Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA

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Abstract

Preserving biodiversity is a global challenge requiring data on species’ distribution and abundance over large geographic and temporal scales. However, traditional methods to survey mobile species’ distribution and abundance in marine environments are often inefficient, environmentally destructive, or resource-intensive. Metabarcoding of environmental DNA (eDNA) offers a new means to assess biodiversity and on much larger scales, but adoption of this approach for surveying whole animal communities in large, dynamic aquatic systems has been slowed by significant unknowns surrounding error rates of detection and relevant spatial resolution of eDNA surveys. Here, we report the results of a 2.5 km eDNA transect surveying the vertebrate fauna present along a gradation of diverse marine habitats associated with a kelp forest ecosystem. Using PCR primers that target the mitochondrial 12S rRNA gene of marine fishes and mammals, we generated eDNA sequence data and compared it to simultaneous visual dive surveys. We find spatial concordance between individual species’ eDNA and visual survey trends, and that eDNA is able to distinguish vertebrate community assemblages from habitats separated by as little as ~60 m. eDNA reliably detected vertebrates with low false-negative error rates (1/12 taxa) when compared to the surveys, and revealed cryptic species known to occupy the habitats but overlooked by visual methods. This study also presents an explicit accounting of false negatives and positives in metabarcoding data, which illustrate the influence of gene marker selection, replication, contamination, biases impacting eDNA count data and ecology of target species on eDNA detection rates in an open ecosystem.

Keywords: biodiversity, eDNA, fish, metabarcoding, monitoring, next generation sequencing

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Introduction

Environmental conservation, management and basic ecology require information on species distributions and trends in abundance. However, for many species and ecosystems—especially in freshwater and marine environments and for mobile organisms—practical methods to monitor biodiversity are often inefficient (e.g. visual surveys), environmentally destructive (e.g. trawls) or require significant person-time and resources (Jones 1992; Baldwin et al. 1996; Wheeler et al. 2004). Furthermore, given the continuous decline in global biodiversity (Butchart et al. 2010), a sampling technique that targets communities instead of individual species—and over larger geographic and temporal scales—could enable more comprehensive management and greatly improved resolution for core ecological research.

Environmental DNA (eDNA) offers a high-throughput, potentially cheaper, vastly more sensitive, and less
invasive approach to survey biodiversity than conventional methods. eDNA is genetic material obtained directly from environmental samples (water, sediment, soil, etc.) that is derived from microbes or shed from multicellular organisms (Taberlet et al. 2012). While eDNA has commonly been used to assess microbial diversity and abundance (Venter et al. 2004; Rusch et al. 2007), only recently has the technique been used to survey higher eukaryotes including fishes (Thomsen et al. 2012a, b; Jerde et al. 2013), mammals (Andersen et al. 2012; Foote et al. 2012), amphibians (Ficetola et al. 2008; Pilliod et al. 2014) and invertebrates (Goldberg et al. 2013; Deiner & Altermatt 2014; Mächler et al. 2014). The majority of these macrobial eDNA investigations to date have been species-specific, but multi-species PCR in combination with high-throughput sequencing (i.e. metabarcoding) can reveal whole-community eDNA. eDNA metabarcoding has been used for such applications as characterizing animal diet content (Deagle et al. 2010; Shehzad et al. 2012), screening for the presence of invasive species in the bait trade (Mahon et al. 2014), and to a lesser extent biodiversity profiling (Thomsen et al. 2012a, b), but uses of this technique for field ecology and conservation have lagged due to unknown error rates of detection and spatial resolution of eDNA in the field.

Small volumes of water contain eDNA sufficient to reliably detect target organisms—including invasive, endangered and rare species—in freshwater environments (Jerde et al. 2011, 2013; Thomsen et al. 2012b; Goldberg et al. 2013; Takahara et al. 2013; Mächler et al. 2014; Laramie et al. 2015; Spear et al. 2015), but few such marine studies exist (Foote et al. 2012; Thomsen et al. 2012a; Kelly et al. 2014; Miya et al. 2015). The ocean imposes an additional set of physical and chemical constraints affecting eDNA distribution and detection probability; currents, tides, wind and salinity all impact eDNA degradation rates and persistence in seawater (Thomsen et al. 2012a; Barnes et al. 2014). Nevertheless, where organisms’ generation of eDNA outpaces the combined forces of degradation and transport we expect to see a significant biological signal of species living in sampled habitats.

Here, we present data from a field survey of marine vertebrates in a coastal ecosystem in Monterey Bay, CA using both eDNA and conventional visual methods. The study area spanned a diversity of distinct habitat types, including kelp forests, which are highly productive epicentres of biodiversity and are quintessential sites of marine ecological research and conservation (Dayton 1985; Steneck et al. 2002). Our objectives were: (i) to compare the performance of eDNA relative to conventional visual surveys, (ii) to test for trends in abundance of taxon-specific eDNA across multiple habitats, (iii) to compare eDNA community composition among habitats and (iv) to estimate the relevant spatial scale of community-level eDNA sampling.

Materials and methods

Visual fish surveys

We conducted visual fish SCUBA surveys and collected seawater samples within the Lovers Point-Julia Platt State Marine Reserve in Monterey Bay adjacent to Hopkins Marine Station of Stanford University on November 13, 2013 (Fig. 1). The 12 survey sites were located within a 2.5 km cross-shore swath that spanned a depth range of 2–70 m and encompassed a diverse range of habitat types including Phyllospadix spp. beds (seagrass), Macrocystis pyrifera dominated rocky reef (kelp forest), deeper rocky reef free of M. pyrifera (rocky reef) and sandy bottom (Table 1). Within each habitat type, surveys and water sampling were conducted at multiple locations, which in some cases represented distinct microhabitats. Open water sites were outside of safe diving limits and thus only surface water was taken as it could be compared with well-studied pelagic communities. Surveys and sampling were performed over a 4-h window from 9:30 to 13:30 (local time). Sites were surveyed using a modified version of the roving diver technique (Schmitt & Sullivan 1996) where experienced divers trained in visual surveys for these habitats (Thompson & Mapstone 1997) ‘roved’ a given site for a predefined period (10 min) while maintaining a constant depth and staying within the prescribed habitat type. Fish were identified at the family level and sized to the nearest 10 cm. Estimated abundance was recorded in one of four log_{10} categories: single (1), few (2–10), many (11–100) and common (101–1000). This technique has been widely used to provide a rapid assessment of the abundance and spatial distribution of fish taxa and has proven robust at providing data on relative abundance and identifying rare species (Schmitt et al. 2002), although cryptic and highly mobile species are probably underrepresented. For data analysis, the median count values for each abundance category were used.

Sample collection

At the midpoint of each survey, divers collected a 3-L water sample at 1 m above the bottom for eDNA using a collapsible plastic bottle (Cubitainer LDPE insert, Hedwin Co., USA). The collection bottles were purchased new for this study and acid washed before use, and each bottle was assigned to a separate sampling site. In addition, water samples were collected at 1 m depth at three sites offshore of the visually surveyed
Table 1 Site-specific survey details and associated metadata

| Site name | Habitat description                  | Latitude (N) | Longitude (W) | Distance from shore (m) | Depth (m) | Species richness (eDNA) |
|-----------|-------------------------------------|--------------|---------------|-------------------------|-----------|-------------------------|
| SGin      | Inner seagrass                      | 36°37′13.82″ | 121°54′11.01″ | 44                      | 2         | 16.7 ± 0.6              |
| SGout     | Outer seagrass                      | 36°37′14.65″ | 121°54′8.82″  | 97                      | 3         | 11.0 ± 1.0              |
| KFin      | Inner kelp forest                   | 36°37′17.72″ | 121°54′9.29″  | 140                     | 5         | 14.0 ± 1.0              |
| KFmid     | Mid kelp forest                     | 36°37′19.5″  | 121°54′7.85″  | 206                     | 9         | 13.0 ± 1.7              |
| KFout     | Outer kelp forest                   | 36°37′19.85″ | 121°54′4.932″ | 262                     | 12        | 11.3 ± 1.2              |
| SBshallow | Shallow sandy bottom                | 36°37′22.31″ | 121°54′2.71″  | 355                     | 18        | 8.0 ± 1.0               |
| RR1       | Rocky reef                          | 36°37′19.74″ | 121°53′57.24″ | 421                     | 21        | 3.7 ± 0.6               |
| RR2       | Rocky reef                          | 36°37′24.84″ | 121°53′59.29″ | 470                     | 24        | 4.7 ± 0.6               |
| SBdeep    | Deep sandy bottom                   | 36°37′28.48″ | 121°53′56.68″ | 596                     | 30        | 9.7 ± 1.5               |
| OW1       | Open water                          | 36°37′37.77″ | 121°53′46.54″ | 978                     | 48        | 5.3 ± 0.6               |
| OW2       | Open water                          | 36°37′49.87″ | 121°53′35.72″ | 1436                    | 55        | 5.7 ± 1.1               |
| OW3       | Open water                          | 36°38′29.28″ | 121°53′19.92″ | 2665                    | 70        | 3.0 ± 0.0               |

1Sampling depth was 1 m from the bottom for all sites except the open water locations where samples were taken 1 m from the surface.

2Mean observed species richness ± SD for the three filter replicates per site.
filtration blanks (1-L deionized water run through blank filters) \((n = 3)\) were included to monitor for contamination.

**Laboratory environment**

Sample processing was performed in a laboratory (A. Boehm laboratory, Stanford University) predominantly used for bacterial work. Benchtops were cleaned with 10% bleach and pipettes UV-irradiated and wiped with RNASE AWAY before beginning any molecular work. Filter tips were used for pipetting to further reduce contamination risks. We employed rigorous controls to monitor for contamination at each step of the process, including field, filtration, extraction and PCR blanks. DNA extractions were conducted on a dedicated bench, separated from PCR and post-PCR work. PCR mastermixes were prepared in a DNA-free hood and DNA template from PCR and post-PCR work. PCR mastermixes were modified by the addition of specific tags on the 5’ ends to allow for the assignment of sequence reads to the correct sample during bioinformatic processing (Valentini et al. 2009). Tags were designed using the Oligotag program (Coissac 2012) and consisted of six nucleotides with a Hamming distance of at least three bases between tags. Tags were preceded by \(\text{NNN} \) (De Barba et al. 2014), and the forward and reverse primers for a given sample had identical tags. PCR reactions were carried out using 5 \(\mu\)L DNA extract (1:10 dilutions), 12.5 \(\mu\)L HotStarTaq Plus Master Mix (Qiagen, USA), 1 \(\mu\)L of each primer (10 \(\mu\)M) and 5.5 \(\mu\)L molecular-biology-grade water (Sigma-Aldrich, USA). Eight-strip PCR tubes with individually attached lids were used instead of 96-well plates to reduce cross-contamination between samples. Thermal conditions for PCR were 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. Four replicate PCR assays were performed for each filter replicate \((n = 54)\) and then pooled. A no template control (NTC) was included for each PCR replicate to account for mastermixes with different tagged primer sets. The pooled PCR products were run through a 1.5% agarose gel stained with ethidium bromide to confirm the presence of the 12S target band and clean NTCs and absence of any nonspecific amplification. All NTCs were negative. PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA) and then quantified using the Qubit dsDNA HS Assay (Invitrogen, CA, USA).

**DNA extraction**

We extracted DNA from each filter using the PowerWater DNA Isolation Kit (MoBio Laboratories, CA, USA). Extraction blanks \((n = 9)\) were included for all extractions and run in subsequent PCRs. We also extracted triplicate positive controls consisting of tissue from swordfish, *Xiphius gladius* (DNeasy Blood and Tissue Kit; Qiagen, USA), and amplified these to monitor for the presence of false positives during the PCR and sequencing steps. Two additional positive controls for sequencing included a mix of total DNA extract from 10 species of bony fishes in equimolar concentration (mock community 1), and a mix of total DNA from the same fishes in increasing concentration (mock community 2) (Table S1, Supporting information). Tissue extractions were performed 3 months prior to the filter extractions. DNA extract concentrations were determined using the Qubit dsDNA HS Assay (Invitrogen, CA, USA).

**PCR amplification**

We used a published vertebrate-specific primer set targeting a small region of the mitochondrial DNA 12S rRNA gene (Riaz et al. 2011). Primer sequences were F-5’ ACTGGGATTAGATACCC and R-5’ TAGAA- CAGGCTCCTCTAG, amplifying a ca. 106-bp gene fragment. We previously validated this primer set in a seawater mesocosm study and found a low false-negative rate for bony fishes but high false-negative rate for cartilaginous fishes (Kelly et al. 2014). These primers were modified by the addition of specific tags on the 5’ ends to allow for the assignment of sequence reads to the correct sample during bioinformatic processing (Valentini et al. 2009). Tags were designed using the Oligotag program (Coissac 2012) and consisted of six nucleotides with a Hamming distance of at least three bases between tags. Tags were preceded by \(\text{NNN} \) (De Barba et al. 2014), and the forward and reverse primers for a given sample had identical tags. PCR reactions were carried out using 5 \(\mu\)L DNA extract (1:10 dilutions), 12.5 \(\mu\)L HotStarTaq Plus Master Mix (Qiagen, USA), 1 \(\mu\)L of each primer (10 \(\mu\)M) and 5.5 \(\mu\)L molecular-biology-grade water (Sigma-Aldrich, USA). Eight-strip PCR tubes with individually attached lids were used instead of 96-well plates to reduce cross-contamination between samples. Thermal conditions for PCR were 95 °C for 5 min followed by 40 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. Four replicate PCR assays were performed for each filter replicate \((n = 54)\) and then pooled. A no template control (NTC) was included for each filter replicate to account for mastermixes with different tagged primer sets. The pooled PCR products were run through a 1.5% agarose gel stained with ethidium bromide to confirm the presence of the 12S target band and clean NTCs and absence of any nonspecific amplification. All NTCs were negative. PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA) and then quantified using the Qubit dsDNA HS Assay (Invitrogen, CA, USA).

**Library prep and DNA sequencing**

Tagged PCR products for the 54 samples were pooled in equimolar concentration (20 ng DNA per sample). The concentration of the final pool was 2.33 ng/\(\mu\)L. If a sample did not have at least 20 ng of DNA (e.g., blanks), the entire amount of sample DNA available
was added to the pool. 150 ng of DNA from the pool was used for library preparation. Library construction for Illumina sequencing followed the KAPA low-throughput library prep kit with real-time library amplification protocol (KAPA Biosystems, MA, USA) in combination with a single NEXTFLEX DNA barcode (BIOO Scientific, TX, USA) containing the Illumina adapter sequence. A single library was prepared for all tagged PCR products across all samples. Library size and concentration were assessed using a Bioanalyser with High Sensitivity DNA assay (Agilent Technologies, CA, USA). Sequencing was carried out at the Stanford Functional Genomics Facility on an Illumina MiSeq platform using paired-end sequencing and a 20% PhiX spike-in control to improve the quality of low-diversity samples.

**Sequence analysis**

We employed stringent sequence and taxon filtering parameters in an effort to generate a high-confidence data set and to repeatedly classify true positives and remove false positives resulting from: (i) low-quality and spurious reads, (ii) low-confidence annotations and (iii) spurious annotations (Fig. 2). Bioinformatic analyses were implemented with a Unix shell script, which incorporates command line tools as well as calls to third-party software as follows: Pair-end reads were first merged with PEAR v0.9.2 (Zhang et al. 2014) using the following parameters: minimum overlap size = 100, maximum assembly length = 161, minimum assembly length = 151, quality score threshold = 15 and P-value = 0.01. Quality filtering was performed using the fastq_filter command in USEARCH v7.0.1090 (Edgar 2010) with the following parameters: expected number of errors per read = 0.5 and minimum sequence length = 154. Merged reads were demultiplexed by tag sequence using the programming language AWK. To minimize the presence of chimeric sequences and tag jumping, only those reads containing the same tag sequence at both the 5' and 3' ends were retained (Schnell et al. 2015). Reads with homopolymers >7 bases were also omitted. The forward and reverse primers were then removed from the demultiplexed reads using CUTFADAP v1.4.2 (Martin 2011) allowing for two mismatches in the primer sequence. USEARCH was used to dereplicate identical reads (–derep_fulllength), remove singletons (–sortbysize), and then cluster into operational taxonomic units (OTUs) at ≥99% identity while further removing chimeras (–cluster_otsu). OTUs were compared to a local nucleotide database containing mitochondrial sequences from the National Center for Biotechnology Information (NCBI) using BLAST+ (Camacho et al. 2009). This database—deposited in the Dryad Digital Repository—totalled 12 709 sequences and included the complete mitochondrial genomes as well partial 12S rRNA gene fragments of bony fishes (Actinopterygii), cartilaginous fishes (Chondrichthyes), true seals (Phocidae), sea lions (Otariidae), whales (Cetacea), marine dolphins (Delphinidae), sea otters (Enhydra) and birds (Aves) (sequences downloaded September 2014). Default BLAST parameters were used except for the following modifications: e-value ≤1e-20, per cent identity ≥98%, and word size = 24. Taxonomy was assigned to the most specific rank possible (generally family or genus) using the lowest common ancestor (LCA) algorithm in MEGAN v5.5.3 (default settings except for: min score = 150, top per cent = 2) (Huson & Weber 2013). Reads with no matches to sequences in our 12S database were not included in subsequent analyses but were annotated using BLAST against the full NCBI nucleotide database returning up to 500 hits per query sequence at an e-value threshold of ≤1e-20 to determine their probably identities. From these results, we assigned taxonomy from the hits with the lowest e-value at levels of ≤1e-40, ≤1e-35, ≤1e-30, ≤1e-25 and ≤1 e-20 (Table S2, Supporting information).

We identified two types of problematic annotations, which we considered to be ‘false’ positives: low-confidence annotations and spurious annotations. Low-confidence annotations are defined here as high-quality annotated reads passing the previous filtering steps that have high-confidence BLAST matches but may be inaccurate due to being (i) present at frequencies below the low-frequency noise threshold set by the positive controls or (ii) present in only one of the three filter replicates per sample. We sequenced the positive controls included in this experiment to establish filtering parameters for low-frequency noise (De Barba et al. 2014). We set a discard abundance, or low-frequency noise, threshold at 0.02%. This threshold represents the abundance of the spurious taxon (i.e. a taxon other than our control species) with the highest abundance in the positive controls, and for each sample, we discarded all taxa whose frequency was below 0.02% (Fig. 2). The criterion to exclude taxa if present in only one of the three filter replicates balanced the need to identify PCR/sequencing errors and contamination against the desire to detect low-concentration DNA; our analysis assumes that a taxon occurring in only one of three filter replicates is more likely the former than a true (but rare and therefore stochastic) signal. While excluded from the remaining analysis, the identities of low-confidence annotations (taxa and read counts) are shown in Fig. 2.

Spurious annotations are also high-quality reads passing the previous filtering steps that have high-confidence BLAST matches but are probably inaccurate due to being (i) present in the negative controls or (ii) exotic to the survey area and adjacent waters. We additionally
Fig. 2 Data analysis framework for processing the eDNA sequence reads. The 12 data-filtering steps to identify true (green) and false (red) positive assignments are listed along with the number of reads and taxa removed at each step. Steps 7-12 are presented in a decision tree framework to show the classification scheme of spurious annotations, low-confidence annotations, and true positives, as well as to list the taxa omitted at each step. 1Present in the negative controls (extraction, collection or filtration blanks) but removed prior to step 11. 2Probable laboratory contamination but removed prior to step 12. © 2015 The Authors. Molecular Ecology Published by John Wiley & Sons Ltd.

| Data filtering steps | No. of reads | No. of taxa |
|----------------------|--------------|-------------|
| 1) Total sequenced reads | 17 050 588 | -- |
| 2) Merging of paired-end reads | 12 646 715 | -- |
| 3) Fastq quality filtering | 12 634 367 | -- |
| 4) Primer and tag identification | 10 980 746 | -- |
| 5) Dereplication | 10 980 746 | -- |
| 6) Singleton removal | 9 294 094 | -- |
| 7) BLAST (percent identity ≥ 98% to 12S mtDNA database) | 7 836 900 | 99 |
| 8) Removal of positive and negative controls | 5 527 190 | 99 |
| 9) Removal of low-frequency noise | 5 526 777 | 60 |
| 10) Removal of taxa present in only 1 out of 3 filter replicates | 5 188 585 | 36 |
| 11) Removal of taxa present in the dataset after the negative control subtraction | 3 793 712 | 35 |
| 12) Removal of exotic taxa or reclassification to lower taxonomic rank | 3 163 287 | 33 |

**Data filtering steps**

- **1) Total sequenced reads**
- **2) Merging of paired-end reads**
- **3) Fastq quality filtering**
- **4) Primer and tag identification**
- **5) Dereplication**
- **6) Singleton removal**
- **7) BLAST (percent identity ≥ 98% to 12S mtDNA database)**
- **8) Removal of positive and negative controls**
- **9) Removal of low-frequency noise**
- **10) Removal of taxa present in only 1 out of 3 filter replicates**
- **11) Removal of taxa present in the dataset after the negative control subtraction**
- **12) Removal of exotic taxa or reclassification to lower taxonomic rank**

**Data analysis framework**

- **7) eDNA genus/family assignment (n = 99)**
  - Unassigned reads (Table S2)
  - **8) Positive and negative controls**
    - Relative abundance ≥ 0.02%
    - Relative abundance < 0.02%
  - **9) Relative abundance ≥ 0.02%**
    - Present in 1 out of 3 filter replicates
    - Present in > 2 out of 3 filter replicates
  - **10) Present in > 2 out of 3 filter replicates**
    - Taxon not exotic to survey area
    - Taxon exotic to survey area
  - **11) Absent in negative controls or remaining after subtraction**
    - Present in dive survey
    - Not present in dive survey
  - **12) Other genera in same family present in survey/databases**
    - Taxon not exotic to survey area
    - Taxon exotic to survey area
    - Taxon found outside survey area but known to be present in adjacent waters
    - Taxon found outside survey area but known to be present in adjacent waters

**Probable contamination or misannotation**

- **Spurious annotation (n = 2)**
  - Taxon
    - Melanesia
    - Thunnus

**True positives**

- **1) Total sequenced reads**
  - Homo
    - Seabed
  - **2) Merging of paired-end reads**
    - Zanocoraxel
  - **3) Fastq quality filtering**
    - Homo
    - Seabed
  - **4) Primer and tag identification**
    - Homo
    - Seabed
  - **5) Dereplication**
    - Homo
    - Seabed
  - **6) Singleton removal**
    - Homo
    - Seabed
  - **7) BLAST (percent identity ≥ 98% to 12S mtDNA database)**
    - Homo
    - Seabed
  - **8) Removal of positive and negative controls**
    - Homo
    - Seabed
  - **9) Removal of low-frequency noise**
    - Homo
    - Seabed
  - **10) Removal of taxa present in only 1 out of 3 filter replicates**
    - Homo
    - Seabed
  - **11) Removal of taxa present in the dataset after the negative control subtraction**
    - Homo
    - Seabed
  - **12) Removal of exotic taxa or reclassification to lower taxonomic rank**
    - Homo
    - Seabed

**Low confidence annotation (n = 24)**

- **Taxon**
  - Helicolenus
  - Spermaceti
  - Dicytus
  - Coryphaena
  - Acronotus
  - Clupeidae
  - Merluccius
  - Semicossyphus
  - Cottidae
  - Chromis
  - Mirounga
  - Galenus
  - Megaptera
  - Scorpis
  - Serraninae
  - Oncorhynchus
  - Ammodytes

**Spurious annotation (n = 3)**

- **Taxon**
  - Homo
  - Zanocoraxel
  - Seabed
sequenced the negative controls (i.e. field and extraction blanks) included in this experiment to identify sequences that were likely the result of contamination. The number of sequences of each taxon present in the respective negative control was subtracted from the sequence abundance of that taxon in the field sample (Nguyen et al. 2015). All remaining annotated reads were either classified as true positives or spurious annotation due to being exotic to the survey area. Taxa classified as true positives were either also seen in the visual surveys or are likely present in the survey area based on other biological information including the KELPFOREST Database (Beas-Luna et al. 2014) and survey data from historical monitoring at Hopkins Marine Station and California waters (Miller & Lea 1972). The KELPFOREST Database is a repository containing the identities of species present in nearshore kelp forest ecosystems of the eastern Pacific Ocean, with a focus on central and southern California. Taxa not known to be present in the survey area but are present in adjacent waters or have other genera in the same family that are known to be present in the survey area or database were also classified (at the family level for the latter case) as true positives. Remaining taxa were classified as exotic to the survey area and excluded from the analysis. Thus the use of extrinsic natural-history data provided an independent means of assessing eDNA error rates for the study.

**Statistical analyses**

eDNA sequence counts were normalized with the R package DESEQ2 v1.6.2 (Love et al. 2014). This method accounts for differential sample depth (correcting for uneven numbers of reads per tag) and is appropriate for normalizing high-variance data sets from high-throughput sequencing (McMurdie & Holmes 2014). In addition, we created a presence/absence version of the data set to compare to the count data.

We assessed pairwise differences in annotated sequence abundances between all pairs of sample sites and habitat types using a Kruskal–Wallis test with Bonferroni correction. The Kruskal–Wallis test was also used to test for differences in whole-community membership, for example differences in annotated read abundances calculated over all habitats simultaneously, between and within habitat types and between filter replicates. We further analysed beta-diversity and community composition in both versions of the data set (normalized counts and presence/absence) with the betadiver, adonis, hclust and metaMDS functions using the vegan package v2.2.0 of R (Oksanen et al. 2008). We used adonis to do a nonparametric analysis of variance (PERMANOVA) of Bray–Curtis dissimilarities among samples, testing for significance using 200 permutations. Nonmetric multidimensional scaling (NMDS) was performed using the Bray-Curtis dissimilarity index with metaMDS.

**Results**

**Sequence data processing**

Sequencing of vertebrate 12S mitochondrial eDNA for all samples generated over 17 million paired-end reads with a relatively uniform read distribution across primer tags (195 091 ± 24 448 reads per tag for field samples) (Fig. S1, Supporting information). We employed stringent sequence filtering parameters as described above, generating a high-confidence data set consisting of 3.16 million, 106-bp reads (Fig. 2). Sequences excluded from the analysis due to not having any taxonomic matches at the specified BLAST or MEGAN thresholds (n = 860 907) were subsequently annotated against the full NCBI nucleotide database at varying e-value thresholds and consisted mainly of reads annotated as Epinephelus (n = 431 987) and Rhacochilus (n = 108 437) (Table S2, Supporting information). Only two spurious taxa (Gallus and Homo) were present in the positive controls (i.e. mock communities and swordfish tissue), and the higher relative abundance for Gallus (0.02%) was used to set the low-frequency noise threshold across the entire data set (Table S1, Supporting information). The source of these two spurious taxa in the mock communities may be contamination from DNA extraction and/or PCR reagents during production in the manufacturer’s laboratories, or laboratory processing (Champlot et al. 2010). The low-frequency noise filter removed 26 of 99 taxa identified by BLAST (Fig. 2). An additional 24 taxa were removed due to their presence in only one of the three replicates per sample (Fig. 2). After the previous filtering steps, three taxa remained that were also present in the negative controls (Homo, Sebastes and Zoa-roidae, a perciform suborder containing gunnels and similar fishes). Subtraction of the number of sequence reads for each of these genera in the negative controls from their sequence abundances in each field sample resulted in the exclusion of Homo (27.9% of total annotated reads) from the data set. The final filtering steps removed two more taxa that were classified as exotic to the survey area and probable contamination or misannotation (Meleagris and Thunnus). Four genera not known to be in the area (Epinephelus, Etropus, Odontesthes and Plectobranchus) were reassigned to the family level due to other family members being present in the survey area and in the KELPFOREST Database; these cases were probably misannotations due to incomplete coverage of these groups in GenBank. The final filtered data set contained
33 unique taxa, with 26, six and one taxa annotated at the genus, family and suborder levels, respectively.

**eDNA vs. visual surveys**

Visual surveys revealed seven fish groups and two pinnipeds (12 taxa) across all habitats, except the open water habitat where visual data were not collected (Table S3, Supporting information). Of the 12 visually observed taxa, 11 were also detected with eDNA (Fig. S2, Supporting information; false-negative rate = 8.3% relative to visual survey). Gobies were the lone taxon seen visually but not detected with eDNA. eDNA detected 18 additional taxa classified as true positives based on their known presence in the survey area (Fig. 3), more than doubling the total number of vertebrates surveyed.

The spatial trends of visual and eDNA counts were highly concordant for the major fish groups surveyed (Fig. 4 and Fig. S3, Supporting information). We were unable to detect a difference in the spatial distribution of eDNA vs. visual survey counts for any of the taxa occurring in both data sets (Kolmogorov-Smirnov tests, \( P > 0.05 \)), although our power to detect differences was limited by small sample sizes in the visual data. Peak eDNA abundance and survey counts co-occurred within ca. 100 m as species as diverse as rockfish, wrasses, surfperch and seals. Flatfish—which can be difficult to survey visually—had contrasting visual and eDNA counts, with eDNA peaking in the sandy bottom habitat.

To evaluate whether PCR amplification bias among taxa due to primer mismatch may have impacted detection and sequence abundance, we aligned the 12S primer sequences with the primer-binding sites for species known to be present in kelp forest ecosystems of Monterey Bay as well as species in the mock communities (Fig. S4, Supporting information). All of the taxa that we detected (i.e. bony fishes and marine mammals) appear to have fewer than two mismatches and if present are located at the 5' of the reverse primer-binding site which is less likely to result in decreased primer-binding efficiency relative to the 3' end (Bru et al. 2008; Stadhouders et al. 2010). Failure to detect some additional taxa, such as cartilaginous fishes, may be due to known mutations in the primer sites for those taxa (Fig. S4, Supporting information). Despite widespread variation in sequence counts across species in mock community 1 (equimolar) and a weak relationship between DNA concentration and sequence count in mock community 2 (increasing concentration) (Table S1, Supporting information), the mock communities did not show strong evidence for decreased amplification efficiency due to primer bias.

**Spatial distribution of eDNA abundance**

Spatial trends in taxon-specific eDNA were strongly consistent with a priori expectations given known species distributions (Fig. 4 and Fig. S3, Supporting information). Taxa were nonrandomly associated with

![Fig. 3 Heatmap showing the mean normalized counts of taxa detected with eDNA at each sample site. Means represent the average of the three filter replicates per sample site. See Table 1 for descriptions of sample sites. Numbers in front of taxon names correspond to the classification groups for true positives as defined in Fig. 2. SG, seagrass; KF, kelp forest; SB, sandy bottom; RR, rocky reef; OW, open water.](https://example.com/image.png)
habitat and distance from shore [Kruskal–Wallis (KW) test, $P < 0.05$], and many exhibited clear peaks in expected habitats (eight taxa significant at $P < 0.05$; three taxa at $P < 0.01$; 15 taxa at $P < 0.001$) (Table S4, Supporting information, All Habitats). For example, the eDNA of rockfish and other kelp forest species such as greenlings (*Hexagrammos, Ophiodon, Oxylebius*), wrasses (*Oxyjulis*) and sea otters (*Enhydra*) peaked in those

![Spatial trends in eDNA and visual count data across the transect, habitats and microhabitats. Panels are shown for those taxa that were present in the visual surveys in addition to those taxa whose summed proportional abundance across all samples was $>10%$. eDNA counts for the three filter replicates are plotted for each sample site. eDNA counts were significantly associated with habitat for all taxa listed (KW, $P < 0.05$) (see Table S4, Supporting information). Visual counts are included for taxa seen on the dive surveys (no visual surveys for the open water sites). Loess curves for visual counts are only included for taxa with $>15$ counts total across all surveyed sites and are not best-fit lines. Plot background (grey and white shading) distinguishes the following habitat types, moving away from shore: seagrass, kelp forest, shallow sandy bottom, rocky reef, deep sandy bottom and open water. See Fig. S3 (Supporting information) for distance from shore plotted on a log(x) scale; (●) proportion normalized eDNA reads; (△) visual counts.](image-url)
species’ core habitats—kelp forest and rocky reef—and decreased in the seagrass and open water samples. Similarly, nearshore taxa including surfperch (Embiotoca and Rhin submissive) and cormorants (Phalacrocorax) were most abundant in the seagrass and absent in habitats offshore. Pinnipeds peaked in seagrass (Phocidae, seals) and in an open water sample (Zalophus, sea lions). Anchovies (Engraulis) and dolphins (Grampus) were nearly absent inshore but peaked offshore. There was more sampling variation in taxon abundances between habitat types than within habitat types (Table S4, Supporting information).

Patterns of community composition

Between-site differences accounted for 92.2% of variance in the data set [permutational multivariate analysis of variance (PERMANOVA) using distance matrices \( P < 0.005 \)]. Between-habitat, within-habitat and filter replicate eDNA variance was 76.3%, 15.9% and 7.8%, respectively (PERMANOVA, \( P < 0.005 \)). Vertebrate communities associated with microhabitats of seagrass, kelp forest, rocky reef and open water habitats were distinguishable using eDNA (Table S5A, Supporting information); thus this method was able to distinguish among communities separated by as little as ~60 m, the smallest spatial interval sampled. Clustering and ordination of eDNA community composition based on presence/absence data distinguished each habitat type from one another (Fig. 5A), but count data showed overlap between sandy bottom, rocky reef and outer kelp forest communities (Fig. 5B). Of the 15 pairwise habitat comparisons, 14 were significantly different (KW, \( P < 0.05 \), \( R^2 = 0.473–0.771 \)) (Table S5B, Supporting information). Filter replicates from the same location showed low variability in observed species richness (Table 1). Overall, the different habitats displayed unique taxonomic assemblages, and vertebrate species richness decreased with distance from shore (Table 1).

Discussion

Our survey of marine vertebrate diversity sheds light on the spatial resolution and error rates of field-based eDNA studies by comparing to conventional visual surveys and sampling across multiple nearshore habitats. eDNA in seawater samples offered accurate, relatively fine-scale spatial resolution (60–100 m) of vertebrate communities across multiple habitats in a highly dynamic nearshore environment, and compared favourably to traditional visual surveys, often the standard of ecological monitoring.

We found spatially concordant trends in individual species’ eDNA and visual surveys, and taxa detected in each habitat with eDNA reflected highly local community composition. The eDNA is therefore likely to be primarily of local origin and the probability of detecting nonresident species decreases with distance from those species distributions, consistent with the only other field eDNA survey of marine fishes to date (Thomsen et al. 2012a). We found significant differences in eDNA community composition and relative abundance of taxa between adjacent habitat types and by distance from shore within a habitat type, indicating that sampling locations in close proximity (~60 m) had distinct eDNA assemblages. If physical processes (e.g. advection, diffusion) drive the dispersal patterns of eDNA, we would expect the eDNA data to be decoupled from the visual surveys and independent of habitat type; conversely, if biological processes (e.g. generation and degradation of eDNA) are more influential, we would expect species’ spatial patterns to differ systematically. Our results support the latter hypothesis, although the detection probability in this study system may be high due to local oceanography at the time of sampling, the retentive properties of kelp forests (Gaylord et al. 2012), or both. We note, too, that differences in community composition and taxon abundance between the open water vs. nearshore habitats may also be due to depth as open water samples were collected at the surface while all other habitat samples were collected 1 m from the bottom. The greatest depth difference between these samples was 29 m.

This study also illustrates the uncertainties surrounding assessing error rates of detection in an open ecosystem. Factors including the number of sample replicates and gene markers, sequence misannotation, contamination, data-filtering strategy and ecology and hydrodynamics impacted our estimates of error, and we note that most habitats do not have the advantage of being as extensively documented as Monterey Bay. For eDNA to be practical for biological monitoring, the method must have low error rates of detection in the field. We quantified error rates at several key steps (Fig. 2): First, we conducted visual fish surveys in conjunction with eDNA sampling to test for concordance between taxonomic identity, geographic location and sequence data. Second, we sequenced 1-L replicates for each composite 3-L sample to assess sample variability among replicate field samples. Third, we included internal controls to enable removal of low-frequency noise and any contaminant reads from the data set (De Barba et al. 2014). Fourth, we employed stringent sequence filtering parameters to identify false-positive assignments and remove spurious results resulting from PCR errors, sequencing errors or carry-over or cross-contamination, generating a conservative, high-confidence data set. Lastly, we filtered for geographic relevance, removing
any remaining taxa passing the previous filters that were exotic to the survey area or vicinity or not present in the KELPFOREST Database. We obtained a low false-negative rate of eDNA detection relative to the visual surveys (1/12 taxa). eDNA detected more than twice the number found in the visual survey (29 eDNA, 12 visual) due to the inclusion of cryptic species (e.g. flatfish, gunnel, kelpfish) not easily seen with visual methods and/or advection of DNA from species outside the immediate survey area (e.g. medusafish, Pacific Butterfish). While our study design did not allow for occupancy modelling due to one sampling event per location and insufficient replication to detect rarer taxa (Schmidt et al. 2013; Ficetola et al. 2015), this approach may improve low detection rates and the reliability of eDNA metabarcoding studies.

It was not possible—and neither was it our objective—to estimate the number of false-negative or false-positive detections relative to the ‘true’ species composition of the survey area. Given the true species composition is unknown, we acknowledge that our conservative sequence filtering may have decreased the number of species detected. For example, taxa known to be present in and around the survey area including Leptocottus (Pacific staghorn sculpin), Semicossyphus (sheephead wrasse), Merluccius (hake) as well as the whale genera Megaptera (humpback whale) and Balaenoptera (blue, fin or minke whale), were detected with eDNA but

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Fig. 5 Hierarchical clustering dendrograms and NMDS ordination of the similarity in vertebrate eDNA community composition across habitats and microhabitats. Dendrograms and NMDS plots are shown for (A) binary presence/absence data and (B) normalized counts generated using the DESEQ2 package. Clustering is based on group-average linkage from Bray-Curtis similarity. Clusters at similarity levels of 50% (outer solid polygon) and 70% (inner dashed polygon) are superimposed on the NMDS plots. Labels refer to filter replicates (see Table 1 for descriptions) and are coloured by habitat type: SG, seagrass; KF, kelp forest; SB, sandy bottom; RR, rocky reef; OW, open water.
classified as false negatives due to low abundance or presence in only one of three filter replicates. A similar majority-rules approach for replicates has been implemented in other metabarcoding studies (Giguet-Covex et al. 2014; Ficetola et al. 2015). When eDNA is rare variability across replicates may be expected, but our approach aimed to balance capturing sample heterogeneity with minimizing spurious assignments due to PCR or sequencing errors, primer tag bias (J. L. O’Donnell, R. P. Kelly, N. C. Lowell & J. A. Port, unpublished data) and/or contamination. Sample heterogeneity in this study underscores the need for a larger number of replicates (perhaps as much as >8), especially for species with low detection probabilities and field studies lacking ground-truthing data (Ficetola et al. 2015), but see Lahoz-Monfort et al. 2015. However, we emphasize that the goal of this study was to survey and compare whole communities, not to detect rare taxa. For such applications, more sensitive, targeted protocols such as quantitative real-time PCR (qPCR) may be more appropriate. Additionally, multiple gene markers may improve detection rates in metabarcoding studies (Evans et al. 2015) due to increased likelihood of finding matches in sequence databases and reduced primer bias. For example, the absence of cartilaginous fishes in our data set may likely be due to two mismatches present at the 3’ end region of the forward primer-binding site that may inhibit amplification (Fig. 54, Supporting information; Kelly et al. 2014).

Taxa that were present in the eDNA data but absent in the dive surveys and local taxonomic databases may result from: (i) sequence misannotation due to sequencing errors or incompleteness of the NCBI nucleotide database, (ii) DNA derived from field or laboratory contamination, (iii) movement of eDNA sourced from species located outside the survey area, and/or (iv) resuspension of sedimentary eDNA (Turner et al. 2015). The latter two sources were not directly investigated in this study. Because of the shorter length of the 12S locus (~106 bp), sequencing errors are more likely to lead to misannotation for closely-related taxa. This was evident for taxa removed due to low-confidence annotation. For example, true seals (Phocidae) were common in our data set as expected but genera of this family with lower abundance (Pusa, Monachus and Leptonychotes) are polar or subtropical in distribution. Similarly, many of the other low-confidence annotations were uncommon or not known to be in the survey area but had other family members present in the system or KELPFORest Database.

Regarding contamination, Sardinops and Brevoortia (family Clupeidae) were both present in the negative controls and had high sequence counts in the combined data set (212 415 and 63 523, respectively). These genera are potential carry-over contaminants from previous experiments. Our data-filtering steps removed these two taxa before even filtering specifically for probable carry-over laboratory contamination though (Fig. 2). To minimize the level of potential human contamination in our samples, we originally processed the samples in this study using a human blocking primer designed to bind to the target 12S gene region and reduce amplification of human DNA. While human DNA was effectively blocked, there was also unexpected and unpredictable blocking of fishes we expected to find (e.g. rockfish and halibut) based on PCR amplification and sequence data (data not shown). We therefore subsequently amplified and sequenced the samples without a human blocker. Similar amplification biases have been seen in other studies when using blocking primers with universal primers (Pinol et al. 2015). Meleagris and Thunnus passed all data-filtering steps including absence in negative controls, but were not relevant to the geographic scope of the study area and so were classified as false positives stemming from possible contamination or misannotation. Meleagris, like Gallus and Homo, may be due to contamination in DNA extraction or PCR reagents during production in the manufacturer’s laboratories. While Thunnus is not present in the survey area, other scombrids are known to be present which could lead to misannotation due to high sequence similarity across species in the 12S gene region. It is possible that Thunnus and other taxa excluded as false positives may also be sourced from the Tuna Research and Conservation Center or Monterey Bay Aquarium which deposit effluent in proximity to the survey area.

Biological and methodological biases are hurdles to estimating species abundances from eDNA (Pompanon et al. 2012), as evidenced by a weak relationship between DNA concentration and sequence abundance in our mock communities (Table S1, Supporting information). For this reason, we compared sequence abundances between sampling locations only within individual taxa, rather than comparing across taxa. Our analysis assumes that eDNA fragments from a given taxon experience the same extraction, amplification and sequencing biases—and same mtDNA copy number per cell—regardless of the location from which they were collected. One exception was the community-level analysis, which unavoidable required cross-taxon comparisons; however, presence/absence eDNA data differentiated vertebrate communities even more definitively (Fig. 5A). Tissue- or species-correction factors (Thomas et al. 2014), designing and optimizing generic primers (Thomsen & Willerslev 2015), and PCR-free approaches (Zhou et al. 2013) are under investigation and may help to link eDNA abundance to organism abundance in the future. Understanding variability in DNA shedding rates across species will also strengthen © 2015 The Authors. Molecular Ecology Published by John Wiley & Sons Ltd.
quantitative estimates (Maruyama et al. 2014; Klymus et al. 2015).

We have shown that even in a nearshore environment subject to wave action and mixing, eDNA may substantially improve upon traditional survey methods and ecologists’ power to monitor the dynamics of whole animal communities. Of particular management relevance, the technique reveals spatial trends in the presence and abundance of iconic species in kelp forest ecosystems important for fisheries and ecosystem health (e.g. rockfish, lingcod, sea otters, cabezon and striped surfperch). Temporally, this study is only a snapshot; further studies will address the stability of eDNA measurements over time in dynamic systems. Seasonal variation in currents, temperature and species abundance patterns as well as variability in fish fauna diversity over the diurnal cycle and water column are important factors influencing both eDNA and visual detection. In addition, ecological and hydrological factors influencing eDNA detection rates such as eDNA shedding rates, fate and transport, and degradation rates require more attention (Jerde & Mahon 2015). Several studies have already shown eDNA to be more cost-effective than traditional monitoring methods (Thomsen & Willerslev 2015). With further validation and refinement, eDNA holds promise as a more comprehensive approach to large-scale environmental monitoring.

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References

Andersen K, Bird KL, Rasmussen M et al. (2012) Meta-barcoding of ‘dirt’ DNA from soil reflects vertebrate biodiversity. Molecular Ecology, 21, 1966–1979.

Baldwin CC, Collette BB, Parenti LR, Smith DG, Springer VG (1996) Methods and Techniques of Underwater Research. Proceedings of the American Academy of Underwater Sciences Scientific Diving Symposium. Smithsonian Institution, Washington, District of Columbia.

Barnes MA, Turner CR, Jerde CL et al. (2014) Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science & Technology, 48, 1819–1827.

Beas-Luna R, Novak M, Carr MH et al. (2014) An online database for informing ecological network models: http://kelpforest.ucsc.edu. PLoS One, 9, e109356.

Bru D, Martin-Laurent F, Philippot L (2008) Quantification of the detrimental effect of a single primer-template mismatch by real-time PCR using the 16S rRNA gene as an example. Applied and Environmental Microbiology, 74, 1660–1663.

Butchart SHM, Walpole M, Collen B et al. (2010) Global biodiversity: indicators of recent declines. Science, 328, 1164–1168.

Camacho C, Coulouris G, Avsagyan V et al. (2009) BLAST+: architecture and applications. BMC Bioinformatics, 10, 421.

Champlot S, Berthelot C, Pruvost M et al. (2010) An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. PLoS One, 5, e13042.

Coissac E (2012) OligoTag: a program for designing sets of tags for next-generation sequencing of multiplexed samples. Methods in Molecular Biology, 888, 13–31.

Dayton PK (1985) Ecology of kelp communities. Annual Review of Ecology and Systematics, 16, 215–245.

De Barba M, Miquel C, Boyer F et al. (2014) DNA metabarcoding: multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. Molecular Ecology Resources, 14, 306–323.

Deagle BE, Chiaradia A, McHnes J, Jarman SN (2010) Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? Conservation Genetics, 11, 2039–2048.

Deiner K, Altermatt F (2014) Transport distance of invertebrate environmental DNA in a natural river. PLoS One, 9, e88786.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26, 2460–2461.

Evans NT, Olds BP, Renshaw MA et al. (2015) Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources, 16, 29–41.

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. Biology Letters, 4, 423–425.

Ficetola GF, Pansu J, Bonin A et al. (2015) Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. Molecular Ecology Resources, 15, 543–556.

Foote AD, Thomsen PF, Sveegaard S et al. (2012) Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. PLoS One, 7, e41781.

Gaylord B, Nickols KJ, Jurgens L (2012) Roles of transport and mixing processes in kelp forest ecology. Journal of Experimental Biology, 215, 997–1007.

Giguet-Covex C, Pansu J, Arnaud F et al. (2014) Long livestock farming history and human landscape shaping revealed by lake sediment DNA. Nature Communications, 5, 3211.

Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP (2013) Environmental DNA as a new method for early detection of New Zealand mudsnails (Potamopyrgus antipodarum). Freshwater Science, 32, 792–800.

Huson DH, Weber N (2013) Microbial community analysis using MEGAN. Methods in Enzymology, 531, 465–485.

Jerde CL, Mahon AR (2015) Improving confidence in environmental DNA species detection. Molecular Ecology Resources, 15, 461–463.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) “Sight-unseen” detection of rare aquatic species using environmental DNA. Conservation Letters, 4, 150–157.

Jerde CL, Chadderton WL, Mahon AR et al. (2013) Detection of Asian carp DNA as part of a Great Lakes basin-wide
surveillance program. Canadian Journal of Fisheries and Aquatic Sciences, 70, 522–526.

Jones JB (1992) Environmental impact of trawling on the seabed: a review. New Zealand Journal of Marine and Freshwater Research, 26, 59–67.

Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. PLoS One, 9, e86175.

Klymus KE, Richter CA, Chapman DC, Paukert C (2015) Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix. Biological Conservation, 183, 77–84.

Lahoz-Monfort JJ, Guillaum-Arroita G, Tingley R (2015) Statistical approaches to account for false positive errors in environmental DNA samples. Molecular Ecology Resources, in press. doi: 10.1111/1755-0998.12486.

Laramie MB, Pilliod DS, Goldberg CS (2015) Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biological Conservation, 183, 29–37.

Love M, Anders S, Huber W (2014) Differential analysis of count data—the DSESeq2 package.

Mächler E, Deiner K, Steinmann P, Altermatt F (2014) Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshwater Science, 33, 1174–1183.

Mahon AR, Nathan LR, Jerde CL (2015) Meta-genomic surveilling of invasive species in the bait trade. Conservation Genetics Resources, 6, 563–567.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBO Net. Journal, 17, 10–12.

Maruyama A, Nakamura K, Yamanaka H, Kondoh M, Minamoto T (2014) The release rate of environmental DNA from juvenile and adult fish. PLoS One, 9, e114639.

McMurdie PJ, Holmes S (2014) Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Computational Biology, 10, e1003531.

Miller DJ, Lea RN (1972) Guide to the Coastal Marine Fishes of California. California Department of Fish and Game, Fish Bulletin 157, Sacramento, CA, 249 pages.

Miya M, Sato Y, Fukunaga T, Hoshino T, Nishida M, Miura T (2014) MiFish, a set of universal primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society Open Science, 2, 150088.

Nguyen NH, Smith D, Peay K, Kennedy P (2015) Parsing ecological signal from noise in next generation amplicon sequencing. New Phytologist, 205, 1389–1393.

Oksanen J, Kindt R, Legendre P et al. (2008) The vegan Package. R package version 2.2-0. URL: http://CRAN.R-project.org/.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP (2014) Factors influencing detection of eDNA from a stream-dwelling amphibian. Molecular Ecology Resources, 14, 109–116.

Pinol J, Mir G, Gomez-Polo P, Agusti N (2015) Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Molecular Ecology Resources, 15, 819–830.

Pomponio F, Deagle BE, Symondson WO et al. (2012) Who is eating what: diet assessment using next generation sequencing. Molecular Ecology, 21, 1931–1950.

Riaz T, Shehzad W, Viari A et al. (2011) ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Research, 39, e145.

Rusch DB, Halpern AL, Sutton G et al. (2007) The sorcerer II global ocean sampling expedition: Northwest Atlantic through eastern tropical Pacific. PLoS Biology, 5, e77.

Schmidt BR, Kery M, Ursenbacher S, Hyman OJ, Collins JP (2013) Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. Methods in Ecology and Evolution, 4, 646–653.

Schmitt EF, Sullivan KM (1996) Analysis of a volunteer method for collecting fish presence and abundance data in the Florida Keys. Bulletin of Marine Science, 59, 404–416.

Schmitt EF, Sluka RD, Sullivan-Sealey K (2002) Evaluating the use of roving diver and transect surveys to assess the coral reef fish assemblage off southeastern Hispaniola. Coral Reefs, 21, 216–223.

Schnell JB, Bohmann K, Gilbert MT (2015) Tag jumps illuminated – reducing sequence-to-sample misidentifications in metabarcoding studies. Molecular Ecology Resources, 15, 1289–1303.

Shehzad W, Riaz T, Nawaz MA et al. (2012) Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (Prionailurus bengalensis) in Pakistan. Molecular Ecology, 21, 1951–1965.

Spear SF, Groves JD, Williams LA, Waits LP (2015) Using environmental DNA methods to improve detectability in a hellbender (Cryptobranchus alleganiensis) monitoring program. Biological Conservation, 183, 38–45.

Stadhouders R, Pas SD, Anber J et al. (2010) The effect of primer-template mismatches on the detection and quantification of nucleic acids using the 5′ nuclease assay. Journal of Molecular Diagnostics, 12, 109–117.

Steneck RS, Graham MH, Bourque BJ et al. (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation, 29, 436–459.

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Molecular Ecology, 21, 1789–1793.

Takahara T, Minamoto T, Doi H (2013) Using environmental DNA to estimate the distribution of an invasive fish species in ponds. PLoS One, 8, e65684.

Thomas AC, Jarman SN, Haman KH, Trites AW, Deagle BE (2014) Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. Molecular Ecology, 23, 3706–3718.

Thompson AA, Mapstone BD (1997) Observer effects and training in underwater visual surveys of reef fishes. Marine Ecology Progress Series, 154, 53–63.

Thomsen PF, Willerslev E (2015) Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation, 183, 4–18.

Thomsen PF, Kielgast J, Iversen LL et al. (2012a) Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One, 7, e41732.

Thomsen PF, Kielgast J, Iversen LL et al. (2012b) Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology, 21, 2565–2573.

Turner CR, Uy KL, Everhart RC (2015) Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation, 183, 93–102.

Valentini A, Miqiel C, Nawaz MA et al. (2009) New perspectives in diet analysis based on DNA barcoding and parallel

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Supporting information

Additional supporting information may be found in the online version of this article.

**Fig. S1.** Number of reads per tag assigned to field samples, positive controls (i.e. mock communities and swordfish tissue) and negative controls (i.e. filtration and extraction blanks) after demultiplexing.

**Fig. S2.** Venn diagram of bony fish taxa and marine mammals as detected by eDNA vs. visual survey for the combined sample sites.

**Fig. S3.** Spatial trends in eDNA and visual count data across the transect and habitats shown on a log(x) scale.

**Fig. S4.** Sequence alignment of the 12S rRNA primer-binding sites for taxa present in Monterey Bay and the mock community.

**Table S1.** (A) Composition of the artificial communities used as positive controls. The two communities contained either equal (community 1) or increasing (community 2) concentrations of tissue DNA. (B) Sequence counts for each taxon present in the three positive controls.

**Table S2.** Taxonomic annotation and read counts for sequences not matching species in our 12S database at specified BLAST and MEGAN thresholds.

**Table S3.** Visual fish survey counts for each sample site.

**Table S4.** Pairwise-comparisons of taxon abundance between (A) habitat types and (B) sample sites within the same habitat.

**Table S5.** Statistical tests of beta-diversity (A) between sites within the same habitat and (B) between habitats ($R^2$ values).