Spatial distribution of environmental DNA in a nearshore marine habitat

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Abstract

In the face of increasing threats to biodiversity, the advancement of methods for surveying biological communities is a major priority for ecologists. Recent advances in molecular biological technologies have made it possible to detect and sequence DNA from environmental samples (environmental DNA or eDNA); however, eDNA techniques have not yet seen widespread adoption as a routine method for biological surveillance primarily due to gaps in our understanding of the dynamics of eDNA in space and time. In order to identify the effective spatial scale of this approach in a dynamic marine environment, we collected marine surface water samples from transects ranging from the intertidal zone to 4 kilometers from shore. Using ~~massively parallel sequencing of~~ PCR primers that target a diverse assemblage of metazoans, we amplified a region of mitochondrial 16S ~~amplicons, we identified a diverse community of metazoans and quantified~~ rDNA from the samples and sequenced the products on an Illumina platform in order to detect communities and quantify their spatial patterns using a variety of statistical tools. We find evidence for multiple, discrete eDNA communities in this habitat, and show that these communities decrease in similarity as they become further apart. Offshore communities tend to be richer but less even than those inshore, though diversity was not spatially autocorrelated. Taxon-specific relative abundance coincided with our expectations of spatial distribution in taxa lacking a microscopic, pelagic life-history stage, though most of the taxa detected do not meet these criteria. Finally, we use carefully replicated laboratory procedures to show that laboratory treatments were remarkably similar in most cases, while allowing us to detect a faulty replicate, emphasizing the importance of replication to metabarcoding studies. While there is much work to be done before eDNA techniques can be confidently deployed as a standard method for ecological monitoring, this study serves as a first analysis of diversity at the fine spatial scales relevant to marine ecologists and confirms the promise of eDNA in dynamic environments.

Introduction

The patterns and causes of variability in ecological communities across space are both seminal and contentious areas of study in ecology (Hubbell, 2001; Anderson et al., 2011). One consistently observed pattern of community spatial heterogeneity is that communities close to one another tend

2

29 to be more similar than those that are farther apart (Nekola and White, 1999). This decrease
30 in community similarity with increasing spatial separation is called distance decay and has been
31 reported from communities of tropical trees (Condit, 2002; Chust et al., 2006), ectomycorrhizal fungi
32 (Bahram et al., 2013), salt marsh plants (Guo et al., 2015), and microorganisms (Martiny et al.,
33 2011; Chust et al., 2013; Wetzel et al., 2012; Bell, 2010). Typically, this relationship is assessed by
34 regressing a measure of community similarity against a measure of spatial separation for a set of
35 sites at which a set of species' abundances (or presences) is calculated. Yet no existing biodiversity
36 survey method completely censuses all of the organisms in a given area. The lack of a single 'silver
37 bullet' method of sampling contributes inconclusiveness to the study of spatial patterning in ecology
38 (Levin, 1992), and leaves open the possibility of new and more comprehensive methods.

39 From a boat or aircraft, scientists can count whales by sight, but not the krill on which they
40 feed. For example, towed fishing nets can efficiently sample organisms larger than the mesh and
41 slower than the boat, but overlook viruses and have undesirable effects on charismatic air-breathing
42 species. However, DNA-based surveys show great promise as an efficient technique for detecting a
43 previously unthinkable breadth of organisms from a single sample.

44 Microbiologists have used nucleic acid sequencing to quantify the composition and function of
45 microbial communities in a wide variety of habitats (Handelsman et al., 1998; Tyson et al., 2004;
46 Venter et al., 2004; Iverson et al., 2012). To do so, microorganisms are collected in a sample of
47 environmental medium (e.g. water), their DNA or RNA is isolated and sequenced, and the identity
48 and abundance of sequences is considered to reflect the community of organisms contained in the
49 sample, which indirectly estimates the quantity of organisms in an area.

50 Macroorganisms shed DNA-containing cells into the environment (environmental DNA or eDNA)
51 that can be sampled in the same way (Ficetola et al., 2008; Thomsen et al., 2012). Potentially, eDNA
52 methods allow a broad swath of macroorganisms to be surveyed from basic environmental samples.
53 However, the accuracy and reliability of indirect estimates of macroorganismal abundance has been
54 debated because the entire organisms are not contained within the sample (Cowart et al., 2015).
55 Concern surrounding eDNA methods is rooted in uncertainty about the attributes of eDNA in the
56 environment relative to actual organisms (Shelton et al., 2016; Evans et al., 2016). Basic questions
57 such as how long DNA can persist in that environment and how far DNA can travel remain largely
58 unknown (but see Klymus et al. (2015); Turner et al. (2015); Strickler et al. (2015); Deiner and

Altermatt (2014)) and impede inference about local organismal presence from an environmental sample. As a result, estimating the spatial and temporal resolution of eDNA studies in the field is a key step in making these methods practical.

The relationship between local organismal abundance and eDNA is further complicated in habitats where the environmental medium itself may transport eDNA away from its source. We know that genetic material can move away from its source precisely because organisms can be detected indirectly without being present in the sample (Kelly et al., 2016b). One might reasonably expect eDNA to travel farther in a highly dynamic fluid such as the open ocean or flowing river than it would through the sediment at the bottom of a stagnant pond (Deiner and Altermatt, 2014; Shogren et al., 2016). Yet even studies of extremely dynamic habitats such as coastlines with high wave energy have found remarkable evidence that eDNA transport is limited enough that DNA methods can detect differences among communities separated by less than 100 meters (Port et al., 2016).

While rigorous laboratory studies have investigated the effects of some environmental factors on eDNA persistence (Klymus et al., 2015; Barnes et al., 2014; Sassoubre et al., 2016) and the transport of eDNA in specific contexts (Deiner and Altermatt, 2014), we suggest that field studies comparing the spatial distribution of communities of eDNA with expectations based on prior knowledge of organisms' distributions are also critical to developing a working understanding of eDNA in the real world. Research to date has documented the non-random spatial distribution of meiofaunal (Fonseca et al., 2014; Guardiola et al., 2016), microbial (Lallias et al., 2015), and extracellular (Guardiola et al., 2015) eDNA of marine and estuarine sediments, and of microscopic plankton in open ocean waters (de Vargas et al., 2015). These studies conducted targeted sampling at intermediate (thousands of meters) to global (thousands of kilometers) scales. Here, we use a grid-based environmental sampling strategy to assess spatial variability of eDNA in a coastal marine environment at a fine scale (tens to thousands of meters), using molecular methods that focus on macrobial metazoans.

We apply methods derived from community ecology to understand spatial patterns and patchiness of eDNA. The underlying mechanism thought to drive the slope of the distance decay relationship in ecological communities is the rate of movement of individuals among sites, which may be driven by underlying processes such as habitat suitability. Because eDNA is shed and transported away from its source, the increased movement of eDNA particles should homogenize community similarity, and thus erode the distance decay relationship of eDNA communities.

Puget Sound is a deep, narrow fjord in Washington, USA, where a narrow band of shallow bottom hugs the shoreline and abruptly gives way to a central depth of up to 300 meters. This form allows the juxtaposition of communities associated with distinctly different habitats: shallow, intertidal benthos, and euphotic pelagic (Burns, 1985). At the upper reaches of the intertidal, the shoreline substrate varies from soft, fine sediment to cobble and boulder rubble. Soft intertidal sediments are inhabited by burrowing bivalves (Bivalvia), segmented worms (Annelida), and acorn worms (Enteropneusta), and in some lower intertidal and high subtidal ranges by eelgrass (*Zostera marina*) (Kozloff, 1973; Dethier, 2010) . Eelgrass meadows harbor epifaunal and infaunal biota, and attract transient species which use the meadows for shelter and to feed on resident organisms. Hard intertidal surfaces support a well-documented biota including barnacles (Sessilia) and other crustaceans, mussels (Bivalvia:Mytilidae), anemones (~~Actinaria~~Actiniaria), sea stars (Asteroidea), urchins (Echinoidea), ~~Bryzoans~~ Bryozoans (Ectoprocta), crustaceans (Decapoda), and a variety of algae (Dethier, 2010). Hard bottoms of the lower intertidal and high subtidal are home to macroalgae such as Laminariales and Desmarestiales which ~~provides~~ provide habitat for a distinct community of fish and invertebrates. The upper pelagic is home to a diverse assemblage of microscopic plankton including diatoms, copepods, and larvae (Strickland, 1983), as well as transitory fish and marine mammals.

We took advantage of this setting to explore the spatial variation and distribution of marine eDNA communities. Using PCR-based methods and massively parallel sequencing, we surveyed mitochondrial 16S sequences from a suite of marine animals in water samples collected over a grid of sites extending from the shoreline out to 4 kilometers offshore in Puget Sound, Washington, USA. We leverage this sampling design to perform ~~the first~~ an explicitly spatial analysis of eDNA-derived community similarity. We investigate two primary objectives. First we examine the spatial patterning of eDNA and determine the degree to which eDNA community similarity can be predicted by physical proximity. We expect that physical proximity will be a strong predictor of community similarity, and that community differences can be detected over small distances. Second, we examine the distribution of diversity from eDNA data, and compare it to our expectations based on distributions of macrobial communities. We expect that distinct eDNA communities exist in this setting, and that their spatial distribution coincides with that of adult macrobial organisms. Because of the vastly different communities of benthic macrobial metazoans as a function of distance from shore,

we expect that more than one eDNA community is present across our 4 kilometer sampling grid, and that communities change as a function of distance from shore. For this reason, we examine two diversity measures of eDNA communities that have been widely used to reveal broad scale patterns based on macrobiota in many ecological systems. Finally, we identify the taxa represented in the eDNA communities, which span a range of life-history characteristics, and we expect that the spatial distribution of eDNA will most closely resemble the distribution of adults in taxa with low dispersal potential.

Methods

There are seven discrete steps to our methodology: (1) Environmental sample collection, (2) isolation of particulates from water via filtration, (3) isolation of DNA from filter membrane, (4) amplification of target locus via PCR, (5) sequencing of amplicons, (6) bioinformatic translation of raw sequence data into tables of sequence abundance among samples, and (7) community ecological analyses of eDNA. We provide brief overviews of these steps here, and encourage the reader to review the fully detailed methods presented in the supplementary material (Supplemental Material).

Environmental Sampling

Starting from lower-intertidal patches of *Zostera marina*, we collected water samples at 1 meter depth from 8 points (0, 75, 125, 250, 500, 1000, 2000, and 4000 meters) along three parallel transects separated by 1000 meters (24 sample locations total; Figure 1). Samples were collected by attaching bottles to a PVC pole and lowering it over the side of a boat over the span of one hour on 27 June 2014. To destroy residual DNA on equipment used for field sampling and filtration, we washed with a 1:10 solution of household bleach (8.25% sodium hypochlorite; 7.25% available chlorine) and deionized water, followed by thorough rinsing with deionized water. Each environmental sample was collected in a clean 1 liter high-density polyethylene bottle, the opening of which was covered with 500 micrometer nylon mesh to prevent entry of larger particles. Immediately after collecting the sample, the mesh was replaced with a clean lid and the sample was held on ice until filtering.

Filtration

One liter from each water sample was filtered in the lab on a clean polysulfone vacuum filter holder fitted with a 47 millimeter diameter cellulose acetate membrane with 0.45 micrometer pores. Filter membranes were moved into 900 microliters of Longmire buffer (Longmire et al., 1997) using clean forceps and stored at room temperature ~~(?)~~(Renshaw et al., 2015). To test for the extent of contamination attributable to laboratory procedures, we filtered three replicate 1 liter samples of deionized water. These samples were treated identically to the environmental samples throughout the remaining protocols.

DNA Purification

DNA was purified from the membrane following a phenol:chloroform:isoamyl alcohol protocol following Renshaw ~~(?)~~(Renshaw et al., 2015). Preserved membranes were incubated at ~~65C~~ 65 ºC for 30 minutes before adding 900 microliters of phenol:chloroform:isoamyl alcohol and shaking vigorously for 60 seconds. We conducted two consecutive chloroform washes by centrifuging at 14,000 rpm for 5 minutes, transferring the aqueous layer to 700 microliters chloroform, and shaking vigorously for 60 seconds. After a third centrifugation, 500 microliters of the aqueous layer was transferred to tubes containing 20 microliters 5 molar NaCl and 500 microliters 100% isopropanol, and frozen at ~~-20C~~ -20 ºC for approximately 15 hours. Finally, all liquid was removed by centrifuging at 14000 rpm for 10 minutes, pouring off or pipetting out any remaining liquid, and drying in a vacuum centrifuge at ~~45C~~ 45 ºC for 15 minutes. DNA was resuspended in 200 microliters of ultrapure water. Four replicates of genomic DNA extracted from tissue of a species absent from the sampled environment (*Oreochromis niloticus*) served as positive control for the remaining protocols.

PCR Amplification

~~From each DNA sample, we amplified~~ We chose a primer set that amplifies an approximately 115 base pair (bp) region of the mitochondrial ~~gene encoding~~ 16S ~~RNA using a~~ rRNA gene in at least 10 metazoan phyla from this habitat, excludes non-metazoans, and resolves taxonomy to the family level in most cases using a public sequence database (Kelly et al., 2016a). We used a two-step polymerase chain reaction (PCR) protocol described by O'Donnell et al. (2016) to generate 4

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171 [replicate products from each DNA sample.](#) In the first set of reactions, primers were identical in ev-
172 ery reaction (forward: AGTTACYYTAGGGATAACAGCG; reverse: CCGGTCTGAACTCAGAT-
173 CAYGT); primers in the second set of reactions included these same sequences but with 3 variable
174 nucleotides (NNN) and an index sequence on the 5′ end (see [Sequencing Metadata](#)). We used the
175 program OligoTag (Coissac, 2012) to generate 30 unique 6-nucleotide index sequences differing by
176 a minimum Hamming distance of 3 (see [Sequencing Metadata](#)). Indexed primers were assigned to
177 samples randomly, with the identical index sequence on the forward and reverse primer to avoid
178 errors associated with dual-indexed multiplexing (Schnell et al., 2015). In a UV-sterilized hood,
179 we prepared 25 microliter reactions containing 18.375 microliters ultrapure water, 2.5 microliters
180 10x buffer, 0.625 microliters deoxynucleotide solution (8 millimolar), 1 microliter each forward and
181 reverse primer (10 micromolar, obtained lyophilized from Integrated DNA Technologies (Coralville,
182 IA, USA)), 0.25 microliters Qiagen HotStar Taq polymerase, and 1.25 microliter genomic or eDNA
183 template at 1:100 dilution in ultrapure water. PCR thermal profiles began with an initialization
184 step (~~95C~~95 °C; 15 min) followed by cycles (40 and 20 for the first and second reaction, respectively)
185 of denaturation (~~95C~~95 °C; 15 sec), annealing (~~61C~~61 °C; 30 sec), and extension (~~72C~~72 °C; 30 sec).
186 20 identical PCRs were conducted from each DNA extract using non-indexed primers; these were
187 pooled into 4 groups of 5 in order to ensure ample template for the subsequent PCR with indexed
188 primers. In order to isolate the fragment of interest from primer dimer and other spurious frag-
189 ments generated in the first PCR, we used the AxyPrep Mag FragmentSelect-I kit with solid-phase
190 reversible immobilization (SPRI) paramagnetic beads at 2.5x the volume of PCR product (Axygen
191 BioSciences, Corning, NY, USA). A 1:5 dilution in ultrapure water of the product was used as tem-
192 plate for the second reaction. PCR products of the second reaction were purified using the Qiagen
193 MinElute PCR Purification Kit (Qiagen, Hilden, Germany). Ultrapure water was used in place of
194 template DNA and run along with each batch of PCRs to serve as a negative control for PCR; none
195 of these produced visible bands on an agarose gel. In total, four separate replicates from each of
196 31 DNA samples were carried through the two-step PCR process for a total of 124 sequenced PCR
197 products. These were combined with additional samples from other projects, totaling 345 samples
198 for sequencing.

DNA Sequencing

Up to 30 PCR products were combined according to their primer index in equal concentration into one of 14 pools, and 150 nanograms from each were prepared for library sequencing using the KAPA high-throughput library prep kit with real-time library amplification protocol (KAPA Biosystems, Wilmington, MA, USA). Each of these ligated sequencing adapters included an additional 6 base pair index sequence (NEXTflex DNA barcodes; BIOO Scientific, Austin, TX, USA). Thus, each PCR product was identifiable via its unique combination of index sequences in the sequencing adapters and primers. Fragment size distribution and concentration of each library was quantified using an Agilent 2100 BioAnalyzer. Libraries were pooled in equal concentrations and sequenced for 150 base pairs in both directions (PE150) using an Illumina NextSeq at the Stanford Functional Genomics Facility (machine NS500615, run 115, flowcell H3LFLAFXX), where 20% PhiX Control v3 was added to act as a sequencing control and to enhance sequencing depth by increasing sequence diversity. Raw sequence data in fastq format is publicly available (see Data Availability).

Sequence Data Processing (Bioinformatics)

Detailed bioinformatic methods are provided in the supplemental material, and analysis scripts used from raw sequencer output onward can be found in the public project directory (see Analysis Scripts). Briefly, we performed five steps to process the sequence data: (1) Merge paired-end reads, (2) eliminate low-quality reads, (3) eliminate PCR artifacts (chimeras), (4) cluster reads by similarity into operational taxonomic units (OTUs), and (5) match observed sequences to taxon names. Additionally, we checked for consistency among PCR replicates, excluded extremely rare sequences, and rescaled (rarefied) the data to account for differences in sequencing depth. The data for input to further analyses are a contingency table of the mean count of unique sequences, OTUs, or taxa present in each environmental sample.

Ecological Analyses

After gathering the data, we use the eDNA community observed at each location to make inferences about the spatial patterning of eDNA communities. We use statistical tools from community ecology to assess the spatial structure of eDNA communities. We report similarity (1- dissimilarity) rather

226 than dissimilarity in all cases for ease of interpretation.

Objective 1: Community similarity as a function of distance

Distance Decay

229 To address our first objective and determine whether or not nearby samples are more similar than
230 distant ones, we fit a nonlinear model to represent decreasing community similarity with distance.
231 We calculated the pairwise Bray-Curtis similarity (1 - Bray-Curtis dissimilarity) between eDNA
232 communities using the R package vegan (Oksanen et al., 2016) and the great circle distance between
233 sampling points using the Haversine method as implemented by the R package geosphere (Hijmans,
234 2016). This model is similar to the Michaelis-Menten function, but with an asymptote fixed at 0:

$$y_{ij} = \frac{AB}{B + x_{ij}} \tag{1}$$

235 Where the relationship between community similarity (y_{ij}) and spatial distance (x_{ij}) between
236 observations i and j is determined by the similarity of samples at distance 0 (A), and the distance at
237 which half the total change in similarity is achieved (B). This allows for ~~a~~ samples collected very close
238 together (near 0) to have similarity significantly less than one. We assessed model fit using the R
239 function nls (R Core Team, 2016), using the nl2sol algorithm from the Port library to solve separable
240 nonlinear least squares using analytically computed derivatives (http://netlib.org/port/nsg.f). We
241 set bounds of 0 and 1 for the intercept parameter and a lower bound of 0 for the distance at half
242 similarity; starting values of these parameters were 0.5 and $x_{max}/2$, respectively. We calculated
243 a 95% confidence interval for the parameters and the predicted values using a first-order Taylor
244 expansion approach implemented by the function predictNLS in the R package propagate (Spiess,
245 2014).

246 There are other conceptually reasonable forms to expect the space-by-similarlity relationship
247 to take; we present these in the supplemental material along with alternative data subsets and
248 similarity indices (see Supplemental Material).

Objective 2: Spatial distribution of diversity

Community Classification

To determine the spatial distribution and variation of eDNA communities (objective 2), we used multivariate classification algorithms. We simultaneously assessed the existence of distinct community types and the membership of samples to those community types using an unsupervised classification algorithm known as partitioning around medoids (PAM; sometimes referred to as k-medoids clustering) (Kaufman and Rousseeuw, 1990), as implemented in the R package cluster (Maechler et al., 2016). The classification of samples to communities was made on the basis of their pairwise Bray-Curtis similarity, calculated using the function vegdist in the R package vegan (Oksanen et al., 2016). Other distance metrics were evaluated but had no appreciable effect on the outcome of the analysis (Figure 8). In order to chose an optimal number of clusters (K), we evaluated the distribution of silhouette widths, a measure of the similarity between each sample and its cluster compared to its similarity to other clusters. We repeated the analysis using fuzzy clustering (FANNY, (Kaufman and Rousseeuw, 1990); however, the results were qualitatively similar to the results using PAM so we omit them here.

Aggregate Measures of Diversity

We calculated two measures of diversity, richness and evenness, to ask if aggregate metrics of the eDNA community showed evidence of spatial patterning. Richness is a measure of the number of distinct types of organisms present and so ranges from 1 (only one taxon observed) to S, the number of taxa observed across all samples. To calculate the evenness of the distribution of abundance of taxa in a sample, we used the complement of the Simpson (1949) index ($1 - \Sigma p_i^2$, where p_i is the proportional abundance of taxon i). The values of this index ranges from 0 to 1, with the value interpreted as the probability that two sequences randomly selected from the sample will belong to different taxa; thus, larger values of the index indicate more evenly divided communities (Magurran, 2003). We calculated Moran's I for both diversity metrics to test for spatial autocorrelation. We also tested for a linear effect of log-transformed distance from shore on each measure of diversity to ask how diversity changes over this strong environmental gradient.

11

Taxon and Life History Patterns

After assigning taxon names to the abundance data, we plotted the distribution in space of a selection of taxa to compare with our expectations on the basis of adult distributions (objective 2). Our aim was to understand where each taxon occurred in the greatest proportional abundance, and its distribution in space relative to that maximum. Thus, we rescaled each sample to proportional abundance, extracted the data from a single taxon, and scaled those values between 0 and 1. We collated life history characteristics for each of the major taxonomic groups recovered, including dispersal range of the gametes, larvae, and adults, adult habitat type and selectivity, and adult body size. ~~Dispersal range was given as~~ For each life history stage of each taxon group, we made an order-of-magnitude approximation of the scale of dispersal~~: for~~. For example, internally fertilized species were assigned a gamete range of 0 km, while broadcast spawners were assigned a gamete range of 10 km. Similarly, adult range size was approximated as 0 km (sessile), 1 km (motile but not pelagic), or 10 km (highly mobile, pelagic). Variables were specified as 'multiple' for ~~groups~~ life history stages known to span more than 1 magnitude of range size. For groups to which sequences were annotated with high confidence, but for which life history strategy is diverse or poorly known (e.g. families in the phylum Nemertea), we used conservative, coarse approximations at a higher taxonomic rank (see Life History Data). These data were used to contextualize group-specific spatial distributions and inform expectations based on known adult distributions.

Results

Sequence Data Processing (Bioinformatics)

Preliminary sequence analysis strongly suggested that the observed variation among environmental samples reflects true variation in the environment, rather than variability due to lab protocols, for the following reasons (note that all value ranges are reported as mean plus and minus one standard deviation). First, all libraries passed the FastQC per-base sequence quality filter, generating a total of 371,576,190 reads passing filter generated in each direction. Second, samples in this study were represented by an adequate number of reads (333,537.9 $\pm$ 112,200.5), with no individual sample receiving fewer than 130,402 reads. Third, there was a very low frequency of cross-contamination

303 from other libraries into those reported here (5e-05±8e-05; max proportion 0.00034). Fourth, after

304 scaling all samples to the same sequencing depth, OTUs with abundance greater than 178 reads

305 (0.14% of a sample's reads) experienced no turnover among PCR replicates within a sample. Fifth,

306 sequence abundances among PCR replicates within water samples were remarkably consistent. A

307 single sample had low similarity among PCR replicates (0.659) after removing this outlier, the

308 lowest mean similarity among replicates within a sample was 0.966. Overall similarities among

309 PCR replicates within a sample were extremely high (0.976 ± 0.013), and far higher than ~~that of~~

310 ~~than~~ those among samples (0.3 ± 0.16). Across PCR replicates, each sample was represented by at

311 least 781425 reads in the raw data and contained between 111 and 443 rarefied OTUs (Supplemental

312 Figure 10).

Ecological Analyses

Distance Decay

315 Physical proximity is a good predictor of eDNA community similarity: Similarity decreased from

316 0.40 (95%CI = 0.36, 0.45) to half that amount at 4500 meters (95%CI = 2900, 7500) (Figure 2).

Community Classification

318 Despite a clear trend in community similarity as a function of spatial separation, the results from

319 our classification analysis are difficult to interpret. The silhouette analysis indicated the presence

320 of 8 distinct communities; however, the gain in mean silhouette width from 2 was small (0.1), and

321 lacked a distinctive peak (Figure 4), indicating substantial uncertainty in the clustering algorithm.

322 Thus, we present the results of cluster assignment for both $K = 2$ and $K = 8$ to illustrate the

323 range of results (Figure 3). Excluding taxa which occur in only one site had no discernible effect

324 on the outcome of the PAM analysis (number of clusters, assignment to clusters). While there was

325 no distinct spatial divide indicating the presence of an inshore versus an offshore community, one

326 of the two communities (at $K = 2$) occurred in only 2 out of 18 samples inside 1000 meters from

327 shore, and never occurred within 125 meters of shore, suggesting the presence of an inshore and

328 offshore community.

Diversity in Space

Sites offshore tend to be less rich and more even than those inshore (Figure 6). Mean OTU richness declined by 1.42 per 1000 meters from a mean of 17.6 taxa (95%CI = 2.15) inshore to 11.9 taxa (95%CI = 4.31) at offshore locations (p = 0.0415; Figure 6). Evenness ~~, the probability that two reads chosen at random from a sample belong to different species,~~ increased by .0666 per 1000 meters from 0.225 (95%CI = 0.0558) to 0.491 (95%CI = ± 0.112), indicating that sequence reads were less evenly distributed among taxa in offshore samples (p ≪ 0.05; Figure 6). There was no evidence for spatial autocorrelation for any of the diversity metrics (Moran's I, p > 0.05; Figure 5).

Taxon and Life History Patterns

We were able to assign a taxon name with confidence to 136 of 146 OTU sequences. The vast majority of sequences (97.6%) and OTUs (96.9%) were matched to organisms that have high potential for dispersal at either the gamete, larval, or adult stage, making it impossible to determine whether the source of that DNA was adults with well-documented spatial patterns (e.g. sessile nearshore specialists) or highly mobile early life history stages. Of the 6 OTUs for which dispersal is limited during all life history stages, only 2 occurred in more than two samples, precluding a quantitative comparison of spatial dispersion based on life history characteristics. These were assigned to *Cymatogaster aggregata*, a viviparous nearshore fish with internal fertilization, and *Cupolaconcha meroclista*, a sessile Vermetid gastropod with presumed internal fertilization and short larval dispersal (Strathmann and Strathmann, 2006; Phillips and Shima, 2010; Calvo and Templado, 2004). *Cymatogaster aggregata* was distinctly more abundant close to shore, with no sequences occurring in any sample beyond 250 meters (Figure 7). *Cupolaconcha meroclista* showed no such distinct spatial trend, occurring in nearly equal abundance at three sites, 75, 500, and 2000 meters from shore. An additional species that was highly abundant in the sequence data, the krill *Thysanoessa raschii*, has pelagic adults, highly seasonal reproduction, and sinking eggs; their distribution was consistent with our expectations based on a tendency of adults to aggregate offshore. Finally, the two most abundant taxa in the dataset were the mussel genus *Mytilus* and the Barnacle order Sessilia; the adults of both taxa are sessile and occur exclusively on hard intertidal substrata but have highly motile larvae. Because large-scale dispersal could not be ruled out for the vast majority of

357 taxa, subsetting the community data by taxonomic group had no qualitative effect on the spatial
358 patterning or diversity metrics, and we omit those results here.

Discussion

360 Indirect surveys of organismal presence are a key development in ecosystem monitoring in the face
361 of increased anthropogenic pressure and dwindling resources for ecological research. Monitoring of
362 organisms using environmental DNA is an especially promising method, given the rapid pace of
363 advancement in technological innovation and cost efficiency in the field of DNA sequencing and
364 quantification. ~~For the first time in a marine environment, we~~ We document four key patterns:
365 (1) eDNA communities far from one another tend to be less similar than those that are nearby, (2)
366 distinct eDNA communities exist and are distributed in a non-random fashion, (3) diversity declines
367 with distance from shore, and (4) spatial patterning of eDNA is associated with taxon-specific life
368 history characteristics.

(1) Communities far from one another tend to be less similar than those that are nearby

371 We demonstrate that ~~more~~ distant locations have ~~less similar~~ less-similar eDNA communities than
372 ~~more~~ proximate locations in Puget Sound, a dynamic marine environment. Our finding is in line
373 with observations based on traditional surveys of terrestrial plants and fungi (Nekola and White,
374 1999; Bahram et al., 2013; Condit, 2002; Chust et al., 2006) and of microorganisms in freshwater
375 (Wetzel et al., 2012), marine (Chust et al., 2013), and estuarine (Martiny et al., 2011) environments.
376 To our knowledge, it is the first to report such a pattern using massively parallel sequencing of
377 environmental DNA in the marine environment, and the first using any technique to describe this
378 pattern from macrobial metazoans. We note that the theoretical expectation is that samples at very
379 close distance be nearly completely similar, while our samples separated by the 50 meters were only
380 40% similar. We interpret this to reflect the highly dynamic nature of this environment, which could
381 cause DNA to be distributed quickly from its source, eroding the rise in similarity at small distances.
382 At the same time, community similarity decreased to very low levels at larger scales, indicating that
383 DNA distribution is not completely unpredictable. This finding implies that the effectively sampled

area of individual water samples for eDNA analysis is likely to be quite small (<100m) in this nearshore environment. Our estimated distance-decay relationship does indicate that proximate samples are more similar than distant samples, but we suggest this pattern is partially obscured by other factors, including signal from mobile, microscopic life-stages.

(2) Distinct eDNA communities exist and are distributed in a non-random fashion

We demonstrate strong evidence for distinct community types and the non-random spatial patterning of those communities. While the spatial distributions of communities is surprising if one were concerned only with the macroscopic life stages of metazoans, it indeed does align with the broader view that even offshore pelagic communities are comprised of and influenced by nearshore organisms. This result underscores the idea that areas immediately offshore act as ecotones, a mixing zone of taxa characteristic of benthic and pelagic environments. While there was no distinct break in community types between onshore and offshore sites, there was some clustering of community types that may be explained by oceanographic features such as nearshore eddies generated by strong tidal exchange in a steep bathymetric setting (Yang and Khangaonkar, 2010). It would be useful to better understand such features during the period of sampling, by way of oceanographic monitoring devices. Finally, the uncertainty in identification of the number of distinct clusters to best characterize the community underlines the difficulty of identifying community patterns with the number of taxonomic groups considered here. We suspect that the signature of eDNA from microscopic life-stages may explain our inability to easily detect spatial community level patterns that align with our initial expectations.

(3) Richness declines and evenness increases with distance from shore

We ~~detected a general pattern of declining richness and increasing evenness with increasing distance offshore~~found that richness declined while evenness increased with distance from shore. Such a pattern is consistent with many other ecosystems which show strong clines in diversity metrics over environmental gradients. ~~The coastal ocean is a highly productive and diverse ecosystem (Ray, 1988).~~ However, our study is novel in that it corroborates a cline well-known on macroscales for macrobiota on a much smaller spatial scale for microscopic animals, suggesting that there may be a self-similarity across scales in diversity patterning (Levin, 1992). The coastal ocean is a highly

productive and diverse ecosystem, where biomass is concentrated most heavily along the bottom and shoreline (Ray, 1988). This differential in biomass concentration from the shoreline to open waters may contribute to the opposing trends we detected. Where particles (organisms, tissues, and cells) are sparse, fewer would be collected per sample of constant volume, thus decreasing the probability of drawing as many types (richness) and increasing the probability that any two particles originate from the same type (evenness). Intriguingly, the cline in diversity from inshore to offshore was not determined by shared changes in communities as one moved offshore; the classification analysis suggested a fair amount of differences among communities at a given offshore distance (Figure 3). ~~Furthermore, the uncertainty in identification of the number of distinct clusters to best characterize the community underlines the difficulty of identifying community patterns with the number of taxonomic groups considered here. We suspect that the signature of eDNA from microscopic life-stages may explain our inability to easily detect spatial community level patterns that align with our initial expectations.~~

(4) Spatial patterning of eDNA is associated with taxon-specific life history ~~characteristics.~~

In contrast to our expectations, other taxa including species with sessile adult stages restricted to benthic hard substrates (e.g. barnacles, mussels) are among the most abundant taxa at sites furthest from shore. However, the larvae and gametes of these taxa are abundant, pelagic, and can be transported long distances by water movement (Strathmann, 1987). This indicates that we likely detected DNA of their pelagic phase gametes and larvae. It is always possible that DNA of adults was advected over long distances and detected offshore but in light of our results with krill and surfperch, we view this as unlikely. We interpret our results as evidence that the chaotic spatial distribution of eDNA communities (Figure 3) results from our primers' affinity for many species which at some point exist as microscopic pelagic gametes or larvae. Our results emphasize that expected results based on easily visually observed individuals or detectable with traditional sampling gear such as nets may be very different from results using eDNA. This does caution that eDNA surveys may have different purposes and may not be directly comparable to existing surveys (Shelton et al., 2016).

We acknowledge that sampling artifacts may have affected our results. For example if entire multicellular individuals were captured in our samples, their DNA could be in much greater density

than eDNA, affecting the observed community. Our sampling bottles excluded particles larger than 500 micrometers, but gametes and very small larvae could have gained entry. It is possible that even a single small individual, containing many thousand mitochondria, would overwhelm the signal of another species from which hundreds of cells had been sloughed from many, larger individuals. Data on larval size distribution at the time of sampling from each species in our data set would allow us to estimate the frequency of such events. Nevertheless, it is precisely the sensitivity to small particles that makes the eDNA approach powerful, so we are reluctant to recommend that aquatic eDNA sampling use finer pre-filtering. Instead, we emphasize the importance of designing and selecting primer sets that selectively amplify target organisms. In the case of the present study, in order to recover patterns matching our expectations, this would be non-transient, benthic marine organisms lacking any pelagic life stage.

The marker we chose for this study detects a wide variety of metazoans while excluding other more common taxa; however, it does not effectively discriminate among species within a higher group in all cases. Other markers, such as mitochondrial cytochrome c oxidase subunit 1 (COX1, CO1, or COI) may provide adequate species-level resolution in some metazoan groups, but have other shortcomings including taxon dropout (Deagle et al., 2014) and amplification of more abundant non-metazoans, as we discovered in an accompanying study (Kelly et al., 2016a). Both have undesirable effects of biasing estimates of diversity. In our case, it is possible that the lumping of multiple species into one group underestimates the true richness of the group and of the entire sample, in turn obscuring true underlying patterns of diversity. In the case of COX1, well-documented primer biases cause failure to amplify some taxa, particularly in mixed samples, with the same result (Deagle et al., 2014). In fact, even surveys relying on traditional capture techniques (e.g. seine nets) and morphological characteristics are subject to biases imposed by the sampling gear (e.g. mesh size), the observer (e.g. taxonomic expertise), and organisms (e.g. morphologically cryptic species). Similarly, no single molecular marker adequately and effectively samples all taxa without bias (Drummond et al., 2015), and thus the choice of marker is an important and context-dependent one. Until whole-genome sequencing of individual cells is a reality, the tradeoffs between taxonomic breadth and resolution will continue to be problematic for metabarcoding studies, just as they are for more traditional ecological survey methods (Kelly et al., 2016a).

Our results also highlight the need for curated life-history databases. As technological advances

increase the speed and throughput of DNA sequencing and sequence processing, making sense of these data in a timely manner requires that natural history data be stored in standard formats in centralized repositories. The rate at which we can make sense of high-throughput survey methods will be limited by our ability to collate auxiliary data. Databases such as Global Biodiversity Information Facility (GBIF), Encyclopedia of Life (EOL), and FishBase (Parr et al., 2014; Froese and Pauly, 2016) contain records of taxonomy, occurrence, and other rudimentary data types, but there is no centralized, standardized repository for even basic natural history data such as body size. As NCBI's nucleotide and protein sequence database (GenBank) has facilitated transformative studies in diverse fields, an ecological analog would be a boon for biodiversity science.

Surveys based on eDNA are intensely scrutinized because of the danger that the final data are subject to complicated laboratory and bioinformatic procedures. Finding virtually no variability among lab and bioinformatic treatments from the point of PCR onward, we were confident our results represented actual field-based differences among samples. However, we note that one PCR replicate had a clear signal of contamination in that the sequence community was extremely similar to those from a different environmental sample. The source of this error is difficult to identify, but seems most likely to be an error during PCR preparation, either in assignment or pipetting during preparation of indexed primers. While the remainder of our results would be largely unchanged had we sequenced a single replicate per environmental sample, we believe the sequencing of PCR replicates is critical for ensuring data quality in eDNA sequencing studies.

While there is much work to be done before eDNA techniques can be confidently deployed as a standard method for ecological monitoring, this study serves as a first analysis of diversity at the fine spatial scales that are likely to be relevant to eDNA work in the field across a range of study systems.

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Author Contributions

Conceived and designed the experiments: JL O'Donnell, RP Kelly, AO Shelton; Collected the data: JL O'Donnell, NC Lowell, GD Williams, RP Kelly, AO Shelton, JF Samhouri; Conducted the analyses: JL O'Donnell; Wrote the first draft: JL O'Donnell; Edited the manuscript: JL O'Donnell, AO Shelton, RP Kelly, JF Samhouri, GD Williams, NC Lowell

Ethics Statement

The authors declare no conflict of interest. Consistent with the public trust doctrine, waters of the US are public, and therefore no permit was necessary to conduct this research (see Illinois Central Railroad v. Illinois, 146 U.S. 387 (1892)).

Data Availablity

0.1 ~~Sequence Data~~

Sequence Data

All sequence ~~files and metadata~~ data are available from ~~EMBL~~NCBI under BioProject PRJNA338801. Scripts to process raw sequence data into the contingency tables used for ecological analyses can be found at:

`https://github.com/jimmyodonnell/banzai`

0.1 ~~Project Repository~~

Project Repository

The following components are available from the project repository on GitHub:

`https://github.com/jimmyodonnell/Carkeek_eDNA_grid`

`http://dx.doi.org/FIXME`

0.0.1 ~~Sequencing Metadata~~

Sequencing Metadata

Sequencing metadata is available in: `Data/metadata_spatial.csv`

0.0.1 ~~Life History Data~~

Life History Data

Life history data is available in: `Data/life_history.csv`

0.0.1 ~~Analysis Scripts~~

Analysis Scripts

All analyses were performed using scripts available in the Analysis subdirectory of the project's repository on GitHub.

References

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders, N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., and Swenson, N. G. (2011). Navigating the multiple meanings of beta diversity: A roadmap for the practicing ecologist. *Ecology Letters*, 14(1):19–28.

Bahram, M., Kõljalg, U., Courty, P. E., Diédhiou, A. G., Kjøller, R., Põlme, S., Ryberg, M., Veldre, V., and Tedersoo, L. (2013). The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. *Journal of Ecology*, 101(5):1335–1344.

542 Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., and Lodge, D. M.

543 (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental*

544 *Science and Technology*, 48(3):1819–1827.

545 Bell, T. (2010). Experimental tests of the bacterial distance–decay relationship. *The ISME Journal*,

546 4(11):1357–1365.

547 Burns, R. E. (1985). *The shape and form of Puget Sound*. Washington Sea Grant, Seattle, 1 edition.

548 Calvo, M. and Templado, J. (2004). Reproduction and development in a vermetid gastropod,

549 Vermetus triquetrus. *Invertebrate Biology*, 123(4):289–303.

550 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T. L.

551 (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, 10:421.

552 Chamberlain, S. a. and Szöcs, E. (2013). taxize: taxonomic search and retrieval in R. *F1000Research*,

553 2(0):191.

554 Chamberlain, S. A., Szöcs, E., Boettiger, C., Ram, K., Bartomeus, I., Foster, Z., and O'Donnell,

555 J. L. (2016). taxize: Taxonomic information from around the web. R package.

556 Chust, G., Chave, J., Condit, R., Aguilar, S., Lao, S., and Perez, R. (2006). Determinants and

557 spatial modeling of tree beta-diversity in a tropical forest landscape in Panama. *Journal of*

558 *Vegetation Science*, 17(1):83–92.

559 Chust, G., Irigoien, X., Chave, J., and Harris, R. P. (2013). Latitudinal phytoplankton distribution

560 and the neutral theory of biodiversity. *Global Ecology and Biogeography*, 22(5):531–543.

561 Coissac, E. (2012). OligoTag: A Program for Designing Sets of Tags for Next-Generation Sequencing

562 of Multiplexed Samples. In Pompanon, F. and Bonin, A., editors, *Data Production and Analysis in*

563 *Population Genomics SE - 2*, volume 888 of *Methods in Molecular Biology*, pages 13–31. Humana

564 Press.

565 Condit, R. (2002). Beta-Diversity in Tropical Forest Trees. *Science*, 295(5555):666–669.

566 Cowart, D. a., Pinheiro, M., Mouchel, O., Maguer, M., Grall, J., Miné, J., and Arnaud-Haond, S.
567 (2015). Metabarcoding Is Powerful yet Still Blind: A Comparative Analysis of Morphological and
568 Molecular Surveys of Seagrass Communities. *Plos One*, 10(2):e0117562.

569 de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le
570 Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner,
571 L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O.,
572 Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent,
573 F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Acinas, S. G., Bork, P.,
574 Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S.,
575 Raes, J., Sieracki, M. E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P.,
576 and Karsenti, E. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, 348(6237).

577 Deagle, B. E., Jarman, S. N., Coissac, E., Pompanon, F., Taberlet, P., Deagle, B. E., Jarman, S. N.,
578 and Coissac, E. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker :
579 not a perfect match DNA metabarcoding and the cytochrome c oxidase subunit I marker : not a
580 perfect match. (September).

581 Deiner, K. and Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a
582 natural river. *PLoS ONE*, 9(2).

583 Dethier, M. N. (2010). Overview of the ecology of Puget Sound beaches. In Shipman, H., Dethier,
584 M. N., Gelfenbaum, G., Fresh, K. L., and Dinicola, R. S., editors, *Puget Sound Shorelines and
585 the Impacts of Armoring—Proceedings of a State of the Science Workshop*, page 262.

586 Drummond, A. J., Newcomb, R. D., Buckley, T. R., Xie, D., Dopheide, A., Potter, B. C., Heled, J.,
587 Ross, H. A., Tooman, L., Grosser, S., Park, D., Demetras, N. J., Stevens, M. I., Russell, J. C.,
588 Anderson, S. H., Carter, A., and Nelson, N. (2015). Evaluating a multigene environmental DNA
589 approach for biodiversity assessment. *GigaScience*, 4(1):46.

590 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*,
591 26(19):2460–2461.

592 Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., Mahon, A. R.,

593 Pfrender, M. E., Lamberti, G. A., and Lodge, D. M. (2016). Quantification of mesocosm fish and
594 amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources*,
595 16(1):29–41.

596 Ficetola, G. F., Miaud, C., Pompanon, F., and Taberlet, P. (2008). Species detection using envi-
597 ronmental DNA from water samples. *Biology letters*, 4(4):423–425.

598 Fonseca, V. G., Carvalho, G. R., Nichols, B., Quince, C., Johnson, H. F., Neill, S. P., Lambshead,
599 J. D., Thomas, W. K., Power, D. M., and Creer, S. (2014). Metagenetic analysis of patterns of
600 distribution and diversity of marine meiobenthic eukaryotes. *Global Ecology and Biogeography*,
601 23(11):1293–1302.

602 Froese, R. and Pauly, D. (2016). FishBase.

603 Guardiola, M., Uriz, M. J., Taberlet, P., Coissac, E., Wangensteen, O. S., and Turon, X.
604 (2015). Deep-Sea, Deep-Sequencing: Metabarcoding Extracellular DNA from Sediments of Ma-
605 rine Canyons. *PLOS ONE*, 10(10):e0139633.

606 Guardiola, M., Wangensteen, O. S., Taberlet, P., Coissac, E., Uriz, M. J., and Turon, X. (2016).
607 Spatio-temporal monitoring of deep-sea communities using metabarcoding of sediment DNA and
608 RNA. *PeerJ*, 4:e2807.

609 Guo, H., Chamberlain, S. A., Elhaik, E., Jalli, I., Lynes, A. R., Marczak, L., Sabath, N., Vargas,
610 A., Więski, K., Zelig, E. M., and Pennings, S. C. (2015). Geographic variation in plant commu-
611 nity structure of salt marshes: Species, functional and phylogenetic perspectives. *PLoS ONE*,
612 10(5):e0127781.

613 Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., and Goodman, R. M. (1998). Molecular
614 biological access to the chemistry of unknown soil microbes: a new frontier for natural products.
615 *Chemistry & Biology*, 5(10):R245–R249.

616 Hijmans, R. J. (2016). geosphere: Spherical Trigonometry.

617 Hubbell, S. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography.*, volume 32.

618 Iverson, V., Morris, R. M., Frazar, C. D., Berthiaume, C. T., Morales, R. L., and Armbrust,
619   E. V. (2012). Untangling Genomes from Metagenomes: Revealing an Uncultured Class of Marine
620   Euryarchaeota. *Science*, 335(6068):587–590.

621 Kaufman, L. and Rousseeuw, P. J. (1990). *Finding Groups in Data: An Introduction to Cluster*
622   *Analysis.*

623 Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., and Samhouri, J. F.
624   (2016a). Genetic and manual survey methods yield different and complementary views of an
625   ecosystem. *Frontiers in Marine Science*, 3:283.

626 Kelly, R. P., O'Donnell, J. L., Lowell, N. C., Shelton, A. O., Samhouri, J. F., Hennessey, S. M.,
627   Feist, B. E., and Williams, G. D. (2016b). Genetic signatures of ecological diversity along an
628   urbanization gradient. *PeerJ*, 4:e2444.

629 Klymus, K. E., Richter, C. A., Chapman, D. C., and Paukert, C. (2015). Quantification of eDNA
630   shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypoph-
631   thalmichthys molitrix. *Biological Conservation*, 183:77–84.

632 Kozloff, E. N. (1973). *Seashore life of Puget Sound, the Strait of Georgia, and the San Juan*
633   *Archipelago.* University of Washington Press, Seattle.

634 Lallias, D., Hiddink, J. G., Fonseca, V. G., Gaspar, J. M., Sung, W., Neill, S. P., Barnes, N.,
635   Ferrero, T., Hall, N., Lambshead, P. J. D., Packer, M., Thomas, W. K., and Creer, S. (2015).
636   Environmental metabarcoding reveals heterogeneous drivers of microbial eukaryote diversity in
637   contrasting estuarine ecosystems. *The ISME journal*, 9(5):1208–21.

638 Levin, S. A. (1992). The problem of pattern and scale in ecology. *Ecology*, 73(6):1943–1967.

639 Longmire, J. L., Maltbie, M., and Baker, R. J. (1997). Use of lysis buffer in DNA isolation and its
640   implication for museum collections. *Museum of Texas Tech University*, 163.

641 Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., and Hornik, K. (2016). cluster: Cluster
642   Analysis Basics and Extensions.

643 Magurran, A. E. (2003). *Measuring Biological Diversity.* Wiley.

644 Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2014). Swarm: robust and
645   fast clustering method for amplicon-based studies. *PeerJ*, 2:e593.

646 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
647   *EMBnet.journal*, 17(1):10.

648 Martiny, J. B. H., Eisen, J. A., Penn, K., Allison, S. D., and Horner-Devine, M. C. (2011). Drivers of
649   bacterial beta diversity depend on spatial scale. *Proceedings of the National Academy of Sciences*,
650   108(19):7850–7854.

651 Nekola, J. C. and White, P. S. (1999). The distance decay of similarity in biogeography and ecology.
652   *Journal of Biogeography*, 26(4):867–878.

653 O'Donnell, J. L., Kelly, R. P., Lowell, N. C., and Port, J. A. (2016). Indexed PCR Primers Induce
654   Template-Specific Bias in Large-Scale DNA Sequencing Studies. *PLOS ONE*, 11(3):e0148698.

655 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R.,
656   O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H.
657   (2016). vegan: Community Ecology Package.

658 Parr, C. S., Wilson, N., Leary, P., Schulz, K. S., Lans, K., Walley, L., Hammock, J. A., Goddard,
659   A., Rice, J., Studer, M., Holmes, J. T. G., and Corrigan, R. J. (2014). The Encyclopedia of
660   Life v2: Providing Global Access to Knowledge About Life on Earth. *Biodiversity Data Journal*,
661   2(2):e1079.

662 Phillips, N. E. and Shima, J. S. (2010). Reproduction of the vermetid gastropod dendropoma
663   maximum (Sowerby, 1825) in Moorea, French Polynesia. *Journal of Molluscan Studies*, 76(2):133–
664   137.

665 Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J.,
666   Yamahara, K. M., and Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest
667   ecosystem using environmental DNA. *Molecular Ecology*, 25(2):527–541.

668 R Core Team (2016). R: A Language and Environment for Statistical Computing.

669 Ray, G. C. (1988). Ecological diversity in coastal zones and oceans. In Wilson, E. O. and Peter,
670   F. M., editors, *Biodiversity*, chapter 4. National Academies Press (US), Washington, DC.

671 Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., and Lodge, D. M. (2015). The
672   room temperature preservation of filtered environmental DNA samples and assimilation into a
673   phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, 15(1):168–176.

674 Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open
675   source tool for metagenomics. *PeerJ*, 4:e2584.

676 Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., and Boehm, A. B. (2016).
677   Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine
678   Fish. *Environmental Science & Technology*, 50(19):10456–10464.

679 Schnell, I. B., Bohmann, K., and Gilbert, M. T. P. (2015). Tag jumps illuminated - reducing
680   sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*,
681   pages n/a–n/a.

682 Shelton, A. O., O'Donnell, J. L., Samhouri, J. F., Lowell, N., Williams, G. D., and Kelly, R. P.
683   (2016). A framework for inferring biological communities from environmental DNA. *Ecological
684   Applications*, 26(6):1645–1659.

685 Shogren, A. J., Tank, J. L., Andruszkiewicz, E. A., Olds, B., Jerde, C., and Bolster, D. (2016).
686   Modelling the transport of environmental DNA through a porous substrate using continuous
687   flow-through column experiments. *Journal of The Royal Society Interface*, 13(119):423–425.

688 Simpson, E. H. (1949). Measurement of diversity. *Nature*, 163(688).

689 Spiess, A.-N. (2014). propagate: Propagation of Uncertainty.

690 Strathmann, M. F. (1987). *Reproduction and Development of Marine Invertebrates of the Northern
691   Pacific Coast: Data and Methods for the Study of Eggs, Embryos, and Larvae*. University of
692   Washington Press, Seattle.

693 Strathmann, M. F. and Strathmann, R. R. (2006). A Vermetid Gastropod with Complex Intracap-
694   sular Cannibalism of Nurse Eggs and Sibling Larvae and a High Potential for Invasion. *Pacific*
695   *Science*, 60(1):97–108.

696 Strickland, R. M. (1983). *The Fertile Fjord: Plankton in Puget Sound.* University of Washington
697   Press, Seattle.

698 Strickler, K. M., Fremier, A. K., and Goldberg, C. S. (2015). Quantifying effects of UV-B, temper-
699   ature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183:85–92.

700 Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., and Willerslev, E. (2012).
701   Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples.
702   *PLoS ONE*, 7(8):1–9.

703 Turner, C. R., Uy, K. L., and Everhart, R. C. (2015). Fish environmental DNA is more concentrated
704   in aquatic sediments than surface water. *Biological Conservation*, 183:93–102.

705 Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M.,
706   Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., and Banfield, J. F. (2004). Community structure
707   and metabolism through reconstruction of microbial genomes from the environment. *Nature*,
708   428(6978):37–43.

709 Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. a., Wu, D.,
710   Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W.,
711   Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch,
712   C., Rogers, Y.-H., and Smith, H. O. (2004). Environmental genome shotgun sequencing of the
713   Sargasso Sea. *Science*, 304(5667):66–74.

714 Wetzel, C. E., de Bicudo, D. C., Ector, L., Lobo, E. A., Soininen, J., Landeiro, V. L., and Bini,
715   L. M. (2012). Distance Decay of Similarity in Neotropical Diatom Communities. *PLoS ONE*,
716   7(9):e45071.

717 Yang, Z. and Khangaonkar, T. (2010). Multi-scale modeling of Puget Sound using an unstructured-
718   grid coastal ocean model: From tide flats to estuaries and coastal waters. *Ocean Dynamics*,
719   60(6):1621–1637.

720   Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). PEAR: A fast and accurate Illumina

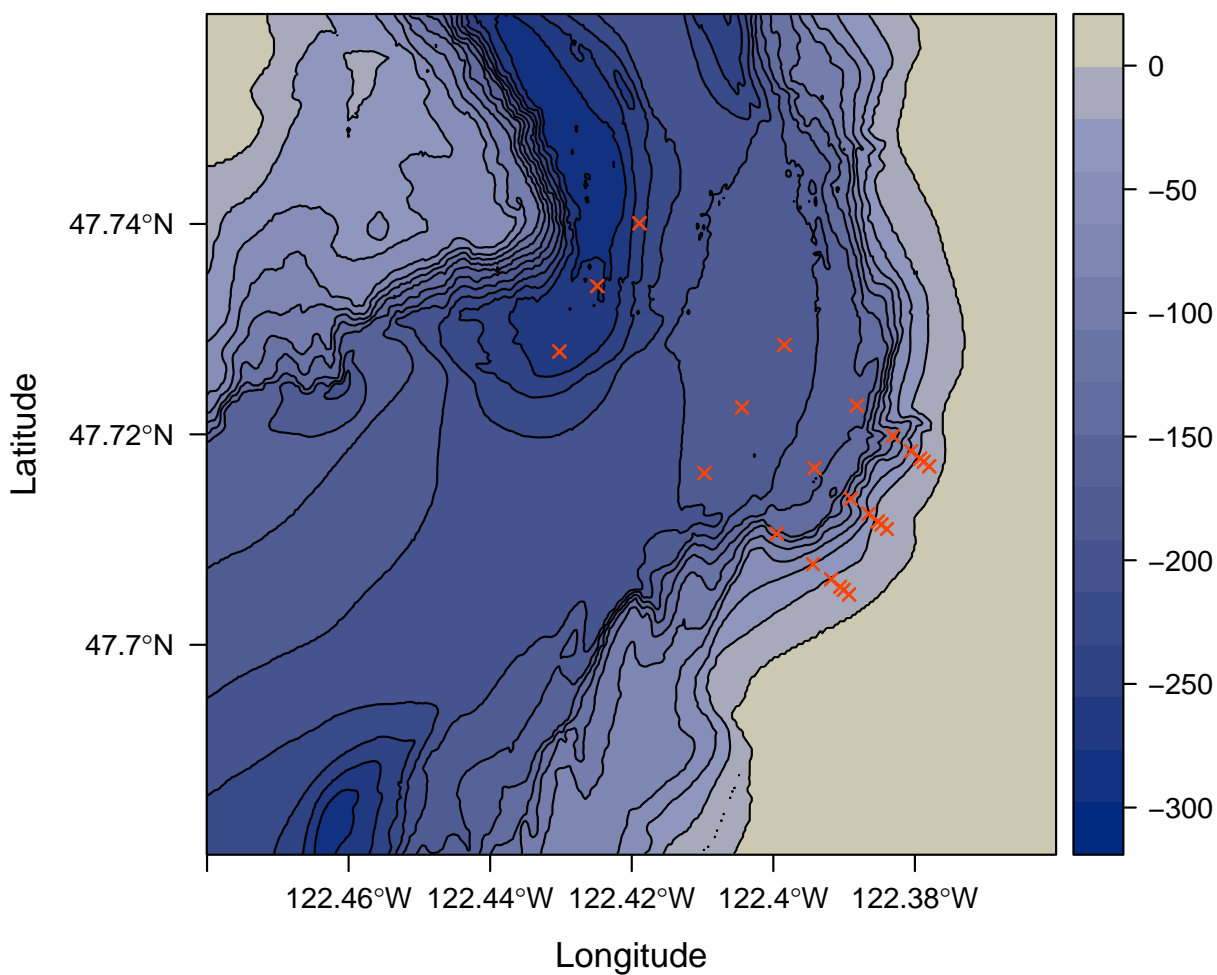721   Paired-End reAd mergeR. *Bioinformatics*, 30(5):614–620.

Figure 1: Map of study area. Depth in meters below sea level is indicated by shading and 25 meter contours. Sampled locations are indicated by red points.
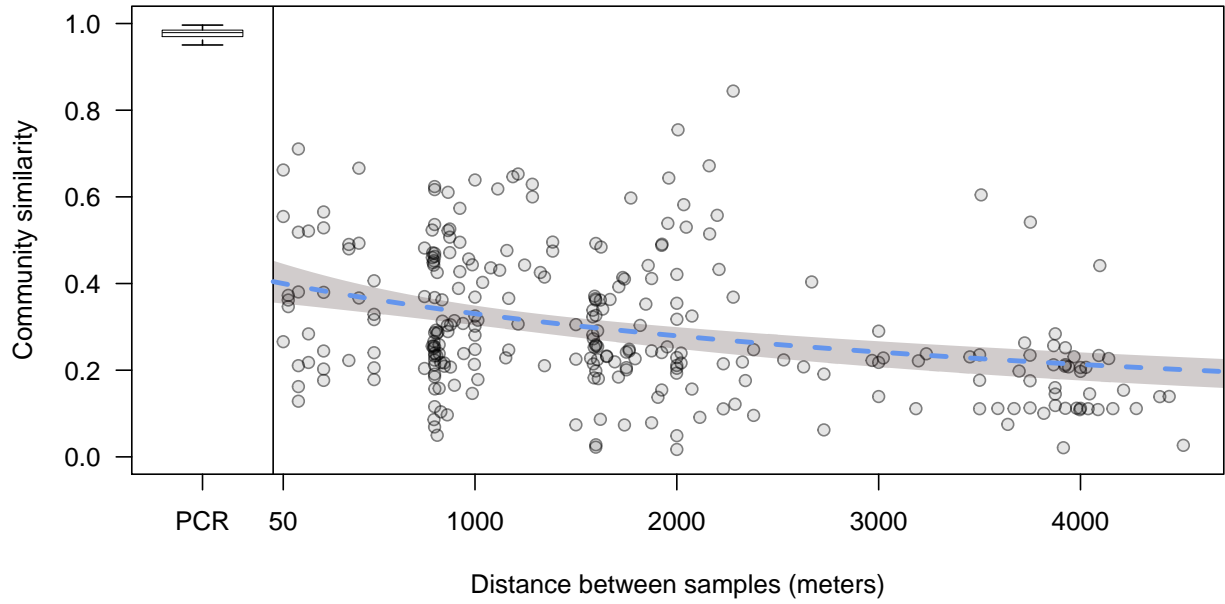
Figure 2: Distance decay relationship of environmental DNA communities. Each point represents the Bray-Curtis similarity of a site sampled along three parallel transects comprising a 3000 by 4000 meter grid. Blue dashed line represents fit of a nonlinear least squares regression (see Methods), and shading denotes the 95% confidence interval. Boxplot is comparisons within-sample across PCR replicates, separated by a vertical line at zero, where the central line is the median, the box encompasses the interquartile range, and the lines extend to 1.5 times the interquartile range. Boxplot outliers are omitted for clarity.
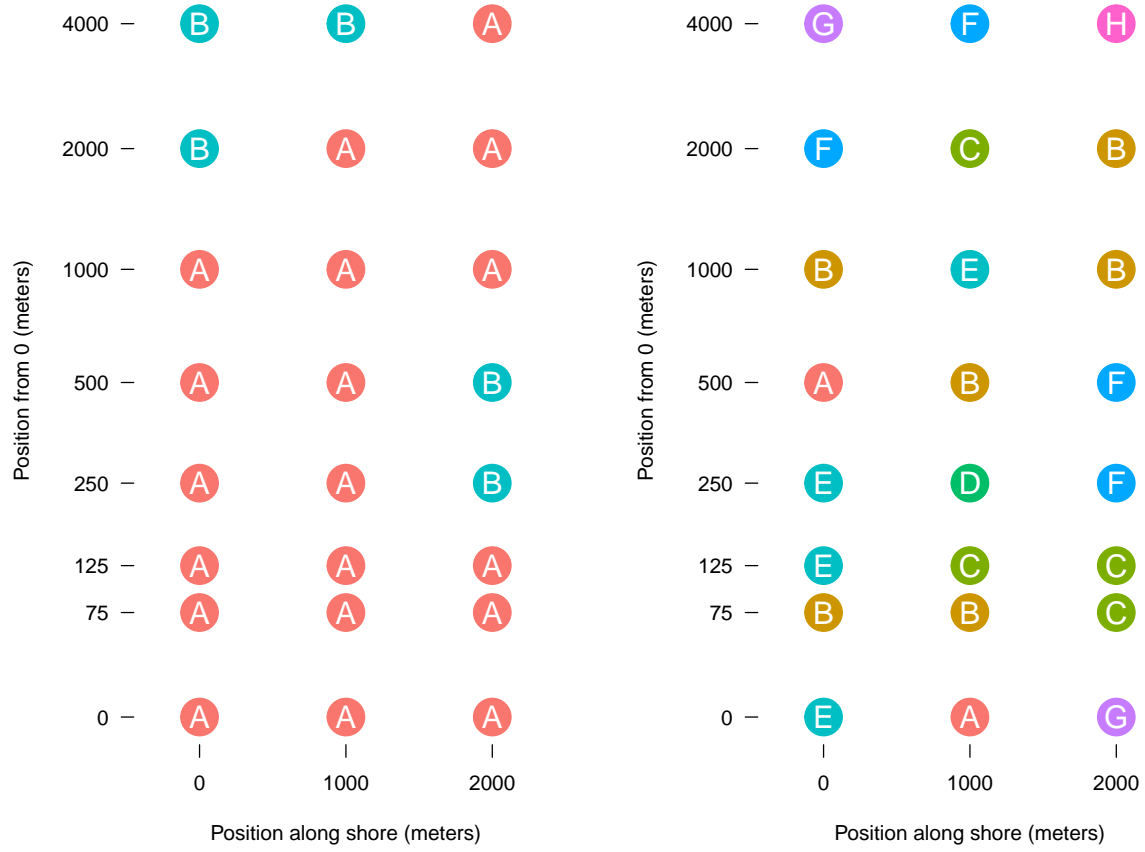
Figure 3: Cluster membership of sampled sites. Distance from onshore starting point is log scaled. Sites are colored and labeled by their assignment to a cluster by PAM analysis for number of clusters (K) chosen based on a priori expectations (2) and mean silhouette width (8).
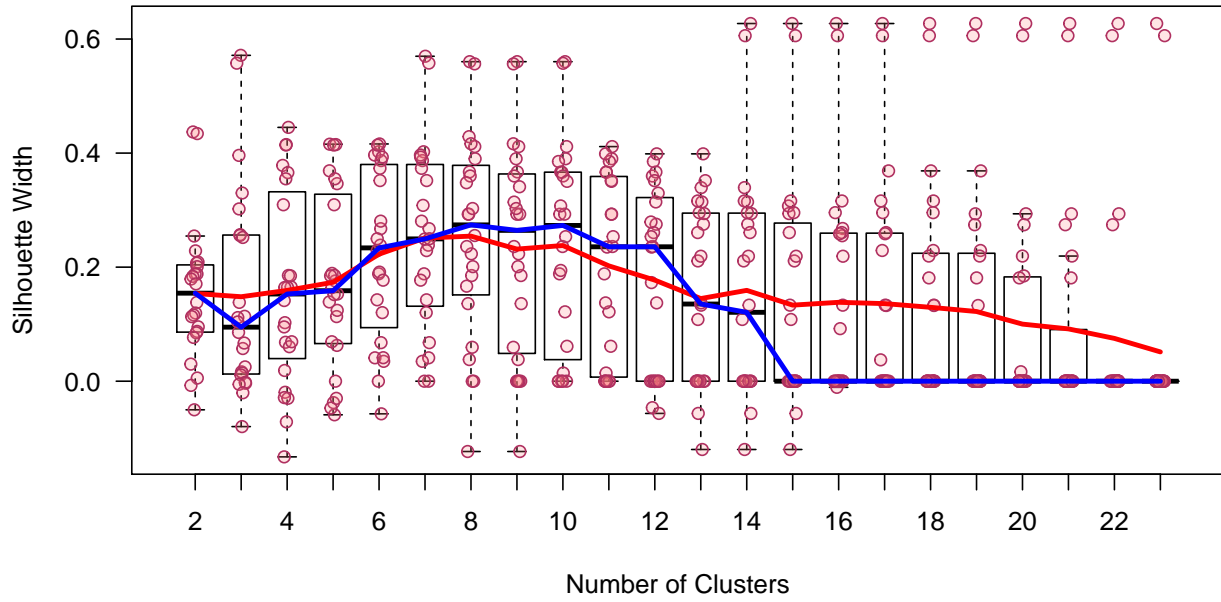
Figure 4: Silhouette widths from PAM analysis. Points are the width of the PAM silhouette of each sample at each number of clusters (K). Red line is the mean, blue line is the median. Boxes encompass the interquartile range with a line at the median, and the whiskers extend to 1.5 times the interquartile range. Boxplot outliers are omitted for clarity.
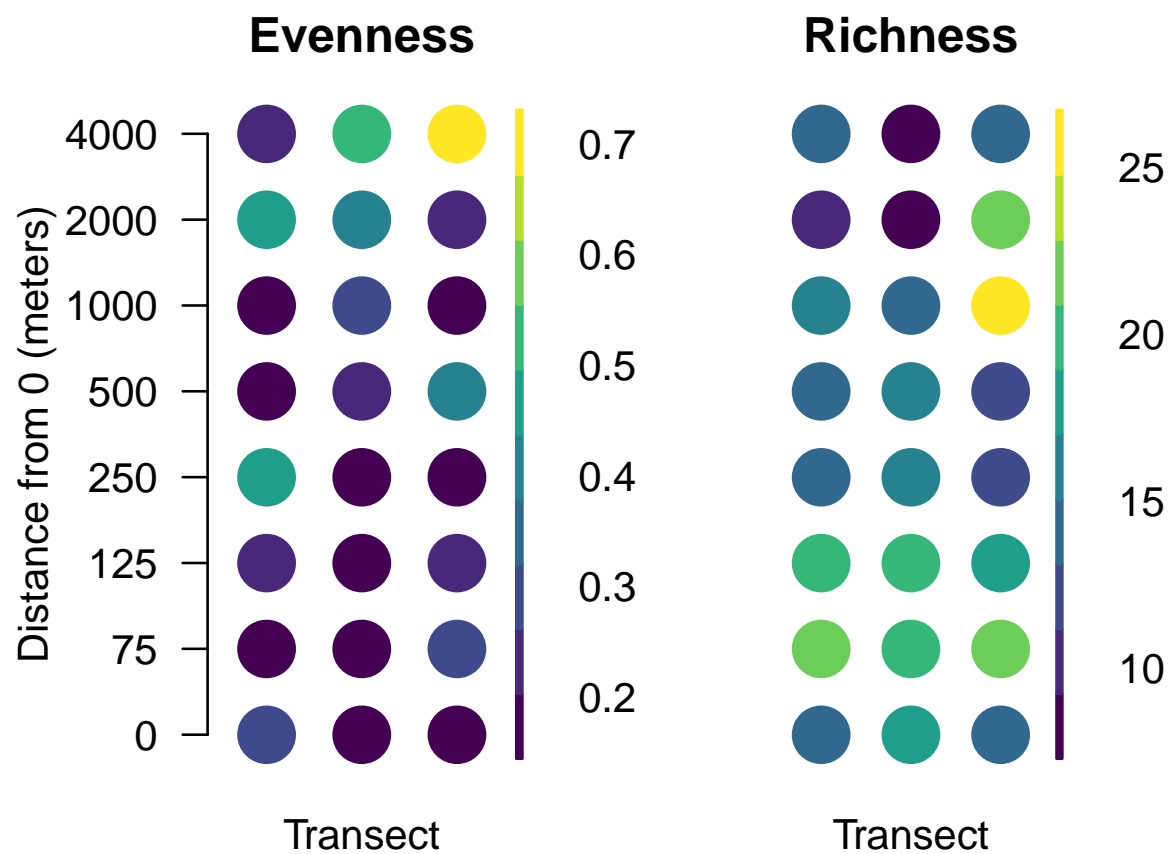
Figure 5: Aggregate measures of diversity at each sample site. Data are rarefied counts of mitochondrial 16S sequences collected from 3 parallel transects in Puget Sound, Washington, USA. Evenness (left) is the probability that two sequences drawn at random are different; richness (right) represents the total number of unique sequences from that location.
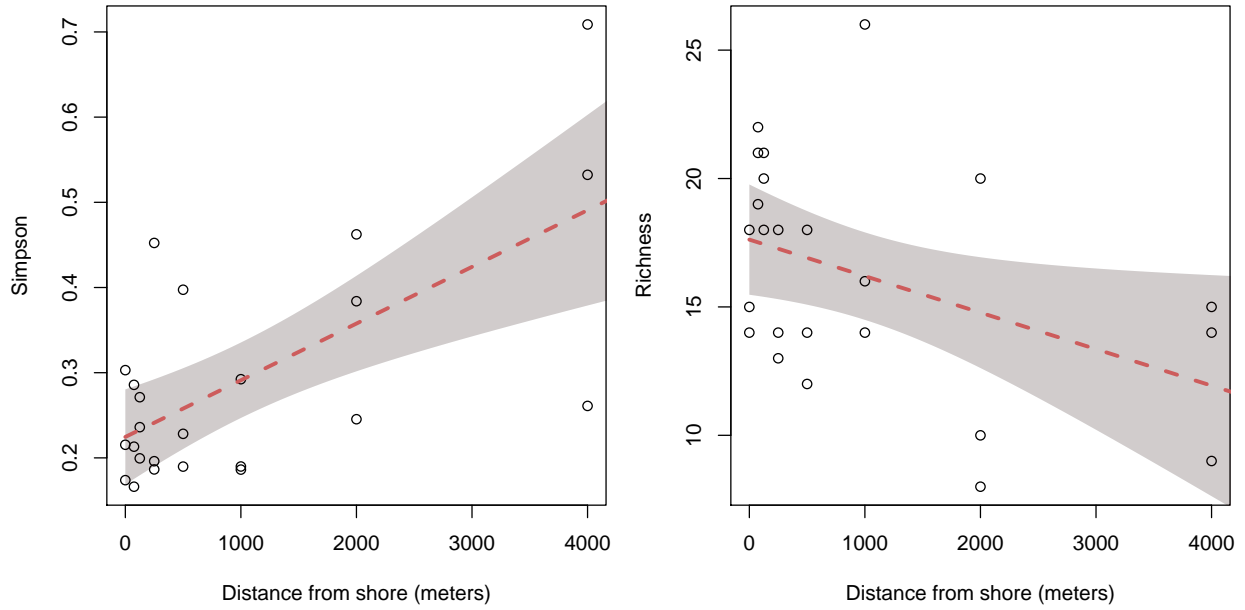
Figure 6: Aggregate diversity metrics of each site plotted against distance from shore. Both Simpson's Index (left) and richness (right) are shown, and have been computed from the mean abundance of unique DNA sequences found across 4 PCR replicates at each of 24 sites. Lines and bands illustrate the fit and 95
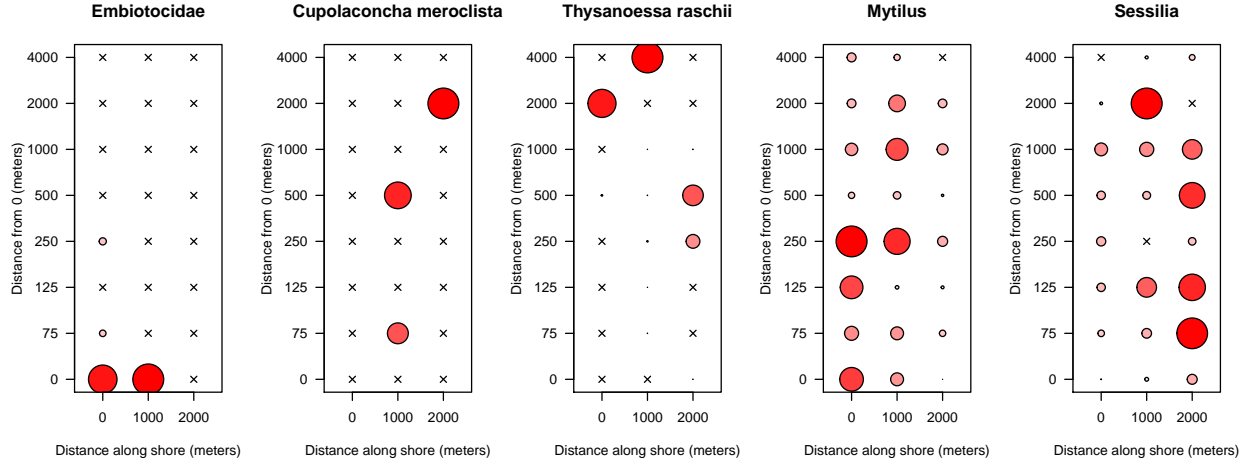
Figure 7: Distribution of eDNA from select taxa. Circles are colored and scaled by the proportion of that taxon's maximum proportional abundance. That is, the largest circle is the same size in each of the panels, and occurs where that taxon contributed the greatest proportional abundance of reads to that sample.

Supplemental Material

Methods

Bioinformatics

726 Reads passing the preliminary Illumina quality filter were demultiplexed on the basis of the adapter

727 index sequence by the sequencing facility. We used fastqc to assess the fastq files output from the

728 sequencer for low-quality indications of a problematic run. Forward and reverse reads were merged

729 using PEAR v0.9.6 Zhang et al. (2014) and discarded if more than 0.01 of the bases were uncalled.

730 If a read contained two consecutive base calls with quality scores less than 15 (i.e. probability of

731 incorrect base call = 0.0316), these bases and all subsequent bases were removed from the read.

732 Paired reads for which the probability of matching by chance alone exceeded 0.01 were not assembled

733 and omitted from the analysis. Assembled reads were discarded if assembled sequences were not

734 between 50 and 168 bp long, or if reads did not overlap by at least 100 bp.

735 We used vsearch v2.1.1 (Rognes et al., 2016) to discard any merged reads for which the sum of the

736 per-base error probabilities was greater than 0.5 ("expected errors") Edgar (2010). Sequences were

737 demultiplexed on the basis of the primer index sequence at base positions 4-9 at both ends using the

738 programming language AWK. Primer sequences were removed using cutadapt v1.7.1 Martin (2011),

739 allowing for 2 mismatches in the primer sequence. Identical duplicate sequences were identified,

740 counted, and removed in python to speed up subsequent steps by eliminating redundancy, and

741 sequences occurring only once were removed. We checked for and removed any sequence likely to be

742 a PCR artifact due to incomplete extension and subsequent mis-priming using a method described

743 by Edgar (2010) and implemented in vsearch v2.0.2. Sequences were clustered into operational

744 taxonomic units (OTUs) using the single-linkage clustering method implemented by swarm version

745 2.1.1 with a local clustering threshold (d) of 1 and fastidious processing (Mahé et al., 2014).

746 Cross-contamination of environmental, DNA, or PCR samples can result in erroneous inference

747 about the presence of a given DNA sequence in a sample. However, other processes can contribute

748 to the same signature of contamination. For example, errors during oligonucleotide synthesis or

749 sequencing of the indexes could cause reads to be erroneously assigned to samples. The frequency

750 of such errors can be estimated by counting the occurrence of sequences known to be absent from

751 a given sample, and of reads that do not contain primer index sequences in the expected position

752 or combinations. These occurrences indicate an error in the preparation or sequencing procedures.

753 We estimated a rate of incorrect sample assignment by calculating the maximum rate of occur-

754 rence of index sequences combinations we did not actually use, as well as the rates of cross-library

755 contamination by counting occurrences of primer sequences from 12S amplicons prepared in a lab

756 more than 1000 kilometers away, but pooled and sequenced alongside our samples. This represents

757 a general minimum rate at which we can expect that sequences from one environmental sample

758 could be erroneously assigned to another, and so we considered for further analysis only those reads

759 occurring with greater frequency than this across the entire dataset.

760   We checked for experimental error by evaluating the Bray-Curtis similarity (1 - Bray-Curtis

761 dissimilarity) among replicate PCRs from the same DNA sample. We calculated the mean and

762 standard deviation across the dataset, and excluded any PCR replicates for which the similarity

763 between itself and the other replicates was less than 1.5 standard deviations from the mean.

764   To account for variation in the number of sequencing reads (sequencing depth) recovered per

765 sample, we rarefied the within-sample abundance of each OTU by the minimum sequencing depth

766 (Oksanen et al., 2016).

767   Because each step in this workflow is sensitive to contamination, it is possible that some se-

768 quences are not truly derived from the environmental sample, and instead represent contamination

769 during field sampling, filtration, DNA extraction, PCR, fragment size selection, quantitation, se-

770 quencing adapter ligation, or the sequencing process itself. We take the view that contaminants

771 are unlikely to manifest as sequences in the final dataset in consistent abundance across replicates;

772 indeed, our data show that the process from PCR onward is remarkably consistent. Thus, after

773 scaling to correct for sequencing depth variation, we calculated from our data the maximum number

774 of sequence counts for which there is turnover in presence-absence among PCR replicates within an

775 environmental sample. We use this number to determine a conservative minimum threshold above

776 which we can be confident that counts are consistent among replicates and not of spurious origin,

777 and exclude from further analysis observations where the mean abundance across PCR replicates

778 within samples does not reach this threshold. For further analyses we use the mean abundance

779 across PCR replicates for each of the 24 environmental samples.

780   In order to determine the most likely taxon from which each sequence originated, the representa-

781 tive sequence from each OTU was then queried against the NCBI nucleotide collection (GenBank;

782 version October 7, 2015; 32,827,936 sequences) using the blastn command line utility (Camacho

783 et al., 2009). In order to maximize the accuracy of this computationally intensive step, we imple-

784 mented a nested approach whereby each sequence was first queried using strict parameters (e-value

785 = 5e-52), and if no match was found, the query was repeated with decreasingly strict e-values (5e-48

786 5e-44 5e-40 5e-36 5e-33 5e-29 5e-25 5e-21 5e-17 5e-13). Other parameters were unchanged among

787 repetitions (word size: 7; maximum matches: 1000; culling limit: 100; minimum percent identity:

788 0). Each query sequence can be an equally good match to multiple taxa either because of invariabil-

789 ity among taxa or errors in the database (e.g. human sequences are commonly attributed to other

790 organisms when they in fact represent lab contamination). In order to guard against these spurious

791 results, we used an algorithm to find the lowest common taxon for at least 80% of the matched

792 taxa, implemented in the R package taxize 0.7.8 (Chamberlain and Szöcs, 2013; Chamberlain et al.,

793 2016). Similarly, we repeated analyses using the dataset consolidated at the same taxonomic rank

794 across all queries, for the rank of both family and order.

795 **Alternative distance decay model formulations**

796 **Linear:** We fit a straight line through the points after log-transforming the spatial distances

797 to estimate the intercept and slope. This model ignores the bounds of our response variable of

798 community similarity.

799 **Michaelis-Menten:** We fit a Michaelis-Menten-like curve to our data. Our formulation can be

800 thought of as a modification of the Michaelis-Menten equation, but with the addition of a parameter

801 in the numerator which modifies the intercept.

$$y = \frac{AB + Cx}{B + x} \tag{2}$$

802 Where C is the asymptote of minimum similarity. This formulation allows us to estimate the

803 maximum similarity in the system, and the rate at which it is achieved. If the value of the parameter

804 (AB) is 0 (i.e. if the intercept is 0), the form is identical to the Michaelis-Menten equation:

$$y = \frac{Cx}{B + x} \tag{3}$$

805   This is conceptually satisfying in that a fit through [0,1] reflects the theoretical expectation that

806 samples at zero distance from one another are necessarily identical. Given an efficient sampling

807 technique, replicate samples taken at the same position in space should be identical, and thus the

808 intercept of the regression of similarity against distance should be 1, and deviation from 1 is an

809 indicator of the efficiency of the sampling method.

810   Finally, we considered a model which estimates an asymptote as the total change in similarity

811 (D):

$$y = \frac{A + Dx}{B + x} \tag{4}$$

812   However, this model failed to converge and produced uninformative estimates of all parameters.
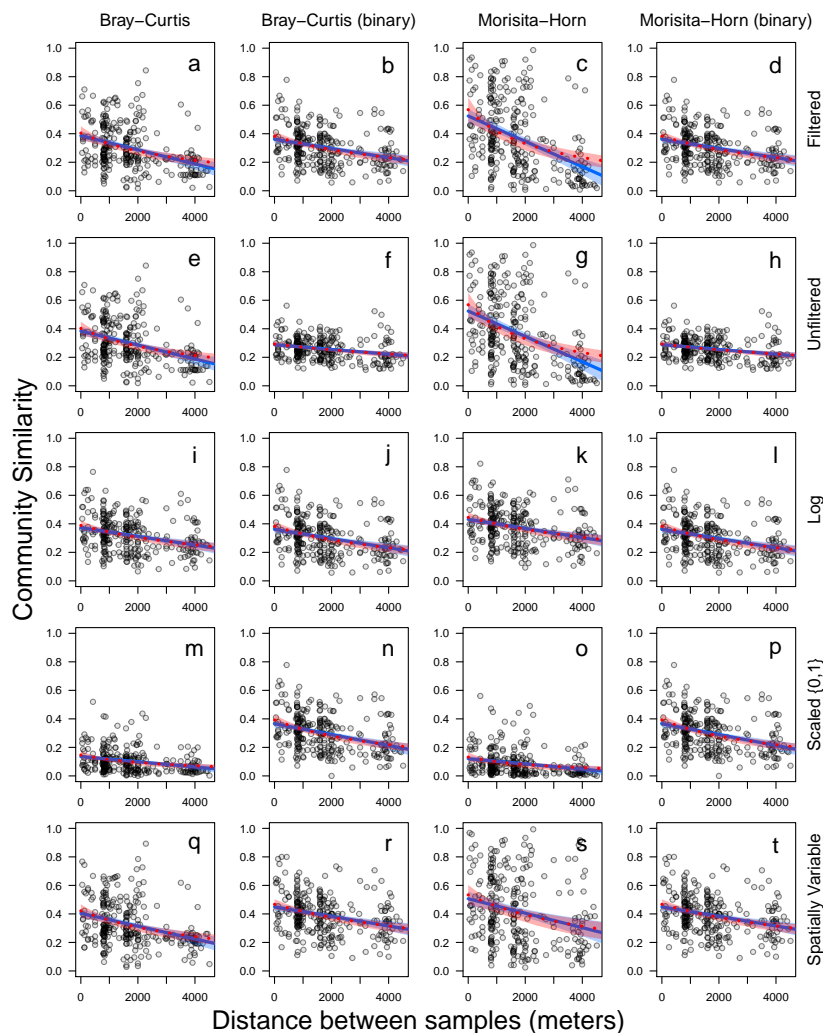
Supplemental Figures

Figure 8: Distance decay relationship of environmental DNA communities using a variety of models, metrics, and data subsets. Each point represents the similarity of a site sampled along three parallel transects comprising a 3000 by 4000 meter grid. Each row of plots represents a different data subset indicated in the right margin, including the final filtered data reported in the main text (a-d), the unfiltered data including all rare OTUs (e-h), log-transformed (log(x+1)) data (i-l), OTU abundance scaled relative to within-taxon maximum (m-p), and exclusion of OTUs found at only one site (q-t). Columns indicate the similarity index used (Bray Curtis or Morisita-Horn) and whether the input was full abundance data or binary (0,1) transformed data. Lines and bands illustrate the fit and 95% confidence interval of both the main nonlinear model (red, dashed line) and a simple linear model (blue, solid line). Results using the Jaccard distance are omitted because of their similarity to Bray-Curtis.
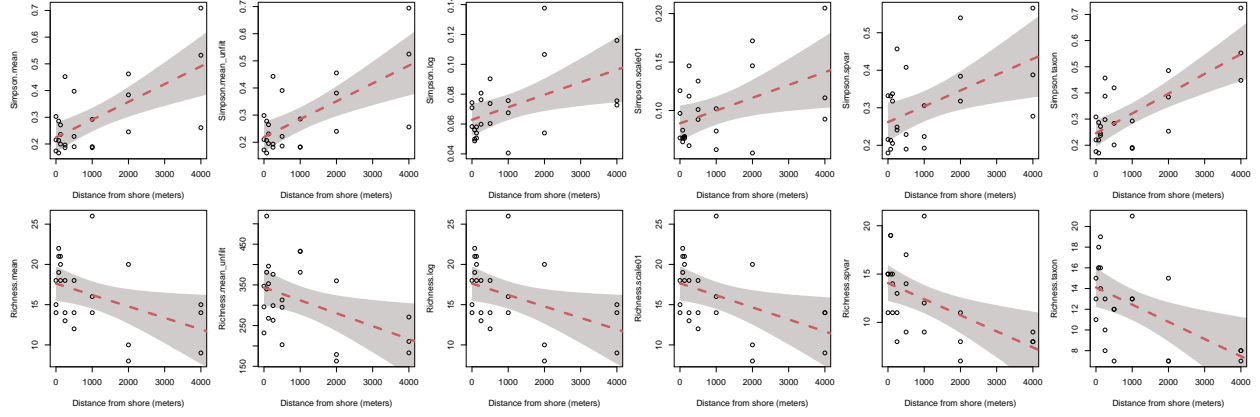
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Figure 9: Aggregate diversity metrics of each site plotted against distance from shore. Both Simpson's Index (top) and richness (bottom) are shown for a variety of data subsets and transformations (left to right: mean, unfiltered mean, $\log(x + 1)$, transformed, scaled, spatially variable, and taxon clustered). Lines and bands illustrate the fit and 95% confidence interval of a linear model. See methods text for detailed data descriptions.
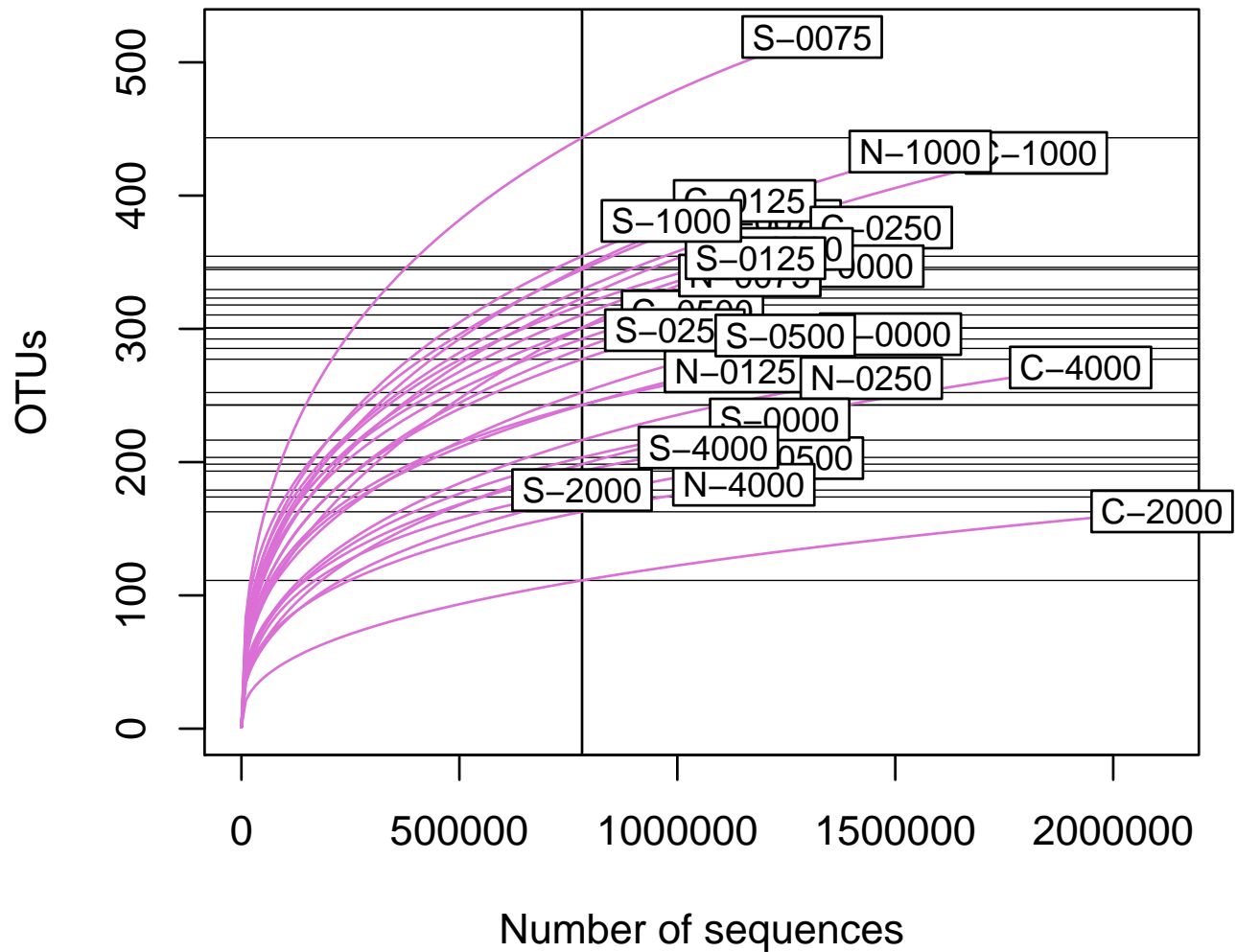
Figure 10: Accumulation of OTUs from 24 environmental samples using randomized rarefaction. Four replicate PCRs were conducted using DNA each environmental sample and independently sequenced, but these are collapsed here to illustrate a single representation of richness. Sample names indicate the position in the sampling grid: south (S), central (C), or north (N), followed by the distance along the transect, in meters (0, 75, 125, 250, 500, 1000, 2000, 4000). Vertical line indicates the minimum combined number of sequence reads per sample. Horizontal lines indicate OTU richness for each sample at the minimum combined number of sequence reads.