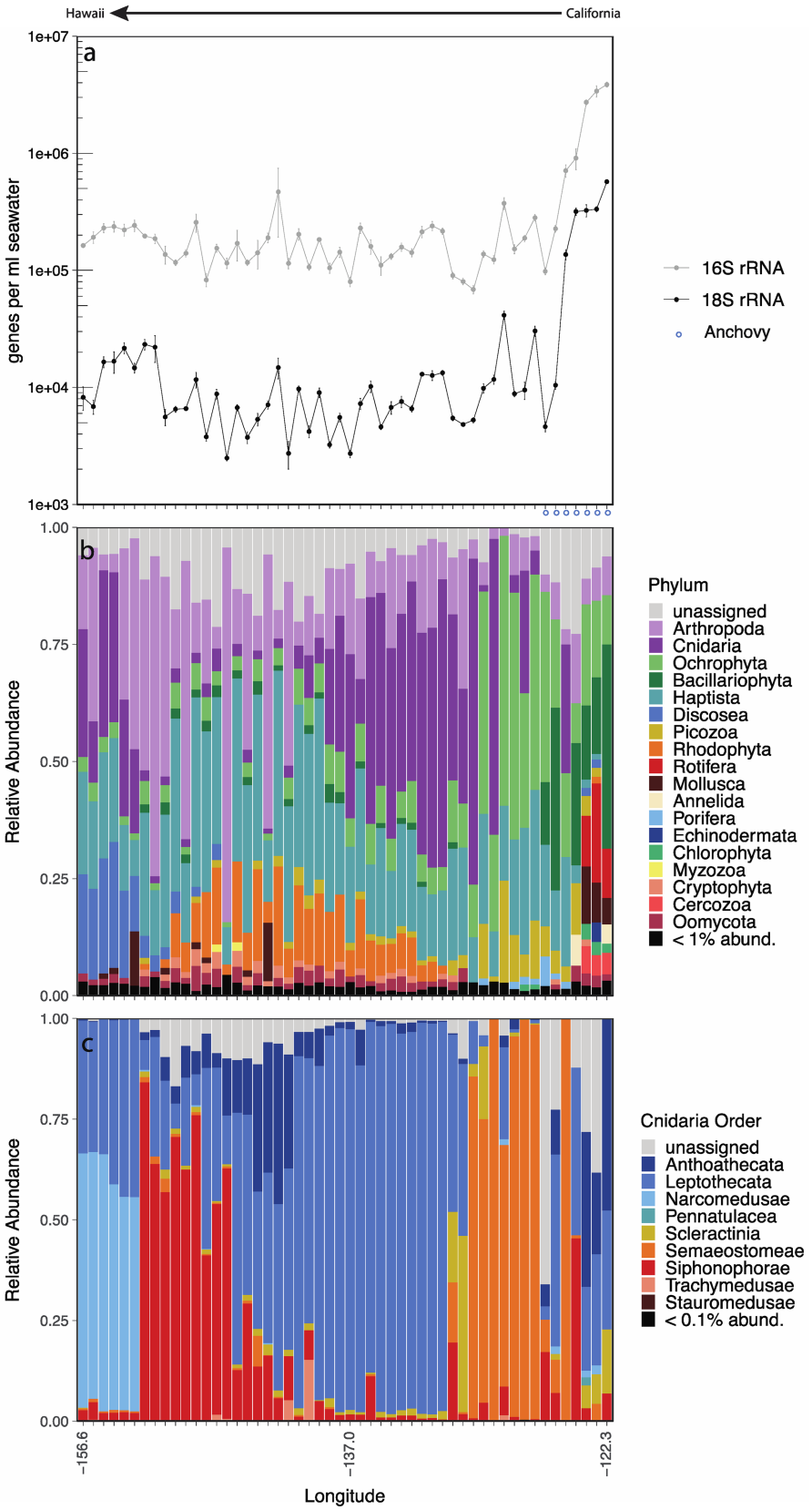

FIGURE 6 Analysis of eDNA from particulate samples collected and preserved by the ESP. The concentrations of targets as determined by qPCR (a) for 16S and 18S rRNA genes, and anchovy over the transit from California (right) to Hawaii (left). Anchovy dLoop genes were detected but not quantifiable in a subset of the samples nearest California (blue circles in panel a). The relative abundances of unrarified CO1 gene sequences affiliated with Eukaryotic phyla (56% of total) and Cnidarian orders are shown in panels b and c, respectively. CO1 gene sequences not identified as Eukarya (e.g., unknown, no_hit, Bacteria, and Archaea) were removed prior to analysis.

average $3.3 \times 10^6 \pm 5.3 \times 10^5$ (95%CI); 18S rRNA gene average $4.1 \times 10^5 \pm 1.3 \times 10^5$ (95%CI)] and along the California coast [$n = 2$, 16S rRNA gene average $8.1 \times 10^5 \pm 1.5 \times 10^5$ (95%CI); 18S rRNA gene average $2.3 \times 10^5 \pm 1.3 \times 10^5$ (95%CI)]. After crossing the California shelf, samples contained lower gene abundances overall. In addition, no clear trend in either rRNA gene abundance were observed for the remainder of the transit to the Hawaiian Islands. 16S rRNA gene abundances per mL seawater ranged from 6.9×10^4 to 4.7×10^5

[$n=47$, average $1.7\times10^5\pm2.1\times10^4$ (95%CI)]. 18S rRNA gene abundances per mL seawater ranged from 2.5×10^3 to 4.1×10^4 [$n=47$, average $1.0\times10^4\pm2.1\times10^3$ (95%CI)]. Anchovy DNA was only detected (albeit below the limit of quantification) at the first seven locations sampled. Anchovy DNA was not detected thereafter in samples collected during the remainder of the transit to HI.

Sequence analysis using the Banzai sequencing pipeline (O'Donnell et al., 2016; Truelove et al., 2022) identified 9167 ASVs in samples collected during the transit to Hawaii. Fifty-six percent (5183 ASVs) of the total ASVs were identified as eukaryotic CO1 genes. Of the eukaryal CO1 genes, 87.7% (4543 ASVs) were assigned to the rank of phylum. More than half of the eukaryal ASVs (56%) were found in fewer than 5 of 52 eDNA samples and 74% were found in fewer than 10 samples. Thirteen eukaryotic ASVs (no sequence minimum per sample) were found in >90% (47 of 52) of the eDNA samples collected. eDNA samples collected in San Francisco Bay and nearer the California coastline contained fewer eukaryotic ASVs compared to samples in oligotrophic waters further westward (Chao1 metric). Samples with the highest diversity, estimated using the Shannon Index, were collected during the day in a western region of the transit from 141° W to 149° W longitude (Figure S4).

The eukaryal ASVs recovered were affiliated with 40 phyla. Thirteen eukaryotic phyla were found in all samples. Of those, Arthropoda, Bacillariophyta, Haptista, Ochrophyta, and Cnidaria were present in all field samples at >0.1% relative abundance. Individual samples contained an average of 22 eukaryotic phyla (range 17–26). Although many of the same eukaryotic phyla were detected in samples across the transit, there was a striking difference in their relative dominance regionally (Figure 6b).

Within San Francisco Bay eDNA samples ($n=3$), 775 eukaryotic ASVs were identified and 81% were assigned to 29 phyla (Figure 6b). The top five eukaryotic phyla, diatoms (Bacillariophyta, 27.2% relative abundance), brown algae (Ochrophyta, 18.7%), Rotifera (14.3%), Mollusca (10.3%), and Arthropoda (7.3%), have historically been observed in the Bay (Ambler et al., 1985; Bollens et al., 2011).

For samples collected from the open Pacific Ocean ($n=49$), 4868 ASVs were identified and 88.4% of those belonged to 37 eukaryotic phyla. Six phyla accounted for the majority of eukaryotic sequences (88%). Those include Arthropoda (23.1%), Haptista (23.3%), Cnidaria (22%), Ochrophyta (11.1%), Rhodophyta (5.7%), and Discosea (3.4%). Haptista was within the top six phyla for every sample collected from open waters with relative abundance range from 4.9% to 48.3% (average 25.5%). The relative proportions of other eukaryotic phyla clearly varied across the transit (Figure 6b). For example, Picozoa and Bacillariophyta had higher relative abundances of sequences in samples collected closer to the California coastline, whereas Discosea made up a greater contribution of sequences recovered nearer Hawaiian Islands. The relative abundance of cnidarian phyla was highly variable, ranging from 0.2% to 71.5%. Samples where cnidarian abundances were >5% of total ASVs included the cnidarian orders Leptothecata, Semaeostomaeae, Narcomedusae, Siphonophorae, Scleractinia, Trachymedusae, and Anthoathecata (Figure 6c). Often particular ASVs were abundant for large distances transit. For example, Rhodophyta ASVs were >1% relative abundance in 28 consecutive samples representing 222 km of Surveyor's cruise track and spanning 2210 km of the Central Pacific.

A number of rarer phyla were represented in relatively small numbers and/or only sporadically (e.g., Mollusca and Chordata) compared to other taxa. Pelagic sea snails, related to Atlantidae (8815 sequences, one ASV) and Pteropoda (9711 sequences, one ASV) were each found in one eDNA sample (26.54 N, 143.61 W, and 21.62 N, 155.63 W, respectively). Squid (*Eucleoteuthis luminosa*, 99.7% identity) were present in two nighttime eDNA samples (32.09 N, 128.76 W and 29.93 N, 132.29 W, one ASV, ~50 sequences in each sample). In a single eDNA sample near the CA coast (37.51 N, 122.91 W), cetacean COI genes (14 sequences) were detected. Noticeably absent from the COI metabarcoding data set were sequences affiliated with fish. Rare anchovy DNA detected using qPCR in the first seven eDNA samples was absent in the COI sequence data.

4 | DISCUSSION

Monitoring and management of the world's oceans require observational scalability beyond what traditional crewed ships can accomplish (cost and availability), and uncrewed vessels have been called upon as a means to meet that need (Jacobs et al., 2021; Meinig et al., 2019). Previous large-scale assessments of marine biodiversity have been exclusively accomplished using crewed research vessels (de Vargas et al., 2015; Ibarbalz et al., 2019; O'Rorke et al., 2022; Raes et al., 2018; Sommer et al., 2017). Here we demonstrated the potential of accomplishing large-scale assessments of biodiversity using an uncrewed system by successfully combining the ESP with the USV Surveyor to remotely collect particulate eDNA samples across a 4200-km region of the East Pacific Ocean over 29 days. Satellite communications with Surveyor were critical to the success of this operation by providing real-time information on the vessel's location, prevailing environmental data, etc., (all displayed via Saildrone's mission portal) as well as operator access to the ESP to review the instrument's logs and interact with the sampler (e.g., alter the sampling schedule). The ESP required minimal intervention during the transit. Of 55 sample collection cartridges used in the field during the 29-day transit, three failed to pass the ESP's leak detection algorithm. The three that failed triggered a "retry" response using another collection cartridge to keep autonomous sampling going so that no loss in eDNA samples occurred at scheduled times. All eDNA samples collected and preserved by the ESP (field and controls) remained at ambient temperatures, the longest for 46 days (including 29 at sea days, 3 port days, and 14 days of instrument storage at MBARI or UH) until recovery and freezing.

Overall, results from comparing the positive control samples indicated that material collected using the ESP fairly represented the diversity of organisms that were present in a sample (Figure S1), even though some portion of the sample was lost due to DNA

degradation (Figure 4). Previous studies (Den Uyl et al., 2022; Truelove et al., 2022; Yamahara et al., 2019; Zhang et al., 2020) making the same comparisons using metabarcoding, metagenomics, and qPCR have shown that RNAlater-preserved samples processed by the ESP are stable and equivalent to their manually processed counterparts for at least a month. However, in those cases, the ESP was deployed underwater aboard an AUV and operated under relatively cooler, less variable temperatures for shorter durations. Here, the ESP was mounted in the aft hull of Surveyor where daily temperature fluctuations (delta temperature 9°C average and 25°C maximum) and occasionally hot spikes (maximum of 38°C) occurred over ~1.5 months. To our knowledge, the eDNA sample storage conditions encountered in this study are unlike any attempted previously. These results show that the method of sample preservation used in this study at the temperatures experienced is not optimal and that some targets or samples may be more sensitive to those conditions than others. Nevertheless, measures of biodiversity based on COI metabarcoding were remarkably similar between material that was filtered manually and flash-frozen versus that processed using the ESP and stored onboard the instrument for 46 days. Comparisons of the number of sequences of each ASVs recovered from manually and ESP-processed positive control samples were also similar but with one notable exception: the higher abundance of ascomycete COI sequences in one of the ESP positive control samples (Figure S1), which we interpret as being postpreservation fungal growth (see below).

COI ASVs recovered from control samples and during the transit suggest that a low level of cross-contamination does exist, but it does not significantly alter the community diversity of the field study. Eukaryal COI ASVs recovered in the negative controls (Figures S2 and S3) accounted for less than 300 sequences present in the previous seawater sample, indicating a very low level of sample-to-sample carryover. In addition, contamination in the sample path observed here although not eliminated, was significantly reduced by backflushing the sample loop of the ESP with weak bleach, compared to previous studies where no toxicant was used (Truelove et al., 2022; Yamahara et al., 2019).

Results also suggest that an unusually high relative abundance of ascomycetes could serve as indicators of significant biofouling or potentially compromised samples where indigenous fungi have grown postcollection. Ascomycetes are found naturally within marine eDNA samples, so their presence is not unexpected. However, it is notable that for two ESP eDNA samples (a single ESP predeployment positive and the postdeployment negative), they accounted for a much greater proportion of the COI sequences. The high relative abundance in postdeployment negatives suggests that either the fungi are not very sensitive to the bleach solution used to clean the sample intake, or that the toxicant (0.2% sodium hypochlorite) became less effective at suppressing fungal fouling over time. The unexpected high percentage of ascomycete COI sequences in a single ESP predeployment positive control suggests fungi may be able to grow if a sample is not adequately preserved. In the field samples collected during this study, ascomycete COI sequences were present, but only at low relative abundances (0–169 total sequences, representing 0%–0.4% of the relative abundance). Thus, we interpret that the RNAlater preservation protocol sufficiently stabilized material acquired onboard Surveyor for subsequent metabarcode analysis.

Cytochrome oxidase subunit I metabarcoding results clearly revealed patterns of major taxa shifts in dominant eukaryotic phyla across the transit representing a wide range of eukaryotic lifestyles, including pelagic (e.g., Narcomedusae and Trachymedusae), pelagic-polyp (e.g., Semaeostomeae and some Leptothecata), and sessile. Eukaryotic phyla identified with a dominant sessile lifestyle included Rhodophyta, some Ochrophyta (class Phaeophyceae), Cnidaria (orders Scleractinia, Anthoathecata, and some Leptothecata), and Echinodermata. The ocean bottom depth for the majority of sample collections that occurred aboard Surveyor was greater than 3500 m, with only one seamount (32.44 N, 127.80 W, 500 m bottom depth) within 50 km of the transit path. Members of these phyla have been described as biofouling organisms (Edmiston et al., 2021; Leary et al., 2014; Pochon et al., 2015). Possible sources of DNA from these organisms include planktonic larvae from benthic taxa, biofouled marine debris in surface waters, or biofouling of the vessel's hull.

4.1 | Lesson learned

The two objectives of this study were to (1) evaluate the utility of the ESP as means for collecting and storing eDNA samples aboard a long-endurance USV, and (2) determine if the eDNA recovered reflected the expected biodiversity of the biomes the vessel traversed. Our results demonstrate that the ESP can be used to collect samples over extended times and distances, and with a few exceptions (see below) the DNA recovered appears to represent the types of biological assemblages that one would have expected based on COI metabarcoding analysis. However, controlled tests also showed that sample degradation and contamination can occur. This reveals the importance of incorporating the appropriate controls (positive and negative) when conducting eDNA studies utilizing new technologies. Consequently, work remains to improve sample processing/storage protocols, identify alternative preservatives, and better understand the efficacy of various methods for interpretive purposes.

The potential for using eDNA samplers for extended operations at sea highlights the critical need to account for conditions that samples must endure. For example, ambient temperatures that the ESP experienced during Surveyor's transit were unlike anything encountered previously, both in terms of diurnal swings and peak temperatures. Mimicking those conditions in a series of controlled laboratory experiments would be informative. Looking forward, the potential extent and range of possible temperature fluctuations onboard a USV will also vary depending on the specific vessel used, where the sampler is mounted within the vessel, if any means of temperature control within the payload bay is available, transit times/tracks, and delays associated with port stops where servicing of the eDNA collection device is not possible. Anticipating such a wide range of

possible conditions to inform extensive laboratory tests is not practical in the near term. Gaining a greater understanding of how different preservation methods and storage conditions effect sample stability aboard USVs under real-world conditions, and definitively determining if DNA from certain species is better preserved than others, for example, will take time. Nevertheless, additional laboratory experimentation based on empirical data obtained during this study, combined with repeating the type of field operation done here under various conditions (e.g., in warmer and colder regions, longer and shorter durations), would be very informative in the near term.

Developing standardized proxy measures for assessing sample integrity is another area worthy of further study. For example, inference of whether samples appropriately represent the biodiversity of waters collected can be based on the relative abundance of certain taxa in the context of the environment being sampled. In that regard, ideally, material collected from hull scrapings pre- and postdeployment in relation to the location of the sample intake should also be included as to provide insights into the role hull biofouling may play in potentially confounding eDNA biodiversity assessments. The latter is especially important in cases where the sample intake is hull-mounted, as it was here.

For the CA to HI transit, Surveyor was configured for seafloor mapping as its primary mission objective. Operationally, the ESP was unaware of its location and environment and sampling occurred at a designated time of day. Aside from satellite images providing an overview of regional changes (e.g., temperature, Figure 3), sensors that collect continuous suites of environmental measurements such as chlorophyll, oxygen, etc., were not available during eDNA sample collection. As USVs are increasingly tasked for autonomous eDNA sample acquisition, the sampler and platform must communicate directly and a greater suite of environmental sensors should be included. Direct communication between the two permits access to actionable data (e.g., latitude and longitude) without a human-in-the-loop. Platform sensors provide high-resolution measurements during and between each sample; thus, they provide actionable data that can drive where or when the sampler acquires eDNA samples (e.g., see Zhang et al., 2020, 2022). Along with concurrent collection of prevailing environmental data and its use to drive eDNA sampling, an ability to sample water from a variety of depths would also be very useful since eDNA signals are known to vary throughout the water column (Chavez et al., 2021). Understanding the effect that time of day has on the diversity of sequences recovered is yet another area that must be explored further since diel vertical migration can impart changes in eDNA profiles in near-surface waters (Lo, 2004; Sommer et al., 2017; Suter et al., 2021).

Although there is much work to be done, there is no doubt that automated eDNA collection devices offer a means to greatly expand the spatial and temporal scales over which material is acquired. However, automated eDNA collection identifies the need for processing large numbers of samples once material is returned to shore. Regardless of how quickly material can be handled onshore, the time required to extract, prepare, and analyze samples will always result in a lag between collection events and the availability of actionable information, which, in turn, limits the utility of eDNA analytics for time-sensitive resource management applications. For this reason, consideration should be given to fully automating eDNA analysis in situ, from live sample acquisition to processed data transmission. Even if and when that is achieved, traditional means of preserving samples should still occur simultaneously to allow for laboratory-based studies to verify results of data obtained in situ, as well as for conducting additional analyses that are not yet possible or practical to accomplish autonomously at sea.

All of the considerations noted above must also be viewed from the lens of the specific question or scientific objective that is at-hand. In some cases, sample mission requirements may be more demanding than others given the environment being sampled, the duration of the mission, the organisms of interest, and the need (or not) for immediate actionable data return. In any case, establishing standardized operating procedures so that material collected using a variety of samplers and USVs can be directly compared through some type of validation procedure would be beneficial in helping to advance this new era of autonomous eDNA ocean monitoring and exploration.

AUTHOR CONTRIBUTIONS

CS, JB, RJ, and BC designed the study. DP, SJ, BR, and RJ integrated the instrumentation to enable eDNA sample collection. CP, BR, and RJ oversaw the acquisition of samples. CP, CW, KY, and JR performed the data analysis. CP, JB, KY, and CS wrote the initial manuscript and all authors provided feedback.

ACKNOWLEDGMENTS

We thank the engineers, technicians, machinists, and R/V *Paragon* crew at MBARI for their skill and dedication supporting Environmental Sample Processor (ESP) instrumentation, as well as the engineers and pilots at Saildrone for their expertise and assistance with installing the ESP aboard the Surveyor SD 1200, and for piloting the vessel. We thank Larry Mayer for the opportunity to participate during Surveyor's voyage to autonomously map the seafloor. Maximilian D. Cremer and the University of Hawaii Marine Center generously provided laboratory space for storing the ESP and recovering eDNA samples following its transit from CA to HI. We thank Jacoby Baker, N. Kobun Truelove, and Kathleen Pitz for many helpful discussions on COI metabarcoding and analysis. Lastly, we thank the anonymous reviewers for comments that improved the manuscript. This work was supported by funds from the David and Lucile Packard Foundation, and NOPP grant (NA19OAR0110409).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequence data have been submitted to NCBI's SRA database under accession number PRJNA977706. Scripts for generating figures

can be found in GitHub at https://github.com/cmpmbari/Surveyor_ESP_eDNA_CAtoHI_2021. Other data available from authors upon request.

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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: Preston, C., Yamahara, K., Pargett, D., Weinstock, C., Birch, J., Roman, B., Jensen, S., Connon, B., Jenkins, R., Ryan, J., & Scholin, C. (2024). Autonomous eDNA collection using an uncrewed surface vessel over a 4200-km transect of the eastern Pacific Ocean. *Environmental DNA*, *6*, e468. https://doi.org/10.1002/edn3.468