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Rapid assessment of coral cover from environmental DNA in Hawai'i

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Abstract

Coral reefs support the most diverse assemblages of marine life on Earth, yet are declining due to local and global stressors. Rapid and widespread monitoring is essential for tracking ecosystem responses, but assessment of coral communities traditionally relies on time-consuming visual estimates of coral cover, the percentage of substrate occupied by living corals. The analysis of environmental DNA (eDNA) offers fast and efficient insights into the abundance and distribution of species, yet it remains untested to monitor coral biomass. Here, we demonstrate that visual estimates are highly correlated with the abundance of coral eDNA on reefs in Hawai'i measured with a relatively simple, rapid, but replicated PCR-based metabarcoding approach. Target sequence length was also tested by amplifying short (~120 base-pairs) and long (~400 base-pairs) fragments from the same region of two mitochondrial DNA genes, 16S ribosomal DNA, and cytochrome oxidase-1 using primers designed to preferentially amplify Hawaiian coral genera. Careful primer selection and target sequence lengths play an important role in determination of coral abundance from eDNA biomass. Given its broad applicability and ease of use, eDNA metabarcoding can provide complementary analytical support for biomonitoring programs and management initiatives tracking changes in coral cover caused by climate change and other disturbances on coral reefs.

KEYWORDS
biomonitoring, coral reefs, environmental DNA, molecular ecology

1 | INTRODUCTION

Coral reefs are among the most threatened ecosystems on Earth (Baker, Glynn, & Riegl, 2008; Brown, 2016; Burke, Reytar, Spalding, & Perry, 2011; De'ath, Fabricius, Sweatman, & Puotinen, 2012; Edmunds et al., 2014; Erftemeijer, Riegl, Hoeksema, & Todd, 2012; Hughes et al., 2017; Pandolfi et al., 2003) and there is an urgent need to increase the frequency and scale of monitoring (Parrish, Braun, & Unnasch, 2003; Roberts et al., 2002) in the face of global change. Currently, biodiversity and ecosystem functioning on reefs are surveyed by numerous complementary methods (Brown et al., 2004; Levy, Hunter, Lukacazyk, & Franklin, 2018; Mumby, Green, Edwards, & Clark, 1999; Rodgers, Jokiel, Bird, & Brown, 2010; Williams et al., 2016). Underwater visual surveys are most widely conducted using SCUBA, which can be both time consuming and logistically challenging. As an efficient alternative, the analysis of environmental DNA (eDNA), DNA shed or expelled from organisms into the environment, has been used to assess species diversity, primarily in aquatic environments (Andruszkiewicz, Starks, et al., 2017; Boussarie et al., 2018; DiBattista et al., 2017; Everett & Park, 2018; Jerde, Mahon,

Chadderton, & Lodge, 2011; Klymus, Marshall, & Stepien, 2017; Li et al., 2018; Parsons, Everett, Dahlheim, & Park, 2018; Port et al., 2016; Stat et al., 2017; Thomsen, Kielgast, Iversen, Moller, et al., 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012; Ushio et al., 2017). Despite the growing use of eDNA to catalog the presence and absence of species, the quantitative description of the relative abundance of species (or guilds of functionally similar species) with eDNA remains an open question (Bakker et al., 2017; Elbrecht & Leese, 2015; Evans et al., 2016; Hänfling et al., 2016; Kelly, Port, Yamahara, & Crowder, 2014; Kelly, Port, Yamahara, Martone, et al., 2014; Klobucar, Rodgers, & Budy, 2017; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Piñol, Senar, & Symondson, 2019; Port et al., 2016; Sigsgaard et al., 2016; Stoeckle, Soboleva, & Charlop-Powers, 2017; Taberlet, Bonin, Zinger, & Coissac, 2018).

In principle, individual taxa should be represented in the eDNA pool in proportion to their biomass if DNA sloughing and degradation rates are similar across taxa. Because eDNA supply, transport, and degradation dynamics are poorly understood (Andruszkiewicz, Sassoubre, & Boehm, 2017; De Souza, Godwin, Renshaw, & Larson, 2016; Deiner & Altermatt, 2014; Dell'Anno & Corinaldesi, 2004; Jane et al., 2015; Jerde et al., 2016; Jo et al., 2017; Kelly, Gallego, & Jacobs-Palmer, 2018; Pilliod, Goldberg, Arkle, & Waits, 2014; Shogren et al., 2017; Turner, Uy, & Everhart, 2015), researchers have focused on the development and critical assessment of a variety of eDNA methods that have different strengths and weaknesses. At one end of the analytical spectrum is quantitative PCR (qPCR), a highly precise and sensitive way to monitor template eDNA abundance in real-time PCR reactions (Murray, Coghlan, & Bunce, 2015; Taberlet et al., 2018). qPCR has become the standard for detection of rare species (Lacoursière-Roussel et al., 2016; Nevers et al., 2018; Wilcox et al., 2013), and can provide information about species abundances based on eDNA biomass (Tillotson et al., 2018; Yamamoto et al., 2016). However, qPCR is limited in that fewer species can be studied at a time with species-specific primers, making community-wide abundance data more technically challenging, costly, and time consuming to ascertain (Harper et al., 2018).

At the other end of the methodological spectrum is community-wide metabarcoding (i.e., PCR combined with next-generation DNA sequencing) with a single (or small number) of universal DNA primer pairs designed to target a relatively wide range of taxa, but not any one group. The disadvantage of metabarcoding with universal primers is that it typically creates uneven PCR amplification across taxa due to the varying quality of the match between primers and a diverse template DNA pool (Bista et al., 2018; Elbrecht & Leese, 2015; Murray et al., 2015; Piñol et al., 2019; Taberlet et al., 2018). In the middle of this spectrum are taxon-specific primers, that, by targeting a narrow range of taxa sharing conserved PCR priming sites, have the potential to recover estimates of abundance (Hänfling et al., 2016; Harper et al., 2018; Pont et al., 2018; Stoeckle et al., 2017; Thomsen et al., 2016; Ushio et al., 2017) as well as to detect greater diversity (Bakker et al., 2017; Balasingham, Walter, Mandrak, & Heath, 2018; Boussarie et al., 2018; Port et al., 2016; Stat et al., 2017; Valentini et al., 2016; Yamamoto et al., 2017) by limiting amplification of unwanted groups and maximizing amplification of target taxa.

Here, we utilize eDNA metabarcoding with primers developed to preferentially amplify two mitochondrial DNA genes (16S ribosomal DNA, or 16S and cytochrome oxidase-1, or COI) from corals on shallow-water reefs of Hawai'i to determine whether or not eDNA surveys could accurately predict coral relative abundance based on percent cover from visual surveys. Our primary objective was to address whether accurate abundance data could be ascertained from an eDNA metabarcoding approach for coastal field sites. Scleractinian corals are ideal subjects for eDNA metabarcoding and are amenable to this approach because they possess highly conserved mitochondrial genomes which allow for targeted amplification of many coral genera with a single pair of primers. Biomonitoring of coral cover using eDNA may also work particularly well given that coral biomass is correlated with surface area, likely a key factor determining the rate at which eDNA is released into the water. We also addressed factors influencing community predictions of abundance, specifically, how target sequence length affects abundance predictions from eDNA. Target sequence length may play an important role in determination of coral abundance from eDNA biomass, as shorter fragments of free eDNA persist longer in marine environments (Dell'Anno & Corinaldesi, 2004; Jo et al., 2017).

2 | METHODS

2.1 | Sampling

Visual surveys of coral cover were conducted during August 2017 around a centroid at each of four sites (Kāne'ohe, Lanikai, Waimānalo, and Maunalua, Figure 1) around the island of O'ahu, Hawai'i using photo-quadrats along three 20 m transects. Transects were aligned perpendicular to shore, spaced 10–15 m apart. A total of 11 (0.5 m × 0.5 m) quadrats were photographed per transect and analyzed using 100 random points per quadrat (for a total of 1,100 points per transect) in CPCe (Kohler & Gill, 2006). Photo-quadrats were randomized and metadata were removed for blind allocation during processing to minimize bias. Water was then collected during August-November 2017 during slack high tide (mean 0.24 m), from the centroid of the three 20 m transects, at each of the same four sites using four sterile 4 L Cubitainers (Hedwin Co.) at depths between 2–4 m, 1 m above the reef. Conditions during collection were partly cloudy, with onshore winds (2.5–7.7 m/s), and an average sea surface temperature of 26°C. Immediately after collection, samples and blanks were placed in the dark on ice and processed within 5 hr of collection.

2.2 | Water filtration and DNA extraction

For each site, we analyzed four different amplification products (two COI primer pairs and two 16S primer pairs) from four biological replicates (multiple water samples) and two negative controls (see Contamination prevention, below). Two technical replicates (duplicate

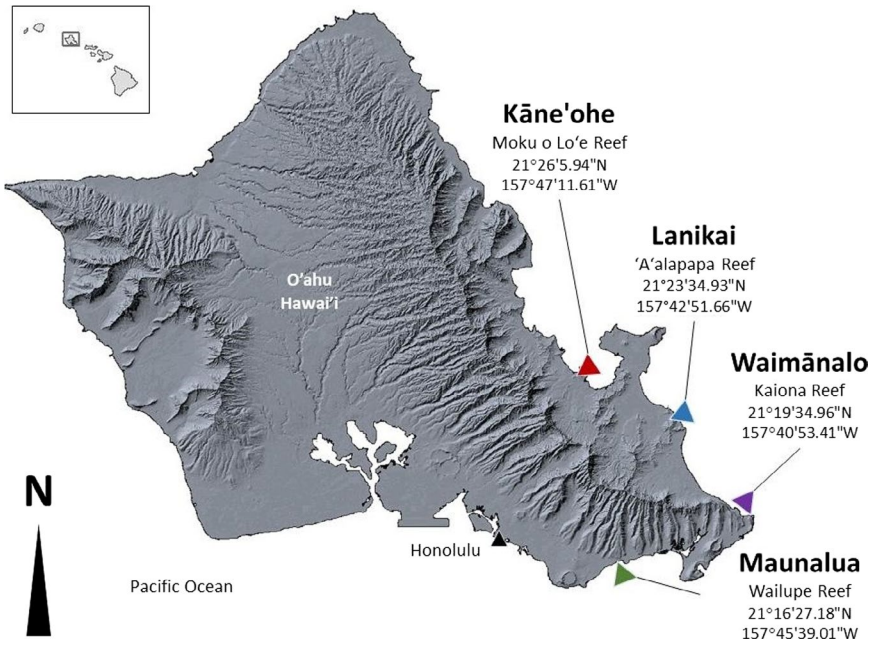

FIGURE 1 Map of the four coastal field sites along the eastern and southern coasts of O'ahu, HI. Reef sites were chosen based on varying degrees of impact, from a marine reserve (Kāne'ohe), popular recreational beaches (Lanikai and Waimānalo), and a developed bay (Maunalua). All sites are easily accessible from shore, with depths ranging from 2 to 4 m. Site centroids are noted with GPS coordinates

extractions and amplifications from individual water samples) were analyzed for each individual water sample for a total of 176 DNA libraries (Figure S1). Water samples (four from each site) and collection blanks (one from each site) were individually homogenized by shaking and inverting collection bottles followed by immediate filtration of a 2 L subsample through sterile analytical test filter funnels (Nalgene) with sterile mixed cellulose esters filters (Millipore; diameter, 47 mm; pore size, 0.22 μm). To maximally and consistently recover DNA from the filters, DNA was then extracted using bead beating of the filter, followed by use of the Qiagen DNeasy Blood & Tissue Kits (Thomsen, Kielgast, Iversen, Moller, et al., 2012). Using sterile forceps and scissors, filters from each water sample were cut in half (two technical replicates) and placed into 2 ml screw-cap microcentrifuge tubes with 0.3 g of 0.5 mm zirconia/silica beads (Biospec Products) and 720 μL ATL cell lysis buffer (Qiagen). Tubes were shaken vigorously for 5 min in a tissue lyser (Retsch) and then incubated at 56°C for 30 min, followed by an additional bead beating and 30-min incubation step. Each filter half (technical replicate) per water sample (biological replicate) was then separately digested with 80 μL Proteinase K (Qiagen) and incubated at 56°C for 2 hr. After transferring 600 μL of the supernatant to new tubes, the manufacturer's protocol for DNeasy Blood & Tissue Kit (Qiagen) was followed with minor adjustments (600 μL AL Buffer, 600 μL ethanol, and two final elution steps of 50 μL AE Buffer). Total eDNA concentrations (mean ± *SE*) extracted from filters were 4.98 ± 0.46 ng*μL^{-1} for Kāne'ohe, 14.69 ± 2.10 ng*μL^{-1} for Lanikai, 5.31 ± 1.43 ng*μL^{-1} for Maunalua, and 5.11 ± 0.48 ng*μL^{-1} for Waimānalo.

2.3 | Primer evaluation

Given that PCR bias (created by primer-template mismatches) is a fundamental concern in eDNA studies (Piñol et al., 2019), amplifications were repeated with two different genes, mitochondrial 16S and COI (Figure S2 and Figure S3, Supporting information). We also assessed the impact of amplicon size, using six primers (Table S1, Supporting information) in combination to amplify short (~120 bp) and long (~400 bp) intervening sequences of each COI and 16S. Novel forward primers were used in both short and long target sequence analyses and two sets of reverse primers for each gene were used to target 120 base pairs and 400 base pairs of the same sequences. All primers were specifically designed in silico in Geneious v11.1.5 (Biomatters Ltd.) to minimize primer binding site differences and maximize among-species sequence differences for coral genera found in the Hawaiian Islands. Phylogenetic resolution was tested using coral sequences in GenBank and trees were constructed using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001) based on consensus sequences for short 16S (Figure S2) and COI (Figure S3) primers. Degenerate positions of the primer sites were limited to two base pairs in 16S and five base pairs for COI (Figure 2). Primers were then validated by amplifying eDNA from aquaria at the Department of Land and Natural Resources Ānuenue Fisheries Research Center (Table S2) with complex invertebrate and vertebrate communities, in which the diversity of corals was known. All coral taxa present in the aquaria were detected in samples from filtered water.

2.4 | PCR metabarcoding and sequencing

Amplifications were conducted in 13 μL volumes consisting of 6.3 μL MyTaq 2× (Bioline), 0.3 μL of forward and reverse primers 0.65 μL BSA (Thermo Fisher Scientific), 4.65 μL nuclease-free water (Growcells), and 0.5 μL template DNA. Thermal cycling parameters were the same for each combination pair of primers, with the only differences being the annealing temperatures. Conditions were: initial denaturation step of 5 minutes at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at the annealing temperature (50°C for 16S and 45°C for COI), and 45 s at 72°C, with a final extension of 10 min at 72°C. The quality of all amplifications were assessed

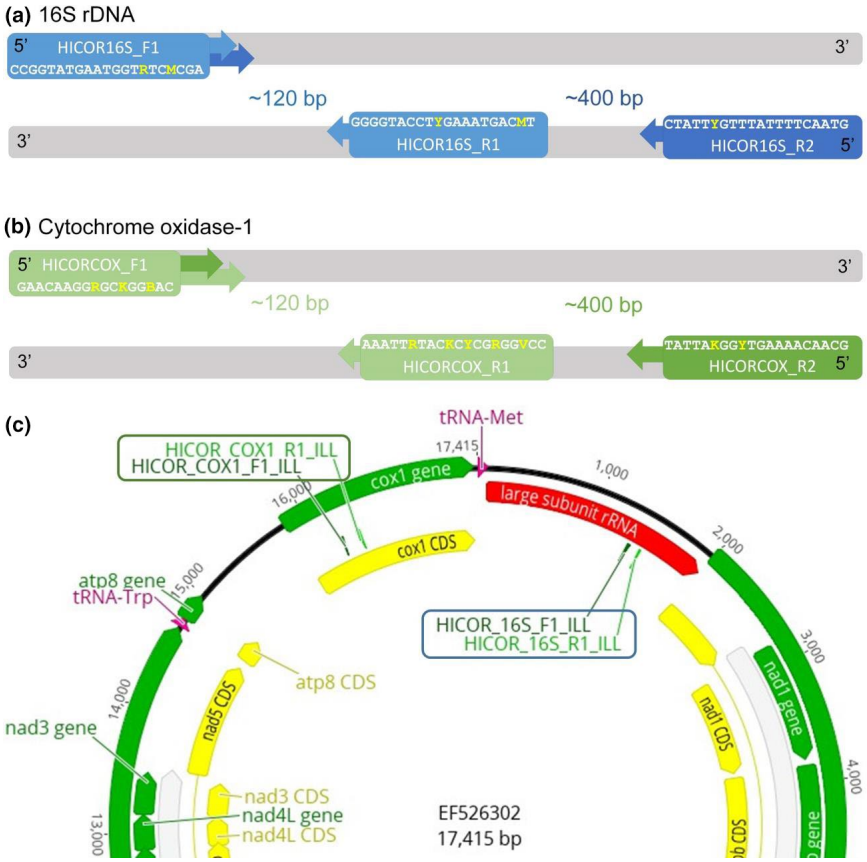

FIGURE 2 Example binding sites for four primer pair combinations, two targeting short (~120 bp) and one targeting long (~400 bp) intervening sequences from each of two mitochondrial DNA genes (16S ribosomal DNA, or 16S and cytochrome oxidase-1, or COI, (a and b) respectively). Forward primers were used in both short and long primer analyses in order to target the same segments of the mitochondrial genome. Primers sites were designed to target short (~120 bp) and highly conserved regions of the 16S rDNA (HICOR16S) and cytochrome oxidase-1 (HICORCOX1) mitochondrial genome, with intervening sequences containing enough variation to distinguish among genera. Degenerate positions of the primer sites (text marked in yellow) were limited to two base pairs in HICOR16S and five base pairs for HICORCOX1. (c) Annotated regions of the coral mitochondrial genome (*Pocillopora damicornis*, accession #EF526302, image created in Geneious v11, Biomatters) can be seen below, with forward and reverse primers marked for both 16S and COI

using gel electrophoresis, running PCR products through a 1.5% agarose gel stained with GelRed (Biotium), and visualized on an ultraviolet imaging platform. Amplification products (positives and negative controls, see Deiner et al., 2017) were prepared for sequencing in a post-PCR work area physically separated from pre-PCR areas (using dedicated post-PCR pipettors, plastics, and reagents) to prevent contamination. Extractions from both filter halves were used as technical replicates from each of the four biological (water sample) replicates within each site. Libraries were prepared for sequencing with Nextera XT v2 (Illumina) index adapters (following the manufacturer's protocol) followed by purification with a 1:1.12 ratio (DNA:beads) of Agencourt© Ampure XP beads (Beckman Coulter). Amplicons were then assessed for DNA concentration in a Qubit fluorometer (Thermo Fisher Scientific) and were pooled to equimolar concentrations. Pooled amplicons (including negative controls) were then (pair-end) sequenced on an Illumina MiSeq platform using the V3 600-cycle reagent kit in a MiSeq flow cell. Short and long amplicon sequences were processed on two separate sequencing runs at the Advanced Studies in Genomics and Proteomics facility at the University of Hawai'i at Mānoa.

2.5 | Bioinformatics

In total, 3.2 million reads were generated and sequence analysis of demultiplexed reads was conducted by pairing, trimming primers and adapters, quality filtering (Q = 20), and merging of overlapping reads with Geneious v11.1.5 (Biomatters Ltd.). First, paired and merged reads (contigs) from the entire project (~2.2 million after quality filtering) were pooled so that singletons and chimeras could be identified and removed prior to clustering (with –unoise3) in USEARCH v11 (Edgar, 2010). Second, zero-radius operational taxonomic units (ZOTUs) were discovered from a pool of all libraries, followed by construction of ZOTU tables to identify lineages within individual libraries. Typically, similar sequences are grouped based on a 97%–99% similarity threshold (Edgar, 2018; Stackebrandt & Goebel, 1994; Yarza et al., 2014), however the denoising pipeline in USEARCH groups sequences into ZOTUs using a superior algorithm

when compared to traditional grouping based on 97% sequence similarities (Edgar, 2016a,b). For each of the two gene regions, correct biological sequences (where certain species may be split into multiple ZOTUs) were recovered using the denoising pipeline in USEARCH (Appendix S1). ZOTUs were then compared with Blastn to the NCBI nucleotide database with a conservative e-value cutoff (e^{-30}) so that ZOTU tables could be constructed only for coral from individual libraries. ZOTU tables were generated by assembling and tabulating reads from individual amplicon libraries with USEARCH. Reads from the entire project were then mapped to the Midori reference database for metazoan mitochondrial DNA (Machida, Leray, Ho, & Knowlton, 2017) to predict taxonomy for COI and 16S using the SINTAX classifier algorithm (Edgar, 2016a) in USEARCH (-sintax) with an 80% bootstrap cutoff.

2.6 | Statistical analyses

Due to differences in total number of coral DNA reads obtained among biological replicates at each site, direct comparisons among sites could only be made after standardizing the total reads among biological replicates within each site. For each of the four water samples from within each site (biological replicates), reads from the two technical replicates were pooled (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018; Leray & Knowlton, 2015) and then standardized so that the total reads from each biological replicate equaled the mean of the four biological replicates per site. A generalized linear model was then performed on log-transformed standardized eDNA reads for both gene loci across sites in R v3.3.3 (R Development Core Team, 2011). Linear regressions in R were used to determine the log-linear relationship between coral reads as a predictor for coral abundance based on percent cover for each of the taxa present in both survey methods, assuming independent observations of coral cover, as space is likely not a limiting factor on Hawaiian coral reefs.

2.7 | Contamination prevention

Filtration was performed in a lab physically isolated from all other molecular work using equipment sterilized in a 10% bleach solution for at least 24 hr. All lab surfaces were sterilized using a 10% bleach solution before and after processing. Negative controls at each step were used to monitor contamination. First, collection blanks that consisted of sampling containers filled with tap water (Turner et al., 2015, also see Figure S4) were taken into and returned from the field. Second, 1 L of tap water was filtered through sterilized filtration equipment as an equipment blank before sample filtration. Both types of tap water blanks were analyzed as negative controls in the same manner as the field-collected water samples. PCR negatives were sequenced alongside other blanks as a necessary precaution to monitor cross contamination of samples and carry-over from equipment contamination. The number of sequence reads that were present in negative controls and matched field ZOTUs were subtracted from the sequence abundances in the corresponding samples (Nguyen, Smith, Peay, & Kennedy, 2015; Port et al., 2016).

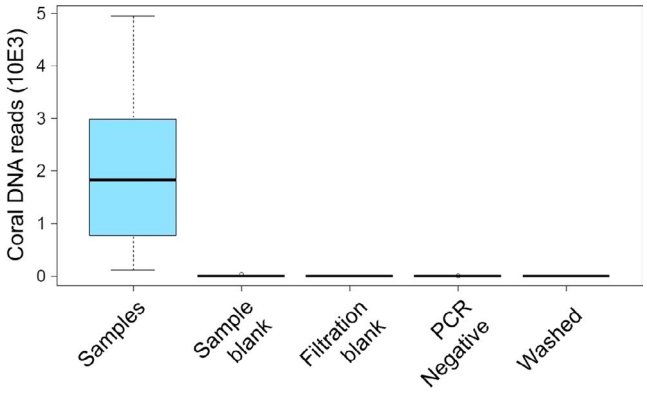

FIGURE 3 Average coral sequence reads from amplifications of eDNA per collection container for field samples (brand new containers used once for sampling, $n = 32$), where contamination was monitored through the use of collection sample blanks (containers filled with tap water brought into the field, $n = 8$), filtration blanks (tap water run through filtration equipment, $n = 10$), PCR negatives ($n = 8$), and washed containers (previously used to collect field samples then washed and used as negative controls, $n = 8$). Multiple negatives are required to help monitor the eDNA workflow for contamination at each step, in order to help pinpoint the source of contamination. The total number of reads for each ZOTU appearing in filtration blanks, collection blanks, and PCR negatives were subtracted from the total number of matching ZOTUs in environmental samples

The protocol for cleaning equipment and containers for reuse was adapted from Thomsen et al. (2016) with the following adjustments: after the collection containers were emptied, each was sterilized with a 10% commercial bleach solution (containing <3% sodium hypochlorite) for at least 24 hr, followed by a nylon bristle brush scrub, and then a second bleach bath for at least 24 hr. Containers were then air dried in the clean room, free of molecular work. Containers used to collect field samples were rotated after cleaning to be used as collection blanks (filled with tap water) and our analyses of negative controls indicate that cleaning was effective at removing amplifiable DNA from the containers before reuse (Figure 3).

3 | RESULTS

3.1 | Visual surveys

Data from visual surveys yielded percent cover and relative cover of coral taxa that are consistent with previous studies of the same sites (Coles & Brown, 2007; Minton, Amimoto, Friedlander, Stamoulis, & Conklin, 2015; NOAA Coral Reef Watch, 2016; Rodgers, Jokiel, Brown, Hau, & Sparks, 2015; Wolanski, Martinez, & Richmond, 2009). Coral cover from photo-quadrats estimated total coral cover to be 59.2% at Moku o Lo'e reef in Kāne'ohe Bay, 34.5% at 'A'alapapa Reef in Lanikai, 15.9% at Kaiona Reef in Waimānalo, and 0.3% at Wailupe Reef in Maunalua Bay. There were significant differences in total coral cover (GLM: $\chi^2 = 39.61$, $p < 0.001$) as well as significant differences in relative abundance of visually surveyed genera

(GLM: $\chi^2 = 27.95$, $p < 0.001$) among field sites. At three of the four sites, *Porites* had the highest relative abundance, but at Waimānalo, *Montipora* was the most abundant coral genus. Two taxa were only recorded in visual surveys at two of the four field sites: *Pavona* at Waimānalo and *Leptastrea* at Kāne'ohe (Figure 4a).

3.2 | eDNA surveys

The total number of eDNA reads (mean ± *SE*) obtained at each site differed, with 74,487 ± 12,498 total reads from Kāne'ohe, 77,858 ± 10,107 total reads from Lanikai, 37,638 ± 9,833 total reads from Maunalua, and 47,595 ± 5,285 total reads from Waimānalo (Table S3). After reads were paired, merged, and quality filtered, 2,202,470 total contigs were analyzed. Of the total generated contigs, 38% were successfully taxonomically assigned using the Midori reference database for marine metazoans. The other 62% of sequences were unable to be assigned with an 80% bootstrap cutoff. Overall, the majority of assigned metazoan contigs were anthozoans (78%), but 16S amplified a greater percentage of anthozoans than COI (61% and 51%, respectively, Figure 5). The remaining metazoans were predominantly assigned to sponges (33.4% 16S and 47.2% COI), followed by arthropods (4.3% 16S and 0.2% COI), and hydrozoans (1.2% 16S and 0.8% COI). Actinopterygii and scyphozoans made up less than 0.1% of assigned metazoan sequences.

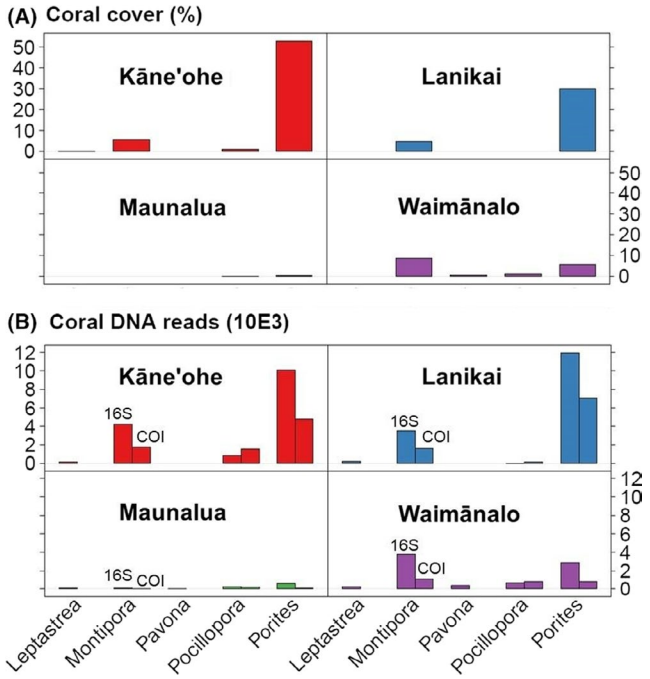

FIGURE 4 (a) Percent coral cover based on visual surveys conducted at each of the field sites (inset) along the eastern and southern coasts of O'ahu, HI. Visual surveys followed those from Walsh et al. (2010); see Methods. Photo-quadrats were allocated blindly and analyzed using 100 random points per quadrat in CPCe. (b) Total coral reads from eDNA samples collected at each of the field sites, grouped by short (~120 bp) target sequence mitochondrial gene loci (16S and COI, left and right bars, respectively). Samples were collected using 2 L sterile containers, filtered, amplified, and sequenced to compare DNA reads to coral abundances. Long sequences (omitted for clarity) targeting 16S were unable to detect *Pocillopora* at any site, whereas long sequences targeting COI were able to detect a cryptic genus, *Cyphastrea*, when short-target primers did not. Both long-target 16S and short-target COI primers were not able to detect less abundant taxa such as *Leptastrea* from Kāne'ohe and *Pavona* from Waimānalo. In all cases, subsequent roving diver surveys between transects, yet outside the photo-quadrats, confirmed the presence of less abundant taxa within 150 m from the site centroid

For primers targeting short (~120 bp) sequences, the number of DNA sequence reads (pooled COI and 16S) present in PCR amplicons explained 91% of the variation in percent coral cover among sites ($R^2 = 0.91$, Figure 6a), indicating a strong predictive relationship between eDNA sequence reads and visual estimates of coral cover. Reads from only the 16S primers were able to explain slightly more of the variation in percent coral cover ($R^2 = 0.95$, Figure 6b) than reads pooled from both genes ($R^2 = 0.91$), and substantially more variation than COI reads alone ($R^2 = 0.71$, Figure 6c). Similarly, coral cover was predicted using primers targeting longer (~400 bp) sequences, but longer sequences explained slightly less (2%) of the variation in coral cover from visual surveys than did shorter sequences of 16S and substantially less variation (33%) than shorter sequences of COI (Figure 7).

As with the visual survey data, the relative abundances of major coral genera inferred from eDNA reads were significantly different across the four sites (Figure 4b) for both short (GLM: $\chi^2 = 35.37$, $p < 0.001$) and long pooled 16S and COI sequences (GLM: $\chi^2 = 35.18$, $p = 0.002$, Table 1). 16S sequences alone detected significant among-site differences in the relative abundance of coral genera, based on eDNA reads for both short (GLM: $\chi^2 = 26.52$, $p = 0.003$) and long (GLM: $\chi^2 = 19.81$, $p = 0.003$) sequences; short COI sequences also detected significant differences in community structure (GLM: $\chi^2 = 15.26$, $p = 0.018$), but long COI sequences did not (GLM: $\chi^2 = 18.46$, $p = 0.102$). A second collection of eDNA from four filtered seawater samples at Waimānalo (one month later) also showed no difference in relative abundance of coral genera from pooled short eDNA reads compared to the original samples (GLM: $\chi^2 = 2.58$, $p = 0.63$, Figure S5).

Pooled data for both eDNA approaches were able to detect more coral genera than visual surveys alone ($n = 4$), with only short COI sequences failing to detect as many coral genera ($n = 3$, Table 2) as visual surveys. Long target sequence primers detected more coral genera ($n = 6$) than short target sequence primers ($n = 5$, Table 2).

4 | DISCUSSION

A metabarcoding approach can be used to infer coral cover from eDNA using filtered seawater. Our data and analyses demonstrate that the number of coral eDNA reads was highly correlated with estimates of percent cover from visual surveys, which allows for rapid documentation of relative abundances in the field. Given this is the first study to quantify coral cover from eDNA, our results suggest that rapid surveys of corals using relatively small volumes of filtered

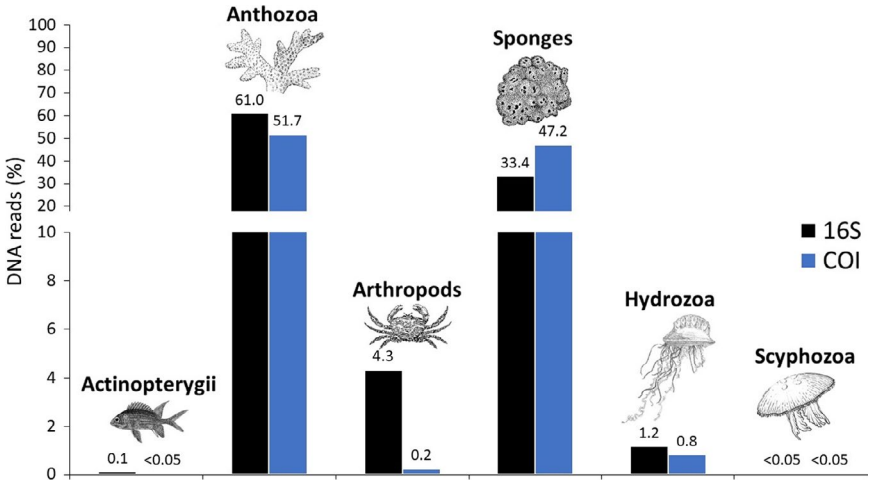

FIGURE 5 Percent total eDNA reads of marine metazoans, collected using primers designed specifically to equally amplify yet distinguish common genera of scleractinian corals from field sites around O'ahu, HI. Primers sites were designed to target short (~120 bp) and long (~400 bp) regions of the highly conserved 16S rDNA (HICOR16S) and cytochrome oxidase-1 (HICORCOX1) mitochondrial genome, with intervening sequences containing enough variation to distinguish among genera. PCR amplification and next-generation sequencing of amplicons resulted in a high percentage of sequence reads from cnidarians, with other taxa being represented to a lesser extent. Taxonomy predictions are based on an 80% bootstrap cutoff using the SINTAX algorithm (Edgar, 2016a) in USEARCH v11 (Edgar, 2010) and sequences that were unable to be assigned have been removed

seawater is possible elsewhere, and can be integrated into existing monitoring efforts, especially in areas where traditional visual-based surveys are logistically challenging.

Primers designed to amplify all genera found in the Hawaiian Islands were also highly effective in detecting differences in community structure (i.e., in terms of the relative abundances of major coral genera) from samples collected at four sites on O'ahu (Figure 4). eDNA surveys were successful in capturing the overall dominance of *Porites* in the Hawaiian archipelago (Coles & Brown, 2007), the relatively high abundance of *Montipora* at Kaiona Reef in Waimānalo, as well as the low overall coral cover in the degraded, but recovering Maunalua Bay (Kittinger et al., 2016; Wolanski et al., 2009). Several less abundant taxa, such as *Leptastrea*, that we know from other observations to be present at some of these well-studied sites (Waimānalo and Lanikai), were almost always detected in eDNA (but not in negative controls), even if not observed in the photo-quadrat data. Similarly, eDNA surveying also detected the scarcity of temperature-sensitive *Pocillopora* at all sites following back-to-back 2014–2015 bleaching events in Hawai'i (Couch et al., 2017), where *Pocillopora* experienced nearly 100% bleaching and mortality (NOAA Coral Reef Watch, 2016); *Pocillopora* was detected by eDNA, but was not captured in transects at 'A'alapapa Reef (offshore from Lanikai Beach). There are several possible reasons for this result, including eDNA transport from corals outside the transect area or eDNA from corals inside the transect area that were not observed, either because they were too small or were positioned between transects and not captured by the photo-quadrats. Subsequent roving diver surveys between transects (but outside the photo-quadrats) confirmed the presence of these less abundant taxa within 150 m from the site centroid. Therefore, our eDNA surveys also provided more accurate estimates of coral richness than visual surveys, as cryptic and rare taxa are often missed using traditional visual-based surveys (Mumby, Green, Edwards, & Clark, 1997; Pearman, Anlauf, Irigoien, & Carvalho, 2016). Visual survey effort in our study was modeled after state agency protocols (Walsh et al., 2010), whereas, presumably, more intensive visual sampling with increased time, effort, and cost would likely yield the missing taxa. Unlike visual surveys which rely on sampling only a subset of the study area, eDNA, in theory, samples more comprehensively from the entire community in a given area with minimal sampling effort. That said, the exact or even approximate volume of water effectively surveyed by eDNA collection remains poorly quantified and varies depending on water movement (Deiner & Altermatt, 2014; Goldberg, Strickler, & Pilliod, 2015; Port et al., 2016; Thomsen, Kielgast, Iversen, Moller, et al., 2012).

The reproducibility of eDNA surveys over time and under fluctuating environmental conditions is not well-established for coastal marine environments. Although samples taken from a single site, 1 month later, produced similar estimates of relative abundance of coral genera, repeated samples over longer periods of time are necessary to characterize temporal variation in eDNA signals on coral reefs. Very little information regarding temporal variation in biological factors exists (such as rates of growth, excretion, and reproduction) and how those factors influence the rate at which DNA is shed by coral reef taxa and maintained in the water column over longer time scales (Bista et al., 2017; Buxton, Groombridge, & Griffiths, 2018). In Hawai'i, the dominance of mounding and massive species of corals and relatively low complexity of colony surface area may provide ideal community structuring that would otherwise lead to overrepresentation in eDNA of branching taxa with greater surface-area-to-volume ratios. Surface area as a proxy for biomass can be tracked with eDNA metabarcoding to measure changes in coral

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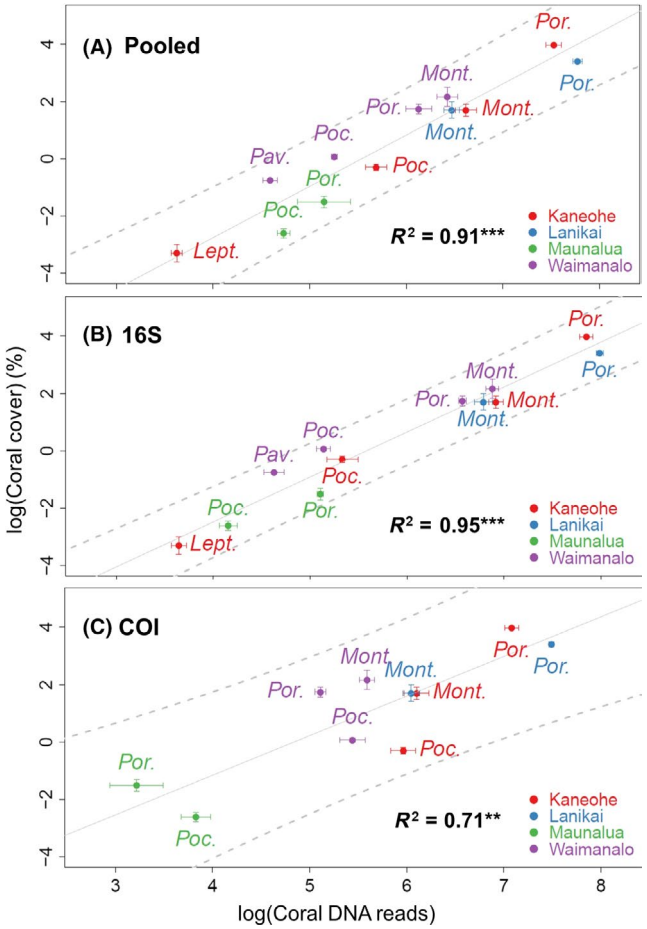

FIGURE 6 Scatterplots of standardized coral DNA reads for short (~120 bp) target sequences from filtered seawater versus percent cover for coral genera surveyed at four field sites from O'ahu, HI. Visual surveys followed those from Walsh et al. (2010); see Methods. Environmental DNA samples were collected using four replicates of 2 L sterile containers, filtered, amplified, and sequenced to compare DNA reads to coral abundances. Field sites are designated by different colors and coral taxa are labeled: *Porites* (*Por.*), *Montipora* (*Mont.*), *Pocillopora* (*Poc.*), *Pavona* (*Pav.*), and *Leptastrea* (*Lept.*). A linear regression and 95% prediction intervals are plotted, showing a significant log-linear relationship for (a) both loci pooled (Regression; $R^2 = 0.91$, $F_{(1,10)} = 107.3$, $p < 0.001$), (b) 16S (Regression; $R^2 = 0.95$, $F_{(1,10)} = 224.5$, $p < 0.001$), and (c) COI (Regression; $R^2 = 0.71$, $F_{(1,10)} = 23.5$, $p = 0.001$). Error bars represent the standard error of the mean. Signif. codes: $p < 0.001$ = ***; <0.01 = **; <0.05 = *.

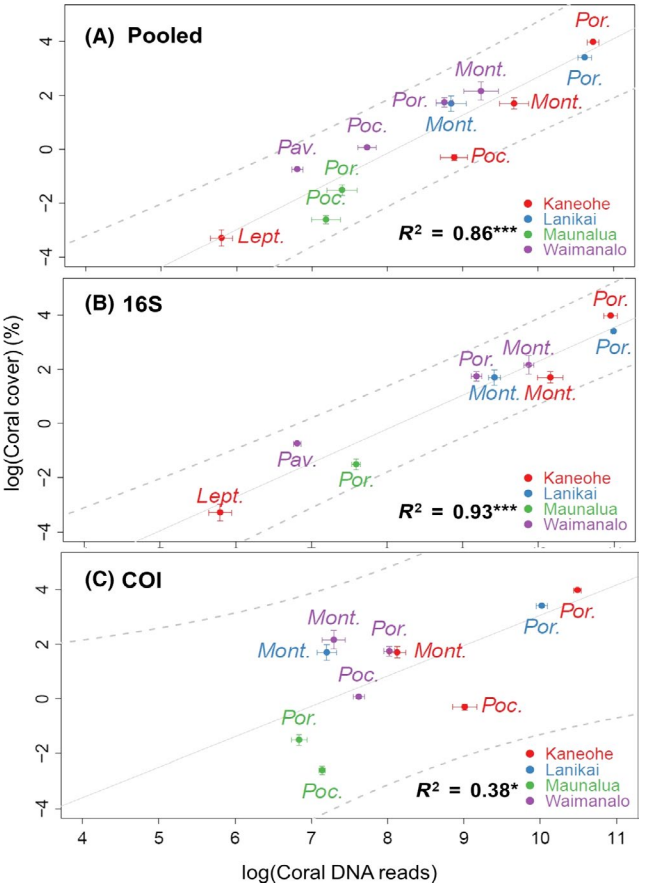

FIGURE 7 Scatterplots of standardized coral DNA reads for long (~400 bp) target sequences from filtered seawater versus percent cover for coral genera surveyed at four field from O'ahu, HI. Visual surveys followed those from Walsh et al. (2010); see Methods. Environmental DNA samples were collected using four replicates of 2 L sterile containers, filtered, amplified, and sequenced to compare DNA reads to coral abundances. Field sites are designated by different colors and coral taxa are labeled: *Porites* (*Por.*), *Montipora* (*Mont.*), *Pocillopora* (*Poc.*), *Pavona* (*Pav.*), and *Leptastrea* (*Lept.*). A linear regression and 95% prediction intervals are plotted, showing a significant log-linear relationship for (a) both loci pooled (Regression; $R^2 = 0.86$, $F_{(1,10)} = 70.1$, $p < 0.001$), (b) 16S (Regression; $R^2 = 0.93$, $F_{(1,7)} = 108.4$, $p < 0.001$), and (c) COI (Regression; $R^2 = 0.38$, $F_{(1,8)} = 6.6$, $p = 0.03$). Error bars represent the standard error of the mean. Signif. codes: $p < 0.001$ = ***; <0.01 = **; <0.05 = *.

cover over time, as well as to potentially increase detection rates of scarce taxa.

The rapid degradation and turnover of eDNA in the water column (Dell'Anno & Corinaldesi, 2004) may be advantageous for capturing a snapshot of biodiversity and abundance of corals on shallow reefs. Due to the large quantity of small, degraded fragments associated with eDNA, we expected short target primers to outperform long target primers in all respects (Taberlet et al., 2018). We used four primer pair combinations, two targeting short (~120 bp) and one targeting long (~400 bp) sequences (in which the forward primer was the same for both short and long amplicons) from each of two mitochondrial DNA genes (COI and 16S), designed to evenly amplify and distinguish major coral genera in Hawai'i. Although each primer combination provided a significant positive correlation between the abundance of sequence reads and coral cover from visual surveys, target sequence length had a clear impact on estimates of coral cover. For both genes, short sequences explained more variation in estimates of coral cover when compared to long sequence counterparts. Short 16S sequence primers yielded the most accurate estimate of coral cover based on visual surveys. In contrast, long COI sequences, yielding PCR amplicons similar in size to those typically generated for barcoding

TABLE 1 Summary of generalized linear mixed model of total coral reads from eDNA samples collected at each of the field sites are based on the interaction of predictors for site and genera (Site*Genus)

	Pooled		16S		COI	
	short	long	short	long	short	long
LR Chisq	35.37	35.18	26.52	19.81	15.26	18.46
df	10	15	10	6	6	12
p-value	0.0001***	0.002**	0.003**	0.003**	0.018*	0.102

Note. Likelihood ratio tests for primer combinations targeting short (~120 bp) and long (~400 bp) amplicons from each of two mitochondrial DNA genes (16S ribosomal DNA, or 16S, and cytochrome oxidase-1, or COI).
Signif. codes: $p < 0.001$ = ***; <0.01 = **; <0.05 = *.

TABLE 2 Summary of HICOR primer combinations targeting short (~120 bp) and long (~400 bp) amplicons from each of two mitochondrial DNA genes (16S ribosomal DNA, or 16S, and cytochrome oxidase-1, or COI), showing the total number of coral genera recovered from PCR amplifications, coefficient of determination (r-squared) values based on comparisons to visual survey data, and mean 95% prediction interval widths (upper PI–lower PI) based on linear regressions (see Figures 6,7)

	Pooled		16S		COI	
	short	long	short	long	short	long
Coral genera detected	5	6	5	4	3	6
Cover/reads regression	0.91***	0.86***	0.95***	0.93***	0.71**	0.38*
Mean 95% PI width	5.30	4.50	5.01	5.06	8.69	14.72

Note. Prediction intervals were calculated using a 95% chance that future observations will fall within a similar distribution based on standard error and sample variability using the predict function in base R v3.3.3 (R Development Core Team, 2011).
Signif. codes: $p < 0.001$ = ***; <0.01 = **; <0.05 = *.

individual animal tissue samples, provided the least accurate estimates of percent cover.

Short metabarcode sequences may provide better estimates of relative abundance for corals over a relatively large area (such as our transect areas) because primers for short sequences will bind to both highly abundant degraded DNA and much less abundant undegraded DNA, yielding estimates of biomass averaged over a larger area (due to mixing) and longer period of time (due to the longer persistence time of degraded DNA). In contrast, primers targeting longer sequences probably only amplify eDNA recently shed from taxa in the immediate vicinity of where the water sample was taken, and therefore may only capture the relative abundances of taxa on smaller spatial scales (Jo et al., 2017).

Although our expectations for target amplicon size were met with respect to the assessment of coral cover, long target sequences performed marginally better at detecting genera, finding more coral genera on Hawaiian coral reefs than short target sequences (6 vs. 5 taxa, respectively). However, that overall result was largely driven by long COI sequences providing the best estimates of species richness (6 vs. 3 taxa, respectively). One possible explanation is that shorter sequences are simply less likely to distinguish some taxa (Coissac, Riaz, & Puillandre, 2012). For example, in highly conserved regions of the coral mitochondrial genome, short fragments may be limited in their taxonomic resolution and estimates of species richness. By using shorter sequences, ZOTUs might be lost and sequence clustering may group taxa not of the same genus. However, analysis of COI short fragments extracted from within their containing long fragments, yield the same number of ZOTUs (none are lost) as long fragments, indicating that short and long COI fragments distinguish coral genera similarly.

A second reason why our short sequences may be less effective at detecting taxa is that the reverse COI primer for the short fragment is a poorer match to some taxa than the reverse primer for the longer fragment. However short, reverse COI primers had only one additional mismatch to some of the scarce taxa (Table S4, Supporting information) that was 18 nucleotides from the 3' end of the primer in two of the taxa's binding sites. Although the impact of a single primer-template mismatch is difficult to assess (Bru, Martin-Laurent, & Philippot, 2008; Rejali, Moric, & Wittwer, 2018; Wu, Hong, & Liu, 2009), particularly given that we have no reference sequences for the rare Hawaiian taxa (*Cyphastrea* and *Leptastrea*) with which to compare to the long-reverse COI primer sequence, reverse primer-template mismatch seems an unlikely explanation for the difference in diversity recovered by short and long sequences. A third explanation is that our long-target reverse COI primer had lower guanine-cytosine (GC) contents than the short-target COI reverse primer (Table S1), although within the recommended 30%–70% GC content range (see Benita, 2003), but which nevertheless may confound fragment abundances (Benjamini & Speed, 2012) due to their lower binding specificity. Lastly, COI primers targeting a smaller fragment may have recovered fewer taxa because amplicons for rare taxa may be less likely to reach the exponential phase of amplification from an enormous pool of degraded template DNA (Golenberg, Bickel, & Weihs, 1996). Although rare taxa may have gone undetected, any bias toward common taxa does not appear to impact the relationship

between eDNA reads and coral cover as rare taxa contribute much less to coral cover in Hawai'i.

Regardless of the reason why, our study suggests that target sequence length must be carefully considered when designing eDNA metabarcoding assays of community abundance, yet sequence length has received little attention in eDNA study design (Goldberg et al., 2016; Taberlet et al., 2018; Wilcox et al., 2018). eDNA metabarcoding approaches have primarily focused on amplifying short (~100 bp) sequences (Hänfling et al., 2016; Harper et al., 2018; Pont et al., 2018; Port et al., 2016; Stoeckle et al., 2017; Thomsen et al., 2016; Valentini et al., 2016) to increase detection rates in systems with (presumably) highly degraded eDNA (Taberlet et al., 2018). While estimates of coral cover are more precise with a particular pair of short sequence primers (16S in our case), assays designed to measure diversity may require different tools than those designed for abundance estimates. Unlike eDNA surveys focused on abundance, using as many genes as possible (e.g., Stat et al., 2017) is likely the best way to increase the detection rate of all taxa (Table 2). Arbitrary selection of gene and sequence length may limit estimates of species richness for short sequences, or reduce the efficacy of obtaining strong predictive relationships between eDNA reads and abundance of taxa for long sequences. For example, had we simply designed short target primers using the standard barcoding gene, COI, we would have detected half as many coral taxa. Sequence length should remain an important consideration when designing assays for detection of environmental DNA (Freeland, 2017), and the role of target sequence length in eDNA transport and degradation dynamics warrants further investigation.

Finally, as a holistic approach to surveying marine biodiversity, previous studies have established that metabarcoding of eDNA from filtered seawater remains unmatched in terms of its cost-effectiveness and sensitivity (Bakker et al., 2017; Boussarie et al., 2018; Port et al., 2016; Stat et al., 2017; Valentini et al., 2016; Yamamoto et al., 2017). However, the results from this study indicate that metabarcoding can also be used under some circumstances as a rapid alternative to qPCR in determining abundance of multiple taxa (Hänfling et al., 2016; Harper et al., 2018; Piñol et al., 2019; Pont et al., 2018; Stoeckle et al., 2017; Thomsen et al., 2016; Ushio et al., 2017). Additionally, this technique may be applicable not only to tracking changes in coral cover, but also to track changes in community composition, as well as to detect cryptic taxa, or new recruits, on coral reefs that may otherwise be missed by traditional visual-based survey methods. Careful selection and/or design of primers for eDNA surveying can provide information for the biomonitoring and management of impacted reef systems, which are facing declines in coral cover on a global scale (Pandolfi, Connolly, Marshall, & Cohen, 2011). The cost of monitoring can be substantially reduced using eDNA when compared to traditional visual survey techniques among accessible nearshore habitats (Brown et al., 2004), but especially in areas where traditional survey techniques are logistically challenging. Environmental DNA may aid in successful biomonitoring and protection of vulnerable marine communities, addressing funding barriers and impacting conservation policies of tropical coral reefs threatened by mass bleaching and other anthropogenic disturbances (Anthony et al., 2017; Baker et al., 2008; Bellwood, Hughes, Folke, & Nyström, 2004; Carpenter et al., 2008; Edmunds et al., 2014; Hughes et al., 2017).

DATA AND MATERIALS AVAILABILITY

All visual survey data, R code, and materials used in the analysis will be made available in some form to any researcher for purposes of reproducing or extending the analysis. Sequence data that support the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA accession PRJNA523257). Shell code for USEARCH v11 can be found in Appendix S1.

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CONFLICT OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

P.K.N. conceived of ideas, conducted all field sampling, laboratory work, and analyses. P.B.M. designed the metabarcoding workflow, provided analytical materials, helped develop the laboratory methods, and supervised all research. Both authors contributed to writing and gave final approval for publication.

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SUPPORTING INFORMATION

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