Environmental DNA Analysis of Marine Biodiversity in the Northern Channel Islands, California

Oceana and Blancpain Expedition

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Cover Photo:
Predatory sea slug, California
Aglaja (*Navanax inermis*) off
Santa Barbara Island.
Credit: Geoff Shester, © Oceana

Executive Summary

- Human impacts on ocean ecosystems continue to compound, from overfishing, bycatch in fishing gear, to plastic pollution and the effects of climate change.
- New technological advances in environmental DNA (eDNA) metabarcoding allow us to non-invasively collect information about the presence and abundance of marine species at unprecedented speed, scale and resolution, allowing us to create baselines of regional biodiversity and monitor changes over time caused by human impacts.
- Oceana launched a five-day expedition to the Northern Channel Islands, CA, where we conducted scuba surveys for fish and macro invertebrates and collected water samples for eDNA analysis. Sample sites included 9 shallow reefs (< 16 m) and 9 paired deep-water samples (60-103 m) at adjacent locations.
- We built and sequenced two libraries targeting fish (12S gene) and eukaryotes (18S gene) from each sample
- From the 18S analysis targeting eukaryotes, we identified high levels of marine biodiversity, represented by 11,211 Operational Taxonomic Units (OTUs) that represent biological entities or molecular eukaryotic species belonging to 43 phyla, 98 classes, 249 orders and 374 families.
- About 20% of all the OTUs from eukaryotes did not have any relevant match in international genetic databases (GenBank), indicating the presence of many species from branches of the tree of life that have been only poorly described and sequenced.
- The recovered 20,725 Amplified Sequence Variants (ASVs) or unique fish sequences of bony and cartilaginous fish, detected 27 orders, 57 Families, 93 Genus and at least 128 Species. About 56% of all the fish sequences did not have any relevant match in international genetic databases (GenBank), suggesting less than half of the fish detected in our samples from California are present in GenBank for the metabarcode used.
- The levels of biodiversity of eukaryotes were similar between shallow and deep sites (average 3,088 and 3,005 eukaryotic OTUs, respectively), while fish diversity was lower at shallow compared to deeper sites for ASVs (average 1,184 and 1,498 fish ASVs, respectively), while shallow sites had more fish taxa taxonomically identified (110 taxa), compared to deeper sites (70 taxa).
- Shallow and deep communities had very different species compositions from each other, with 25% of all the eukaryotes and fish taxa found exclusively in the deep ecosystems.
- The study sheds light on the massive scale of marine biodiversity present in the region. A large fraction of species that were detected by our analysis are not currently included in the genetic reference databases and their ecological roles are poorly understood.
- Thirteen fish species identified by the eDNA analysis are species that observers have identified as bycatch in the California set gillnet fishery.

Background

Environmental DNA (eDNA) metabarcoding has emerged as a groundbreaking and innovative method designed to identify multiple species simultaneously. This technique involves the collection of traces of eDNA found in environmental samples, including water, air, and sediments. In the ocean, the interaction of organisms with their aquatic environment results in the release of eDNA due to shedding of cells from epithelial tissues, excretion, and release of gametes or cells into the water during reproduction or predation, among many other sources. The eDNA is usually collected by filtration of water samples, and then subjected to Polymerase Chain Reaction (PCR) amplification with universal primers and high-throughput sequencing of conserved genomic regions, which are referred to as metabarcodes. Some examples of commonly used metarbacodes are the 12S ribosomal subunit for amplifying DNA from bony fish and elasmobranchs, and the cytochrome oxidase subunit I (COI) or the18S ribosomal subunit for capturing DNA from all eukaryotes (i.e., all living things, excluding bacteria and viruses).

Once the DNA sequences are generated, they can either be analyzed by each unique sequence independently as an Amplified Sequence Variant (ASV), or the complexity of the dataset can be reduced by grouping sequences into clusters at a certain identity level (e.g. 97% similarity) to create Operational Taxonomic Units (OTUs). These OTUs could represent different taxonomic units or different species, but there are many documented cases where multiple OTUs represent the same species (e.g., an old species with large geographic range and plenty of genetic variation), or cases where multiple species shared the same OTU (e.g. recently diverged and closely related species). They are compared against comprehensive genetic reference databases to match the identity of the eDNA sequences with known species, or if an exact match is not present then determine the nearest known common ancestor of the detected genetic material. For the purposes of this study, we assume that each OTU represents a distinct species.

Some databases contain sequences that are exclusive to a single metabarcode, such as the Barcode of Life Database (BOLD) for COI, which is maintained by the International Barcode of Life Consortium. There are also public sequence repositories where the international scientific community shares a diverse array of DNA sequences that can be utilized for generating custom made metabarcode reference libraries, including GenBank maintained by the National Center of Biotechnology Information (NCBI) and the European Molecular Biology Laboratory Nucleotide Sequence Database (EMBL). Since the number of species that have been sequenced and deposited in GenBank or EMBL is very limited compared to the number of species present on the planet, it is not uncommon that many eDNA sequences do not have close matches in the databases. In these cases, the eDNA sequences are left as "not assigned", or are indeed assigned but just to higher taxonomic levels (e.g. Phylum, Class or Order). eDNA metabarcoding is increasingly recognized as a cost-effective and replicable tool for complementing biodiversity assessments, not only for specific taxa but also across the entire tree of life. By facilitating the identification of various organisms in different ecosystems, eDNA metabarcoding plays a crucial role in ecological monitoring and conservation efforts.

The conservation organization Oceana, in partnership with the Swiss watchmaker Blancpain, is working to reduce bycatch in California state-managed fisheries. The incidental catch and discarding of marine animals (known as bycatch) is widely considered among the top ecological impacts of fisheries. The state-managed set gillnet fishery has high rates of bycatch and is allowed to operate in state waters surrounding the Channel Islands. The ocean waters around the Channel Islands are known to have unusually high biodiversity resulting from complex seafloor topography and the confluence of cold and

warm water currents. Non-selective gear types such as gillnets that are fished in diverse ecosystems like the Channel Islands region have the potential to significantly impact the diversity, function, and resilience of the ecosystem if not thoughtfully managed. Biodiversity is a key component in stable ecosystems which are facing unprecedented stressors and defaunation from warming ocean temperatures, habitat loss, and other anthropogenic impacts. While biodiversity has been generally characterized using visual scuba methods, little research has been done using environmental DNA in this region.

Methods

Field Sampling

With the goal of describing the marine biodiversity present in the Channel Islands, California, USA, Oceana organized a scientific expedition on board the diving vessel "Peace" that departed from Ventura, CA on the morning of Monday April 29th, 2024. The expedition visited 3 of the 8 islands - Santa Cruz, Anacapa and Santa Barbara Island - during the five days at sea (Fig. 1), before returning to Ventura on the evening of Friday May 3rd. We collected water samples for eDNA analyses with 4 liter (L) collapsible water bags during scuba dives at shallow sites near the coast (< 16 m deep), and at paired deep offshore sites (< 105 m) with an oceanographic Niskin bottle of 6L capacity.

During the five days of sampling, we collected a total of 40 individual samples (Table 1), including 18 shallow samples at 9 shallow sites, 18 deep samples at 9 paired deep sites, and four field negative controls (running water from the boat, used to monitor potential contamination during field work). Each water sample consisted of 2L of seawater that was filtered through a 0.45 mm filter using a vacuum pump and a filtering unit. We collected and filtered two independent 2L replicates at each site. The shallow samples were collected between 7-16 m deep at 12º C, and the deep samples were collected between 60 and 103 m and 9.9 - 12.4 º C.

Table 1. Details of 40 individual eDNA samples collected in the field, including sample number, site ID, collection date, site name, replicate number, sample depth and water temperature (measured while scuba diving or with a sensor attached to the Niskin bottle), latitude and longitude, island, sampling method and eDNA concentration.

Sample #	Site ID	Date	Site	Replicate	Depth (m)	Temp (C)	Latitude	Longitude	Island	Method	eDNA (ng/mL)
1	1	2024-04-29	Yellowbanks	1	12.0	12	33.99122	-119.55264	Santa Cruz	scuba	3.54
2		2024-04-29	Yellowbanks	2	12.0	12	33.99122	-119.55264	Santa Cruz	scuba	1.3
3	2	2024-04-29	Offshore Yellowbanks	1	63.0	11.0	33.97865	-119.55618	Santa Cruz	niskin	1.27
4		2024-04-29	Offshore Yellowbanks	2	63.0	11.0	33.97865	-119.55618	Santa Cruz	niskin	0.937
5	3	2024-04-29	Flame Reef	1	16.0	12	33.98577	-119.58672	Santa Cruz	scuba	1.97
6		2024-04-29	Flame Reef	2	16.0	12	33.98577	-119.58672	Santa Cruz	scuba	0.911
7		2024-04-29	Field Negative Control								0.344
8	4	2024-04-30	Offshore Flame Reef	1	81.0	10.3	33.97282	-119.58337	Santa Cruz	niskin	0.583
9		2024-04-30	Offshore Flame Reef	2	81.0	10.3	33.97282	-119.58337	Santa Cruz	niskin	0.47
10	5	2024-04-30	Cat Rock	1	7.0	12	34.00404	-119.42205	Anacapa	scuba	4.01
11		2024-04-30	Cat Rock	2	7.0	12	34.00404	-119.42205	Anacapa	scuba	4.2
12	6	2024-04-30	Offshore Cat Rock	1	103.0	10.8	33.99333	-119.39922	Anacapa	niskin	0.546
13		2024-04-30	Offshore Cat Rock	2	103.0	10.8	33.99333	-119.39922	Anacapa	niskin	0.28
14	7	2024-04-30	Cathedral Cove	1	12.0	12	34.01105	-119.44130	Anacapa	scuba	1.21
15		2024-04-30	Cathedral Cove	2	12.0	12	34.01105	-119.44130	Anacapa	scuba	3.7
16	8	2024-04-30	Anacapa Passage	1	39.0	12.4	34.01427	-119.51863	Santa Cruz	niskin	2.81
17		2024-04-30	Anacapa Passage	2	39.0	12.4	34.01427	-119.51863	Santa Cruz	niskin	0.771
18		2024-04-30	Field Negative Control								5.52
19	9	2024-05-01	Three Sisters Rocks	1	8.0	12	33.46469	-119.04248	Santa Barbara	scuba	3.15
20		2024-05-01	Three Sisters Rocks	2	8.0	12	33.46469	-119.04248	Santa Barbara	scuba	2.3
21	10	2024-05-01	Offshore Santa Barbara	1	102.0	10.0	33.44059	-119.03874	Santa Barbara	niskin	0.559
22		2024-05-01	Offshore Santa Barbara	2	102.0	10.0	33.44059	-119.03874	Santa Barbara	niskin	1.23
23	11	2024-05-01	Ranger Point	1	11.0	12	33.47661	-119.02703	Santa Barbara	scuba	0.871
24		2024-05-01	Ranger Point	2	11.0	12	33.47661	-119.02703	Santa Barbara	scuba	0.793
25	12	2024-05-01	Offshore Ranger Point	1	60.0	10.0	33.48210	-118.98860	Santa Barbara	niskin	0.337
26		2024-05-01	Offshore Ranger Point	2	60.0	10.0	33.48210	-118.98860	Santa Barbara	niskin	0.251
27	13	2024-05-02	Little Scorpion	1	8.0	12	34.04698	-119.54537	Santa Cruz	scuba	2.67
28		2024-05-02	Little Scorpion	2	8.0	12	34.04698	-119.54537	Santa Cruz	scuba	3.57
29	14	2024-05-02	Offshore Scorpion	1	88.0	10.0	34.07153	-119.50130	Santa Cruz	niskin	3.7
30		2024-05-02	Offshore Scorpion	2	88.0	10.0	34.07153	-119.50130	Santa Cruz	niskin	1.55
31	15	2024-05-02	Heart Attack Reef	1	9.0	12	33.98499	-119.61339	Santa Cruz	scuba	3.89
32		2024-05-02	Heart Attack Reef	2	9.0	12	33.98499	-119.61339	Santa Cruz	scuba	13.4
33	16	2024-05-02	Off Heart Attack	1	84.0	10.6	33.96222	-119.56688	Santa Cruz	niskin	1.14
34		2024-05-02	Off Heart Attack	2	84.0	10.6	33.96222	-119.56688	Santa Cruz	niskin	0.59
35		2024-05-02	Field Negative Control								0
36	17	2024-05-03	Rat Rock	1	15.0	12	34.01227	-119.44483	Anacapa	scuba	4.52
37		2024-05-03	Rat Rock	2	15.0	12	34.01227	-119.44483	Anacapa	scuba	4.08
38	18	2024-05-03	Offshore Rat Rock	1	100.0	9.9	33.98188	-119.42063	Anacapa	niskin	0.313
39		2024-05-03	Offshore Rat Rock	2	100.0	9.9	33.98188	-119.42063	Anacapa	niskin	3.28
40		2024-05-03	Field Negative Control								0.198

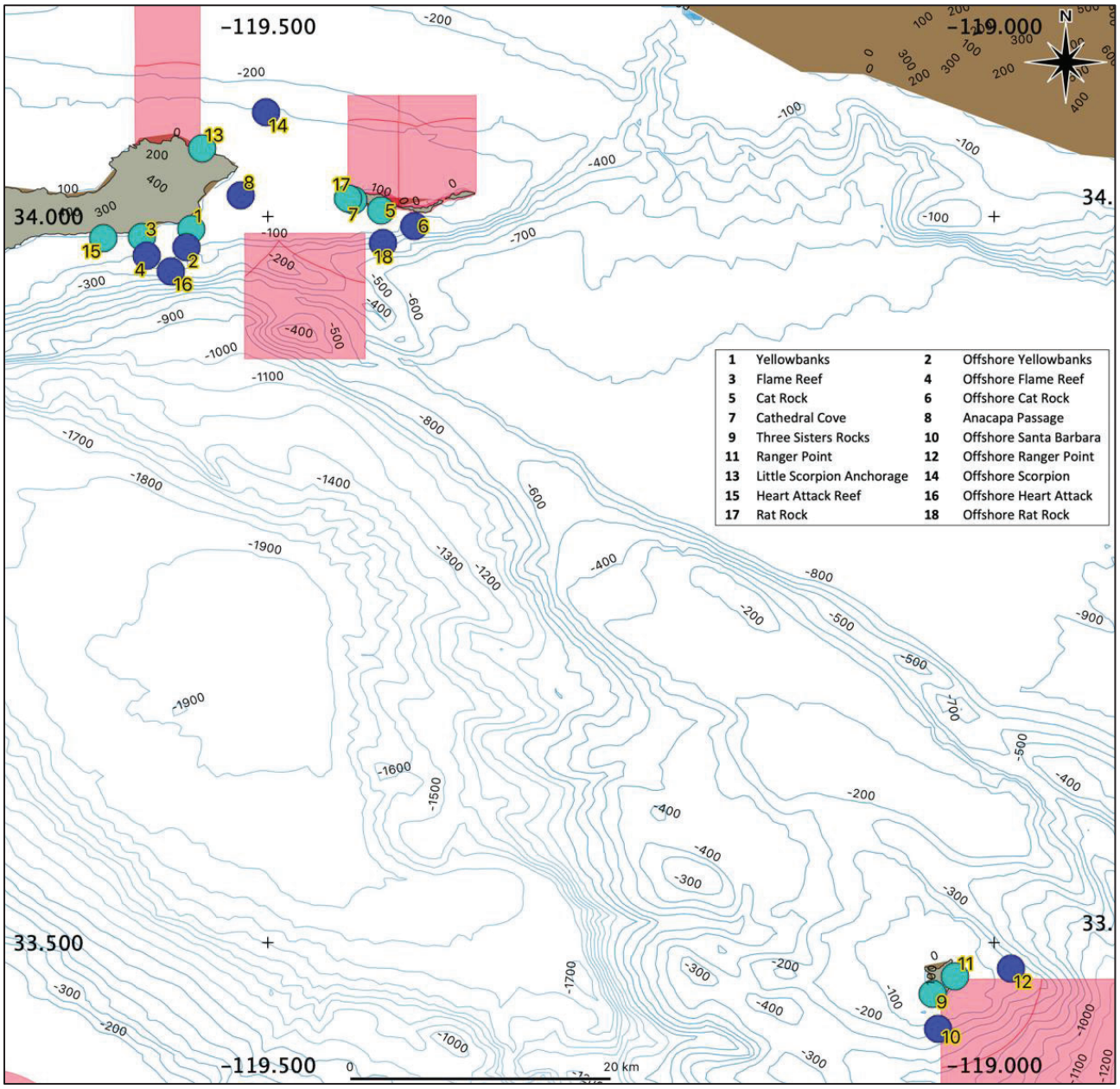

Figure 1. Map of the Northern Channel Islands showing shallow coastal sites sampled while scuba diving (teal blue dots) and offshore deep sites sampled with niskin bottles (dark blue dots). Numbers in yellow correspond to the site number shown in the inset. Local bathymetry is represented by 100 m isobaths. Pink polygons represent Marine Protected Areas.

Laboratory Analyses

We extracted eDNA from the samples with the DNeasy blood and tissue kit (QIAGEN) in a dedicated space and using equipment exclusive for low density DNA. DNA was quantified with a flourometer (QUBIT 2.0 with High Sensitivity Kit, INVITROGEN), resulting in concentrations between 0.251 and 13.4 ng/mL for the field samples, and zero and 5.52 ng/mL for the field negative controls (Table 1). Negative controls were included during the two eDNA extraction sessions, but no DNA contamination was detected in these samples with the Qubit assay (<0.1 ng/mL). For each sampled site, we built two amplicon libraries: a) targeting ~130 base pairs (bp) from the V7 18S nuclear ribosomal gene of all Eukaryotic organisms using primers and procedures described recently (Mac Loughlin et al., 2024), including all multicellular animals or metazoa, in addition to fungi, micro and macro algae and microeukaryotes and protists in the sample; b) targeting ~70 bp from the 12S mitochondrial ribosomal gene of fish (both bony and cartilaginous), using primers and protocols published previously (Valdivia-Carrillo et al., 2021).

Genomic libraries were constructed via a 2-step Polymerase Chain Reaction process (PCR) process. The 1st PCR (PCR1) included 2 PCR replicates (25 cycles each) of each field replicate sample and negative controls using primers containing adapters for 2nd PCR and universal primers mentioned above, as explained in detail previously (Mac Loughlin et al., 2024). Every PCR reaction included PCR negative controls. The 2nd PCR (PCR2) primers amplified a pool of 4 PCR1 replicates from each field site and included dual indexing to identify each field sample and Illumina adapters for sequencing. PCR2 replicates of eDNA samples from each library, including all negative controls, were purified (AMPure beads, Beckman), and mixed in equimolar concentrations before sending for sequencing. Each final library included pooled PCR2 reactions from the 18 sites + 3 pooled negative controls (field, DNA extraction and PCR, respectively) = 21 samples. The two eDNA libraries were sequenced in a partial lane of Illumina NextSeq 500 150 x 2 mid-output at the University of Arizona Genetics Core, producing ~25 million reads for both libraries (~300,000 paired reads per field site/barcode).

Bioinformatic Analyses

Analyses for the 18S barcode were performed with the software USEARCH v11 (Edgar, 2010). Raw demultiplexed sequence reads were merged by maximum (300 bp) and minimum (100 bp) lengths and a maximum number of differences of 10. Forward and reverse primers were discarded, and the reads were quality filtered under a maximum expected number of errors 1.0. Reads were dereplicated with a minimum size (2 reads) to get the unique sequences and subsequently clustered (97% similarity threshold) into Operational Taxonomic Units (OTUs), including detection and exclusion of chimeras. The final OTUs were compared with the BLAST algorithm to the NCBI platform (Benson et al., 2013) for taxonomic assignment. XML files of the first 100 best hits obtained for each OTU were analyzed in the MEGAN 6 Community Edition software (Huson et al., 2016) with parameters: Min score of 50.0, Min Percent Identity of 70.0, and Min Support Percent of 0.01. MEGAN used the Last Common Ancestor algorithm (LCA, using the naive approach) where each OTU was statistically assigned to the LCA in the taxonomic tree, where the less consistency of taxonomic assignment, the higher up in the tree the assignment is placed for the OTU until the LCA of all likely assignments is reached.

For the analyses of the 12S barcode we used the ANACAPA pipeline (Curd et al., 2019) to create a custom 12S reference database, obtain amplicon sequence variants (ASVs, or unique sequences

identified in the study) from 12S sequences, and perform taxonomic annotation of ASVs within a Bayesian framework. First, we used the CRUX module with default parameters to create a genetic reference library of the 12S teleo metabarcode. We used the European annotated nucleotide (ENA) vertebrate repository (143rd version, downloaded June 7th, 2021; as seed for ecoPCR in silico amplification (Boyer et al., 2015) and the NCBI annotated nucleotide repository (downloaded February 16th, 2023) for BLASTn search of all available 12S sequences. Some local fish references and associated taxonomy were manually added to the CRUX reference and converted to Bowtie format. Sequencing reads underwent quality control and ASV parsing using ANACAPA's second module. Primers and adapters were removed using cutadapt allowing a 30% mismatch. ASV parsing was performed using DADA2 (Callahan et al., 2016), allowing 40 bp minimum sequence length and 20 bp minimum overlap with a maximum of 2 mismatches for forward and reverse read alignment. The ANACAPA classifier module was then used to assign taxonomy to ASVs using a modified version of the BCLA algorithm in Bowtie 2 v2.3. under default parameters, 100 bootstrap replicates to assess the robustness of phylum to species level annotations, considering a 95% bootstrap support for final analyses. We compared the taxonomic assignments produced against FishBase list of fish from California, United States of America, accessed on Nov 6, 2024 that contained 566 marine species (FishBase, 2024) and Smithsonian databases. In many cases where a species not native to California was identified, we manually curated the assignments indicating the most likely local fish species or higher taxa present instead based on these two databases.

After the taxonomic classifications were completed, we conducted a series of additional quality filters: 1) All the OTUs and ASVs identified in any of the negative controls were completely excluded from the analyses (including field, DNA extraction and PCR steps), 2) OTUs/ASVs that were not marine were excluded, including insects and terrestrial plants, and 3) OTUs/ASVs identified as Bacteria and Human were also excluded.

Results

Eukaryotic Diversity (18S barcode)

The analyses identified 12,521 OTUs/species of Eukaryotes present in the samples, from which 1,288 were found in a negative control sample and were completely excluded, along with 9 bacteria, 6 human, 1 insect and 6 terrestrial plants, leaving 11,211 OTUs/species of marine eukaryotes present in the field samples (Supplementary File 1). From these, 2,209 OTUs/species (19.7% from total) did not have any relevant match in GenBank (728 No hits, 1481 Not Assigned) and thus were not assigned taxonomically.

From 9,002 OTUs that were assigned taxonomically, 720 OTUs (7.9%) were identified as Eukaryotes without further classification, indicating again no significant matches were found to support their assignment even at the phylum level. From 8,282 OTUs of Eukaryotes with additional taxonomic information, we found a dozen major taxonomic ranks (Fig. 2), including 5,741 OTUs members of the SAR group that includes a large diversity of poorly studied microeukaryotes from Stramenopiles (983 OTUs), Alveolata (3,689 OTUs) and Rhizaria (884 OTUs). Other diverse major groups identified included Cryptophyceae (300 OTUs), Haptista (201 OTUs) and Discoba (72 OTUs). A relatively small

fraction of OTUs were assigned to three recognized kingdoms: Fungi (47 OTUs), Viridiplantae [plants] (374 OTUs), and Metazoa [animals] (1,287 OTUs).

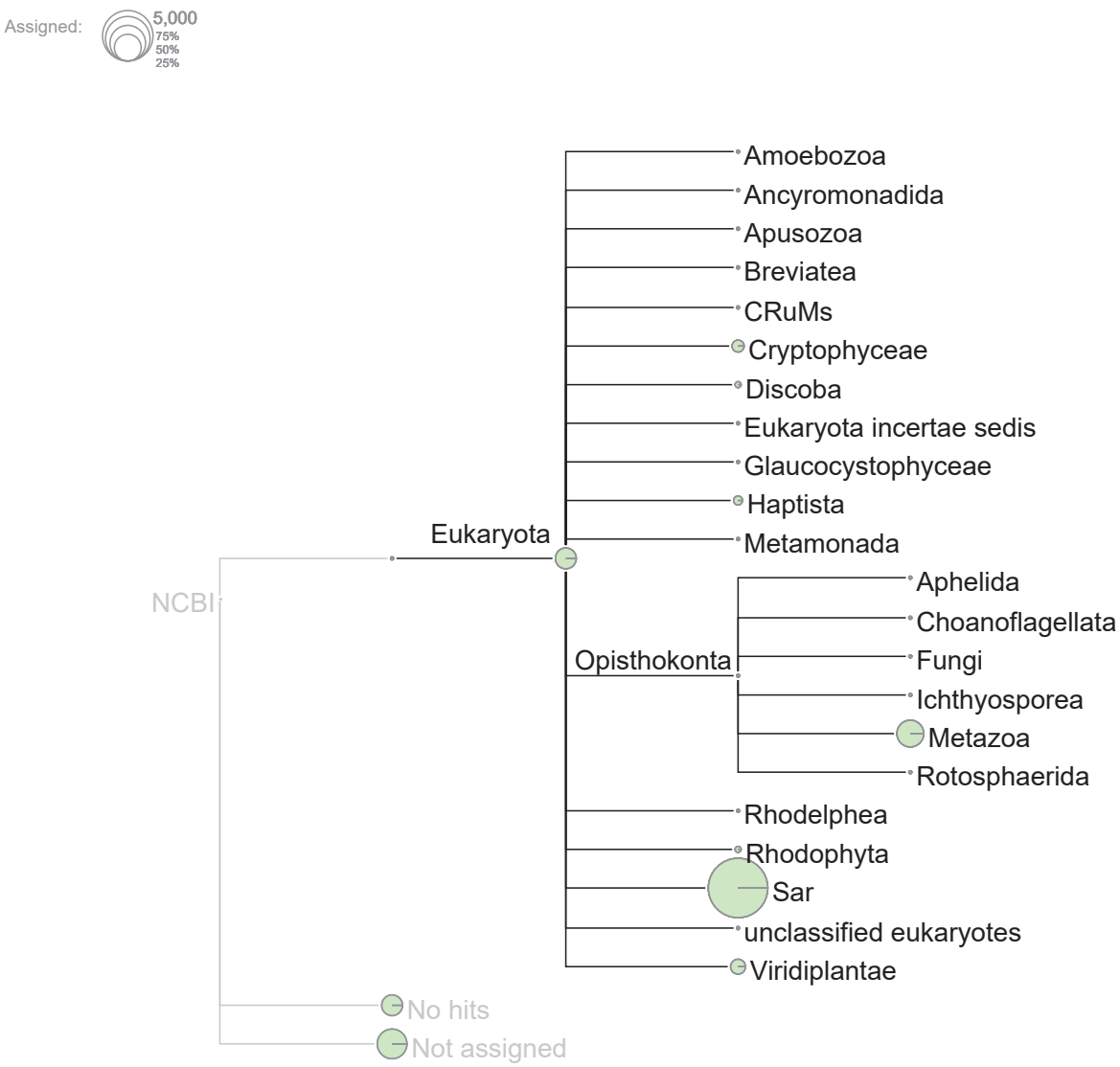

Figure 2. Taxonomic assignments of 11,211 OTUs/species of marine eukaryotes to different kingdoms and other major unclassified taxonomic groups, using the 18S barcode.

Below the kingdom level, the eDNA analysis identified 43 different phyla, 98 classes, 249 Orders, 374 Families, 452 Genus and 421 Species (Supplementary File 1). The most diverse phyla were Ciliophora (499 OTUs), Chlorophyta (367 OTUs), Arthropoda (356 OTUs), Bacillariophyta (343 OTUs), Cercozoa (292 OTUs), Haptophyta (191 OTUs), Apicomplexa (174 OTUs), Annelida (170 OTUs), Cnidaria (117 OTUs), Endomyxa (96 OTUs), Bryozoa (92 OTUs), Rhodophyta (91 OTUs), Chordata (89 OTUs), Euglenozoa (68 OTUs) and Mollusca (61 OTUs). Another 28 phyla had $\leq$ 44 OTUs.

Taxonomic assignments of eukaryotes peaked at the Class level, where 6,059 OTUs were successfully assigned to 98 distinct Classes (Fig. 3, Supplementary File 1). The most diverse classes present were Dinophyceae (2,802 OTUs), Cryptophyceae (300 OTUs), Spirotrichea (298 OTUs), Hexanauplia (267 OTUs), Mamiellophyceae (190 OTUs), Bigyra (184 OTUs), Coscinodiscophyceae (179 OTUs), Polychaeta (162 OTUs), Polycystinea (152 OTUs), Conoidasida (137 OTUs), Oligohymenophorea (89 OTUs), Thecofilosea (88 OTUs), Hydrozoa (85 OTUs), Acantharea (75 OTUs), Ascetosporea (71 OTUs), Bacillariophyceae (66 OTUs), Florideophyceae (66 OTUs), Chloropicophyceae (62 OTUs) and Dictyochophyceae (62 OTUs). Another 79 Classes had $\leq$ 55 OTUs (Supplementary File 1).

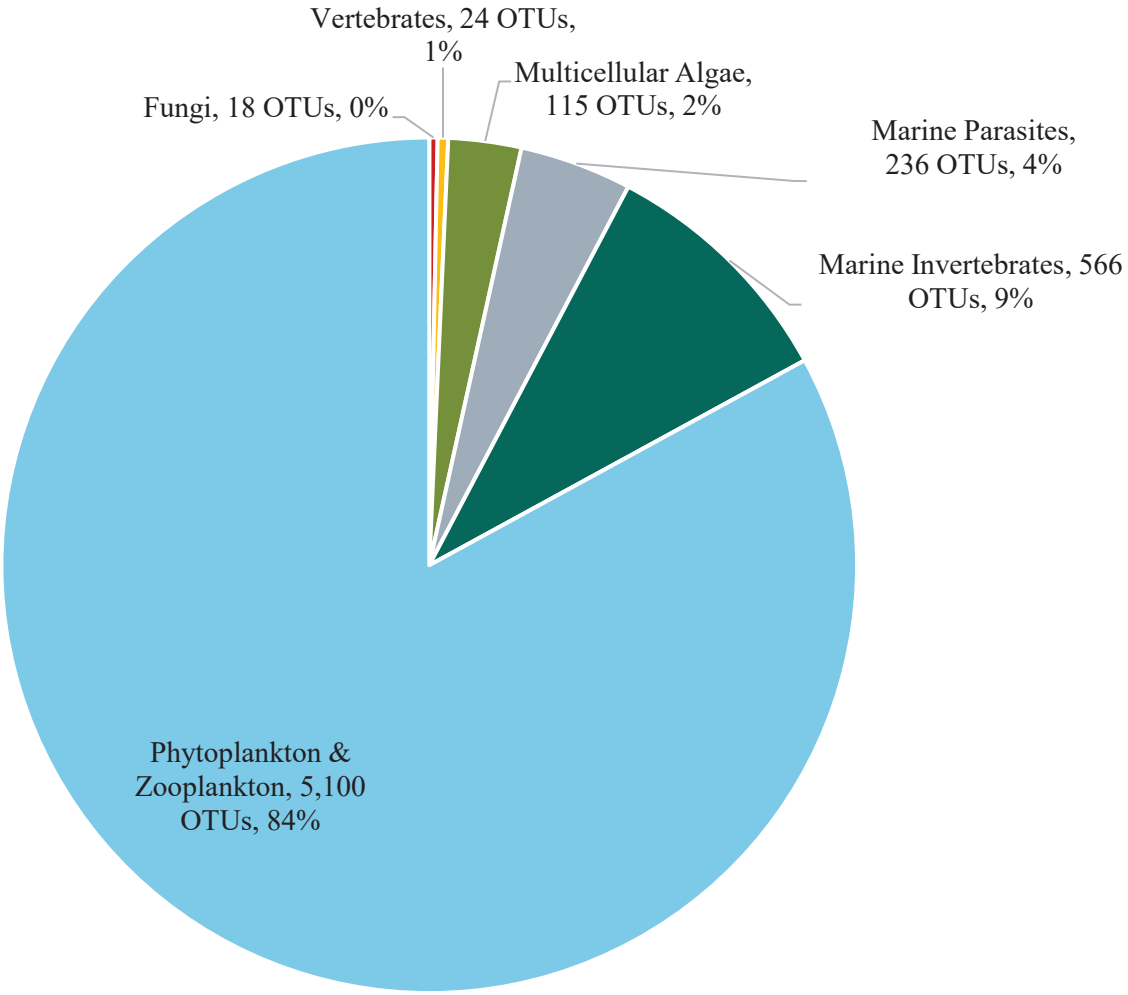

Figure 3. Taxonomic assignment of 6,059 OTUs to 98 different Classes using the 18S barcode, grouped in common categories.

Table 2. Taxonomic assignment of 6,059 OTUs to 98 different Classes using the 18S barcode, grouped in common categories.

Fungi		Vertebrates		Multicellular Algae		Marine Parasites		Marine Invertebrates		Phytoplankton and Zooplankton	
Class	OTUS	Class	OTUS	Class	OTUS	Class	OTUS	Class	OTUS	Class	OTUS
Sordariomycetes	6	Actinopteri	20	Florideophyceae	66	Myxozoa	3	Hydrozoa	85	Dinophyceae	2802
Chytridiomycetes	3	Chondrichthyes	3	Phaeophyceae	41	Aconoidasida	1	Gymnolaemata	55	Cryptophyceae	300
Glomeromycetes	3	Aves	1	Ulvophyceae	8	Conoidasida	137	Anthozoa	25	Spirotrichea	298
Dacrymycetes	1	**Total OTUs**	**24**	**Total OTUs**	**115**	Ascetosporea	71	Stenolaemata	20	Hexanauplia	267
Dothideomycetes	1					Ichthyosporea	24	Bivalvia	38	Mamiellophyceae	190
Eurotiomycetes	1					**Total OTUs**	**236**	Gastropoda	16	Bigyra	184
Pucciniomycetes	1							Holothuroidea	8	Coscinodiscophyceae	179
Saccharomycetes	1							Malacostraca	5	Polycystinea	152
Tremellomycetes	1							Pycnogonida	3	Oligohymenophorea	89
Total OTUs	**18**							Thecostraca	3	Thecofilosea	88
								Ostracoda	2	Acantharea	75
								Scyphozoa	2	Bacillariophyceae	66
								Asteroidea	1	Chloropicophyceae	62
								Crinoidea	1	Dictyochophyceae	62
								Ophiuroidea	1	Pyramimonadophyceae	50
								Polyplacophora	1	Raphidophyceae	24
								Scaphopoda	1	Chrysophyceae	23
								Thaliacea	40	Choanoflagellata	21
								Demospongiae	26	Fragilariophyceae	17
								Ascidiacea	19	Bolidophyceae	16
								Appendicularia	6	Litostomatea	14
								Calcarea	3	Pelagophyceae	13
								Polychaeta	162	Phyllopharyngea	12
								Chromadorea	19	Mediophyceae	11
								Enoplea	5	Centroplasthelida	10
								Palaeonemertea	5	Colpodea	9
								Nassophorea	4	Heterotrichea	8
								Rhabditophora	4	Chlorarachniophyceae	7
								Enopla	3	Pilidiophora	7
								Clitellata	2	Prostomatea	7

								Monogenea	1	Breviatea	4
								Total OTUs	**566**	Chlorophyceae	4
										Bangiophyceae	3
										Chlorodendrophyceae	3
										Elardia	3
										Nephroselmidophyceae	3
										Rhodelphea	3
										Flabellinia	2
										Glaucocystophyceae	2
										Phytomyxea	2
										Stylonematophyceae	2
										Developea	1
										Euglenida	1
										Trebouxiophyceae	1
										Eurotatoria	1
										Pinguiophyceae	1
										Prasinodermophyceae	1
										Total OTUs	**5,100**

We registered the presence of 3,592 OTUs assigned to 249 different orders, from which the most diverse were (Fig. 5): Syndiniales (696 OTUs), Gymnodiniales (225 OTUs), Peridiniales (196 OTUs), Mamiellales (154 OTUs), Prymnesiales (85 OTUs), Eugregarinorida (78 OTUs), Choreotrichida (68 OTUs), Diplonemea (67 OTUs), Cryomonadida (65 OTUs), Poecilostomatoida (65 OTUs), Chloropicales (62 OTUs), Coscinodiscales (53 OTUs) and Thraustochytrida (52 OTUs). Another 236 Orders had $\leq$ 50 OTUs assigned (Supplementary File 1).

The complete list with the 374 Families, 452 Genus and 421 Species detected and the number of OTUs observed within each taxa/sampled site is available in Supplementary File 1.

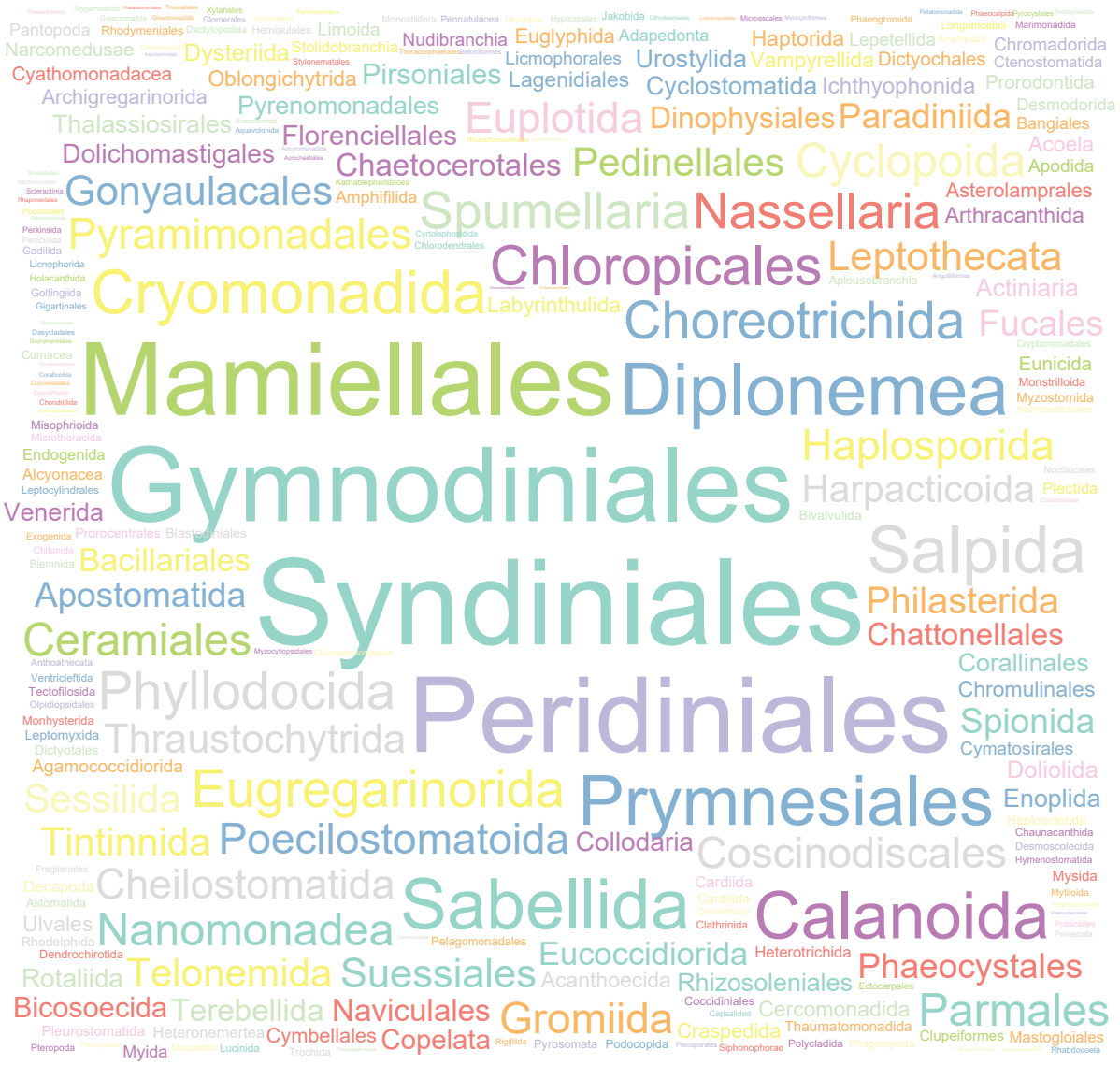

Figure 4. Word cloud with taxonomic assignment of 3,592 OTUs assigned to 249 different orders. Size is proportional to the number of OTUs within each order.

Discussion of 18S Analysis

The presence of 11,211 OTUs/species of marine eukaryotes comprising 43 different Phyla, 98 Classes, 249 Orders, 374 Families (including a dozen of other major taxonomic groups within SAR) indicate high levels of marine biodiversity off the Channel Islands. Nearly 20% were unable to be classified taxonomically using existing databases– even at the highest taxonomic ranks – highlighting the novelty of the sequences obtained in the global context. The lack of reference sequences for many branches of the tree of life for marine eukaryotes indicate many remain poorly described. Notably, about half of all the OTUs/species belong to the group SAR (Stramenopiles, Alveolata and Rhizaria) which are remarkably understudied compared to their diversity, abundance, and key role in recycling nutrients in marine ecosystems (Cohen et al., 2024).

The taxonomic diversity found in the Channel Islands is comparable at higher taxonomic ranks to similar eDNA surveys for the same 18S barcode from a known biodiversity hotspot (Gulf of California, Mexico: 27 Phyla, 95 Classes, 250 Orders, 537 families), with the distinction that the Gulf of California study included only shallow sites (< 25 m) (Mac Loughlin et al., 2024). Other studies using different 18S metabarcodes reported 498 Eukaryotic Families from water and sediment samples in Okinawa Japan, 259 Families in the Black Sea from shallow and deep-water samples, and 287 Families from shallow reefs in Australia (Mac Loughlin et al., 2024).

The diversity of the shallow and deep samples was on average similar, but we observed a larger variation in the number of 18S OTUs/species in the deep samples, including the samples that showed the largest diversity in the entire study (Figure 5). These observations indicate that the deeper oceanic areas around the islands are home to rich biological communities that compare in diversity to the shallow coastal reefs. In fact, the number of OTUs species from all the deep sites (8,412) was larger than those of the shallow sites (7,510, Supplementary File 1). However, the biological communities living in the deep sites are quite distinct from the shallow sites. For example, we found 4,711 OTUs/Species (42%) that were shared between shallow and deep sites, while 3,701 were only found in shallow sites (33%) and 2,799 (25%) were exclusive from deep sites. These results align with similar eDNA surveys where about a third of the diversity from deep sites is not shared with nearby shallow reefs (Cerrillo-Espinosa et al., 2024). The application of eDNA metabarcoding stands out as an efficient method to quickly survey marine biodiversity across the entire tree of life.

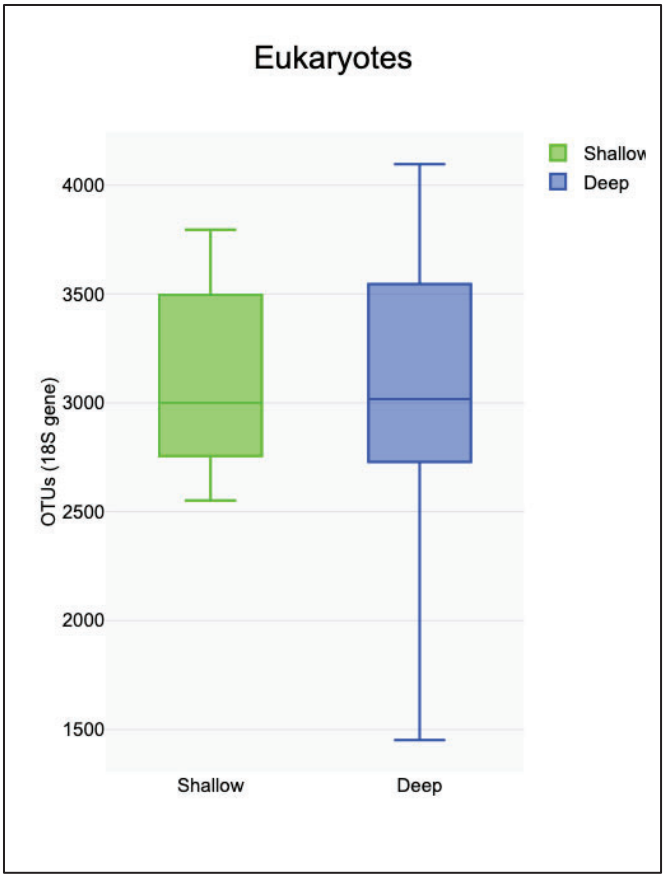

Figure 5. Boxplot showing the distribution of the number of OTUs/species of Eukaryotes observed within each of the nine shallow and nine deep sampled sites.

Fish Diversity (12S barcode)

The eDNA analyses identified 25,249 ASVs or unique sequences total for the 12S barcode. From these, 3,996 ASVs were removed from the analysis because they were found in one or more of the negative control samples (including field, DNA extraction and PCR steps). Other taxa removed included 327 Bacteria, 6 unclassified Eukaryotes, 8 Diatoms, 170 human sequences, 2 amphibians and 13 birds. The final dataset consisted of 20,725 ASVs, (Supplementary File 2) from which more than half, (11,612 or 56.0%) could not be taxonomically assigned because there were not similar to sequences in the custom-made database constructed based on all the fish sequences available worldwide (i.e., GenBank). The remaining sequences were all taxonomically assigned to the phylum Chordata, including two Classes, 27 Orders, 57 Families, 93 Genus and 115 Species (Supplementary File 2). In total at least 128 different taxa were identified at or below the family level (Table 3a & 3b), from which 84 taxa were observed more than once in the study (i.e., excluding singletons or single observations).

Most of the ASVs were identified as bony fish (Class Actinopteri, 9,071 ASVs or 99.5%) and just a few were assigned to sharks and rays (Class Chondrichthyes, 40 ASVs, or 0.5%). The complete list of 27 fish orders detected is available in Supplementary File 2.

From the 57 fish families identified, we conducted analyses (Fig. 6) about the diversity of ASVs or different sequences found (which could be a proxy for taxonomic and phylogenetic diversity within a family), and the number of sequence reads assigned to each family (which could be a proxy about the abundance/biomass of individuals from a particular family, although some known biases exists in the efficiency of PCR amplification for particular species with the universal 12S primers used). The results indicated the family Clupeidae (sardines, anchovies, herring) had the largest number of different sequences (1,526 ASVs, Fig. 6), but a relatively low number of total sequence reads (only 17 K reads), with all of them assigned to the genus *Sardinops* (Table 3a). Here, the mismatch between ASVs and reads could indicate a bias where the primers used show a sub-optimal PCR amplification efficiency for *Sardinops*. In contrast, the family Pomacentridae was second place in terms of ASVs (1,248 Fig. 6), and had the largest number of reads in the entire study (487 K reads), with evidence for the presence of at least 18 different species (including 10 species from the genus *Chromis*, and 3 species from the genus *Stegastes*, among others), and a very large number of ASVs (784) and reads (469 K reads) assigned to the species *Chromis punctipinnis* (Table 3a & 3b).

The third place in terms of diversity of ASVs was the family Sebastidae with 867 ASVs and a high number of reads (267 K reads). Here most reads were assigned to the genus Sebastes (267 K reads), but in total only 176 reads were assigned to four species, suggesting many *Sebastes* species do not have genetic sequences in the reference database. Additionally, for those species that are present in the reference database, their genetic sequences for the 12S barcode could be very similar among species, preventing a taxonomic assignment with 95% confidence.

Table 3a. Summary of fish taxa identified with the 12S barcode taxonomically assigned to the species level, with known ranges covering the Channel Islands and Southern California Bight. Each assignment shows the genus identified and species within each family, as well as the total number of different fish sequences (Amplified Sequence Variants, ASV) and total reads assigned to each taxa.

Family	#ASVs	Total reads	Genus	#ASVs	Total reads	Species identified with known range in Southern CA	Common Name	#ASVs	Total reads
Embiotocidae	207	41,473	*Embiotoca*	28	730	*Embiotoca jacksoni*	Black Perch	10	10
			Phanerodon	1	1	*Phanerodon vacca*	Pile Perch	1	1
Pomacentridae	1,248	487,551	*Abudefduf*	25	50	*Abudefduf troschelii*	Pacific sergeant major	5	5
			Chromis	966	481,223	*Chromis punctipinnis*	Blacksmith	784	469,431
			Hypsypops	5	323	*Hypsypops rubicundus*	Garibaldi*	5	323
Sciaenidae	66	8,735	*Atractoscion*	13	15	*Atractoscion nobilis*	White seabass*	7	7
Bathylagidae	81	1,931	*Lipolagus*	81	1,931	*Lipolagus ochotensis*	Eared blacksmelt	81	1,931
Atherinopsidae	29	4,855	*Atherinopsis*	5	5	*Atherinopsis californiensis*	Jack silverdale	5	5
Carangidae	30	4,575	*Trachurus*	28	4,573	*Trachurus symmetricus*	Pacific jack mackerel*	3	3
Girellidae	32	3,211	*Girella*	32	3,211	*Girella nigricans*	Opaleye	3	13
Kyphosidae	270	68,325	*Medialuna*	200	61,874	*Medialuna californiensis*	Halfmoon*	200	61,874
Clupeidae	1,526	17,841	*Sardinops*	1,526	17,841	*Sardinops sagax*	Pacific sardine*	2	2
Engraulidae	598	223,007	*Engraulis*	598	223,007	*Engraulis mordax*	Northern anchovy*	598	223,007
Merlucciidae	7	7	*Merluccius*	7	7	*Merluccius productus*	North pacific hake*	7	7
Gobiidae	189	35,337	*Typhlogobius*	13	733	*Typhlogobius californiensis*	Blind goby	13	733
Labridae	439	24,015	*Halichoeres*	26	1,825	*Halichoeres (Oxyjulis) californicus*	Senorita Wrasse	1	1
			Semicossyphus	158	1,217	*Semicossyphus (Bodianus) pulcher*	California sheephead*	158	1,217
Lutjanidae	715	46,629	*Lutjanus*	245	571	*Lutjanus argentiventris*	Yellow snapper	6	6
Myctophidae	20	931	*Stenobrachius*	20	931	*Stenobrachius leucopsarus*	Northern lampfish	5	5
						Stenobrachius nannochir	Garnet lanternfish	1	1
Cottidae	60	7,074	*Chitonotus*	23	2,296	*Chitonotus pugetensis*	Roughback sculpin	23	2,296
			Clinocottus	1	1	*Clinocottus analis*	Woolly sculpin	1	1
			Oligocottus	23	4,350	*Oligocottus maculosus*	Tidepool sculpin	2	2
						Oligocottus snyderi	Fluffy sculpin	20	4,347
Pholidae	6	698	*Pholis*	3	3	*Pholis ornata*	Saddleback gunnel	6	698
Hexagrammidae	43	10,362	*Ophiodon*	43	10,362	*Ophiodon elongatus*	Lingcod*	43	10,362
Scorpaenidae	3	3	*Epinephelus*	1	1	*Scorpaenodes xyris*	Rainbow scorpionfish	3	3
Sebastidae	867	267,131	*Sebastes*	816	267,069	*Sebastes macdonaldi*	Mexican rockfish	3	3
						Sebastes paucispinis	Bocaccio*	5	5
Serranidae	597	24,494	*Paralabrax*	96	17,242	*Paralabrax nebulifer*	Barred sand bass*	1	1

Diplophidae	81	9,237	*Diplophos*	81	9,237	*Diplophos taenia*	Pacific portholefish	81	9,237
Fistulariidae	72	12,626	*Fistularia*	72	12,626	*Fistularia commersonii*	Bluespotted cornetfish	29	113
Balistidae	649	3,390	*Balistes*	287	644	*Balistes polylepis*	Finescale triggerfish*	79	197
Heterodontidae	12	1,003	*Heterodontus*	12	1,003	*Heterodontus francisci*	Horn shark*	12	1,003

*Taxa identified in the eDNA analysis that are known to be caught in the California set gillnet fishery, documented by federal observers.

Table 3b. Summary of fish taxa identified with the 12S barcode taxonomically assigned at the family level or below to taxa that are not currently known to have ranges covering the Channel Islands and Southern California Bight. Each assignment shows the genus and species within each family, as well as the total number of different fish sequences (Amplified Sequence Variants, ASV) and total reads assigned to each taxa. Bolded taxa are not known to cover this region; possible alternative species closely related to these taxa were manually identified using Fishbase and Smithsonian databases. If no relevant closely related species were identified, that cell was left blank.

Family	#ASVs	Total reads	Genus	#ASVs	Total reads	Species Matched in Database	Possible closely related local species	Common Name of possible closely related local sp.	#ASVs	Total reads
Embiotocidae	207	41,473	*Ditrema*	1	1	N/A	*19 sp.* *Amphistichus argenteus* *Amphistichus rhodoterus* *Hyperprosopon argenteum* *Hypsurus caryi* *Micrometrus aurora* *Micrometrus minimus* *Phanerodon atripes* *Rhacochilus toxotes* *Zalembius rosaceus*	 Barred surfperch Redtail surfperch Walleye surfperch Rainbow seaperch Reef perch Dwarf perch Sharpnose seaperch Rubberlip seaperch Pink seaperch		
Malacanthidae	159	129,158	*Caulolatilus*	354	129,158	***Caulolatilus cyanops***	*Caulolatilus princeps*	Ocean Whitefish	193	657
						Caulolatilus microps			2	2
			Lopholatilus	1	1	***Lopholatilus chamaeleonticeps***	N/A		1	1
Pomacanthidae	14	34	***Holacanthus***	9	28	N/A				
			Pomacanthus	5	5	***Pomacanthus maculosus***	*Pomacanthus zonipectus*	Cortez Angelfish (some documentation in SoCal)	1	1
						Pomacanthus sexstriatus			1	1
						Pomacanthus xanthometopon			1	1
Pomacentridae	1,248	487,551	*Chromis*	966	481,223	***Chromis viridis***	*Chromis alta*	Oval damselfish	1	6
						Chromis alpha			2	166
						Chromis cinerascens			1	1
						Chromis insolata			8	24
						Chromis multilineata			2	2
						Chromis scotochiloptera			1	1
						Chromis scotti			13	14
						Chromis tingting			1	1
						Chromis yamakawai			1	1
			Mecaenichthys	2	2	***Mecaenichthys immaculatus***			2	2
			Pristotis	1	1	***Pristotis obtusirostris***			1	1

			Amphiprion	2	2	***Amphiprion clarkii***			1	1
			Stegastes	228	5,928	***Stegastes leucostictus***			2	2
						Stegastes variabilis	*S. leucorus*	Whitetail major	2	14
						Stegastes flavilatus			32	4,942
Sciaenidae	66	8,735	*Cynoscion*	1	1	***Cynoscion reticulatus***	*C. parvinis*	Shortfin weakfish	1	1
Congridae	2	14	*Gnathophis*	2	14	***Gnathophis longicauda***	*G. cinctus*	Hardtail conger	2	14
Muraenidae	61	48,988	*Gymnothorax*	36	33,246	***Gymnothorax chilospilus***	*G. mordax*	California moray	2	2
						Gymnothorax flavimarginatus	*Muraena argus*	Argus moray	4	4
						Gymnothorax formosus			1	1
Microstomatidae	2	2	*Nansenia*	2	2	***Nansenia boreacrassicauda***	*N. candida*	White pencilsmelt	1	1
Atherinidae	1	1	*Atherinosoma*	1	1	***Atherinosoma microstoma***	*Atherinops affinis*	Topsmelt silverside	1	1
Exocoetidae	6	60	*Parexocoetus*	1	1	***Parexocoetus brachypterus***	*Cheilopogon californicus*	California flyingfish	1	1
							C. hubbsi	Blotchwing flyingfish		
							Fodiator acutus	Sharpchin flyingfish		
Hemiramphidae	2	9	*Hemiramphus*	2	9	N/A	*Hemiramphus saltator*	Longfin halfbeak		
Chaenopsidae	2	2	N/A			N/A	*Chaenopsis alepidota*	Orangethroat pikeblenny		
							3 sp. Neoclinus	Fringehead		
Labrisomidae	113	35,062	*Malacoctenus*	1	1	***Malacoctenus mararitae***	*Alloclinus hoderi*	Island kelpfish	1	1
							Cryptotrema corallinum	Deep-water blenny		
			Xenomedea	112	35,061	***Xenomedea rhodopyga***	*Paraclinus intergripinnis*	Reef finspot	112	35,061
Tripterygiidae	1	1	N/A			N/A				
Cirrhitidae	4	7	***Cirrhitichthys***	4	7	***Cirrhitichthys oxycephalus***			4	7
Kyphosidae	270	68,325	*Kyphosus*	70	6,451	***Kyphosus cornelii***	*Kyphosus azureus*	Zebra-perch Sea chub	2	5
						Kyphosus labriformis	*K. ocyurus*	Bluestriped chub	27	2,507
Clupeidae	1,526	17,841	*Sardinops*	1,526	17,841	***Sardinops ocellatus***	*Clupea pallasii*	Pacific herring	3	3
Dussumieriidae	24	3,302	*Etrumeus*	24	3,302	***Etrumeus teres***	*Etrumeus acuminatus*	red-eye round herring	24	3,302
Fundulidae	17	13,262	*Fundulus*	7	7	***Fundulus dispar***	*Fundulus parvipinnis*	California killifish	1	1
						Fundulus zebrinus			15	13,260
Eleotridae	1	1	*Ophiocara*	1	1	***Ophiocara porocephala***			1	1
Gobiidae	189	35,337	*Bollmannia*	171	34,595	***Bollmannia boqueronensis***	*Lythrypnus dalli*	Bluebanded Goby	1	1
							Ctenogobius sagittula	Longtail goby		
							Eucyclogobius kristinae	Southern tidewater goby		
							E. newberryi	Tidewater goby		
							Gillichthys mirabilis	Longjaw mudsucker		
							Ilypnus gilberti	Cheekspot goby		
							L. zebra	Zebra goby		

			Gobulus	1	1	***Gobulus crescentalis***	*Quietula y-cauda*	American shadow goby	171	34,595
			Ponticola	13	733	***Ponticola syrman***	*Rhinogobiops nicholsii*	Blackeye Goby	1	1
Odontobutidae	1	1	***Perccottus***	4	18	***Perccottus glenii***			1	1
Holocentridae	5	19	***Myripristis***	1	1	***Myripristis adusta***			1	12
			Sargocentron	1	1					
Apogonidae	1	1	***Foa***	152	423	***Foa leisi***	*Apogon atricaudus*	Plain cardinalfish	1	1
							Apogon pacificus	Pink cardinalfish		
Labridae	439	24,015	***Bodianus***	1	1	***Bodianus diplotaenia***			13	13
						Bodianus rufus			134	405
			Macropharyngodon	1	3	N/A				
			Thalassoma bifasciatum	26	1,825	***Thalassoma bifasciatum***			1	3
			Halichoeres	158	1,217	***Halichoeres dispilus***	*H. semicinctus*	Rock wrasse	1	1
						Halichoeres melanotis			7	7
Lophiidae	13	2,667	*Lophiodes*	4	19	***Lophiodes reticulatus***	*Lophiodes caulinaris*	Spottedtail angler	1	1
			Lophius	43	43	N/A	*L. spilurus*	Threadfin angler		
Haemulidae	74	5,215	*Haemulon*	31	5,172	***Haemulon sexfasciatum***	*Anisotremus davidsonii*	Xantic sargo	15	15
							Brachygenys californiensis	Californian salema		
			Haemulopsis	3	3	***Haemulopsis leuciscus***	*Microlepidotus inornatus*	Wavyline grunt	31	5,172
Lutjanidae	715	46,629	*Hoplopagrus*			***Hoplopagrus guentherii***	*Lutjanus colorado*	Colorado snapper	3	3
			Lutjanus	245	571	***Lutjanus monostigma***			1	1
						Lutjanus rivulatus			1	1
						Lutjanus novemfasciatus	*Lutjanus novemfasciatus*	Pacific dog snapper	1	1
			Paracaesio	1	1	***Paracaesio xanthura***			1	1
			Rhomboplites	1	1	***Rhomboplites aurorubens***			1	1
Mugilidae	1	1	*Mugil*			N/A	*Mugil cephalus*	Striped mullet		
Cottidae	60	7,074	*Scorpaenichthys*	43	10,362	N/A	*Scorpaenichthys marmoratus*	Cabezon	5	397
Liparidae	8	533	N/A	3	3	N/A	*20 sp. Acantholiparis, Careproctus, Elassodiscus, Liparis, Nectoliparis, Paraliparis, Psednos, Rhinoliparis*			
Peristediidae	3	3	*Peristedion*	6	698	***Peristedion brevirostre***			3	3
Sebastidae	867	26,7131	*Sebastes*	816	267,069	***Sebastes schlegelii***	*49 sp. Sebastes*		1	1
						Sebastes vulpes			12	167
Serranidae	597	24,494	*Cephalopholis*	25	2,234	***Cephalopholis panamensis***	*Paralabrax maculatofasciatus*	Spotted sand bass	25	2,234
			Epinephelus	1	1	***Epinephelus bontoides***			1	1
			Mycteroperca	5	20	***Mycteroperca rosacea***			5	20
			Paralabrax	96	17,242	***Paralabrax auroguttatus***	*Paralabrax clathratus*	Kelp bass	1	1
			Rypticus	1	1	***Rypticus bicolor***			1	1
			Hyporthodus	1	1	N/A				

Scombridae	98	13,140	*Scomber*	94	13,135	N/A	*Scomber japonicus*	Chub mackerel		
Scombropidae	1	1	*Scombrops*	1	1	***Scombrops oculatus***			1	1
Stromateidae	3	9	*Peprilus*	3	9	***Peprilus snyderi***	*Peprilus simillimus*	Pacific pompano	3	9
Mullidae	15	560	*Mulloidichthys*	15	560	N/A	*Mulloidichthys dentatus*	Mexican goatfish		
Balistidae	649	3,390	*Balistes*	287	644	***Balistes vetula***	*Xanthichthys mento*	Redtail triggerfish	21	21
			Sufflamen	22	2,065	***Sufflamen verres***			5	27
Diodontidae	76	19,376	*Diodon*	73	19,373	***Diodon liturosus***	*Diodon holocanthus* *D. hystrix*	Longspined porcupinefish Spot-fin porcupinefish	1	1
Tetraodontidae	41	5,918	*Canthigaster*	40	5,917	***Canthigaster jactator***	*Sphoeroides annulatus*	Bullseye puffer	2	2
			Sphoeroides	1	1	N/A	*S. lobatus*	Longnose puffer		
Triakidae	28	6,873	*Mustelus*	27	6,872	***Mustelus manazo***	*Mustelus californicus*	Gray smoothhound	1	1
						Mustelus mosis	*M. henlei*	Brown smooth-hound	7	7
						Mustelus norrisi	*M. lunulatus*	Sicklefin smooth-hound	2	9

The families Lutjanidae (Snappers) and Balistidae (Trigger fish) followed in terms of their number of ASVs with 715 and 649, respectively. However, the number of total reads assigned to each family was relatively small (46 K and 3 K reads, respectively), suggesting again a sub-optimal PCR amplification or relatively low densities/biomass of the species present within these two families. Seven species were found for Lutjanidae, with a very small number of reads assigned at the Genus and species levels, suggesting lack of sequences in the reference databases (Table 3a & 3b).

Other fish families with many reads assigned that could be indicative of high biomass were Engraulidae (Anchovies, 223 K reads, all assigned to *Engraulis mordax*), Kyphosidae (Sea Chubs, 68 K reads, mostly unassigned and 6 K reads assigned to *Kyphosus labriformis*) and Malacanthidae (Tilefishes, 128 K reads, mostly assigned to the genus *Caulolatilus*). Other 51 fish families had $\leq$ 597 ASVs, including seven families represented by a single ASV and a single read (i.e., singletons), the presence of which should be interpreted with caution and verified by other independent data. Within the Chondrichthyes (sharks, skates, rays), we detected the presence of the family Trakidae (Houndsharks, 28 ASVs and 6,873 reads, mostly assigned to the genus *Mustelus* (Smooth-hound sharks) found at the site Heart Attack reef, Santa Cruz Island) and the family Heterodontidae (Horn sharks, 12 ASVS and 1,003 reads, all assigned to *Heterodontus francisci* in the site Cat Rock, Anacapa Island).

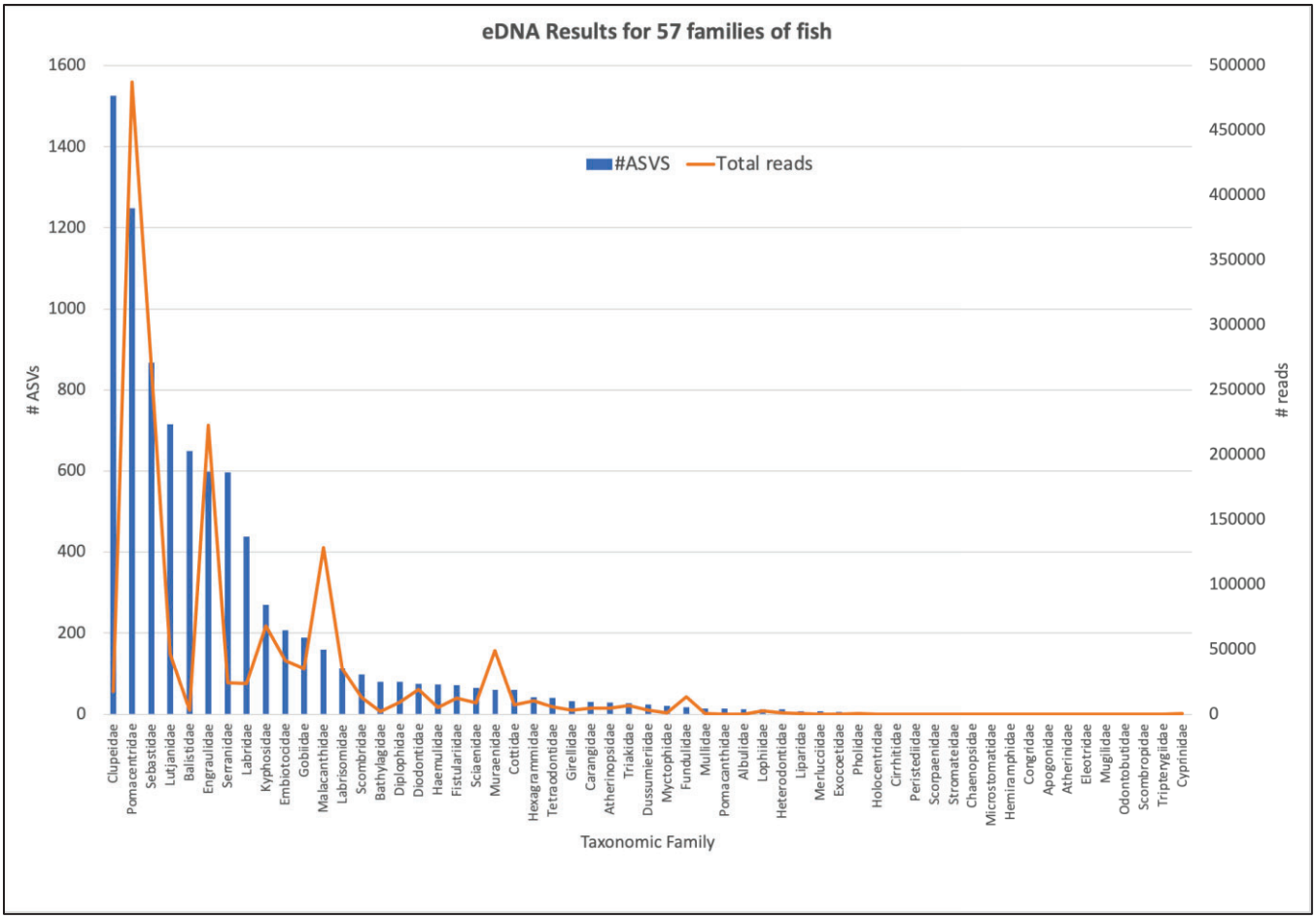

Figure 6. Distribution of unique sequences (ASVs) and number of sequence reads within the 57 fish families detected with the eDNA data.

Discussion of 12S Analysis

Considering we were able to taxonomically assign only 44% of all the ASVs detected (20,725 ASVs), and from these only 34 out of the 128 (26.6%) fish taxa identified with eDNA were correctly assigned to known taxa native to the Channel Islands area (Table 3a), we can roughly estimate that less than a quarter of the fish from the Channel Islands are present in custom made genetic reference databases (including GenBank) for the 12S metabarcode employed. If a species native to the Channel Islands area is not present in the reference databases, then the eDNA sequences are assigned to the best match available in the database, which often represent a closely related species from the same genus or family from elsewhere around the world (highlighted in bold in Table 3b). In total, 94 out of the 128 fish taxa identified were assigned to species not known to be native to California (73.4%) and were manually re-assigned to another local species using FishBase and Smithsonian databases.

While the eDNA survey was able to detect 27 Orders, 57 Families, 93 Genus and 128 different fish taxa, visual scuba surveys of fish conducted simultaneously at each site by three expert divers registered 14 species (Table 4), or 11% of the fish taxa detected with eDNA. In contrast, eDNA detected 11 out of the 14 fish observed with scuba surveys (78.5%), although about half of the taxonomic

assignments were at the species level, and a quarter at the genus and another quarter at the family levels (including the most abundant fish from the scuba surveys Señorita [*Oxyjulis californica*] that was inferred to be present by a high abundance of eDNA sequences assigned to its family, Labridae). These results highlight the value of eDNA data to complement traditional underwater surveys to improve fish assessments, while also confirming that the eDNA surveys are far from perfect and that a few species observed during the scuba surveys might be missed by eDNA (e.g., Kelp greenling *Hexagrammos decagrammus,* Painted greenling *Oxylebius pictus,* California halibut *Paralichthys californicus).* Although these species could be present within half of the fish eDNA data that was not taxonomically assigned due to a lack of genetic references, it is also possible that they were not detected because of their relatively low abundance and limited biomass (and eDNA) in the surveyed sites (Table 4). Low abundance and/or lack of references also could explain the absence in the surveys of species of conservation concern, including the Giant Seabass (*Stereolepis gigas*) and some shark species (Tope shark, *Galeorhinus galeus)*. Other species-specific primers might better detect the presence or absence of particular species of interest, highlighting the value of further research.

Some fish observed at high abundances during the scuba surveys also showed high numbers of eDNA reads and ASVs, but the relationship between the two types of surveys was far from perfect (Table 4). We observed that some of the most abundant species from the scuba surveys had large numbers of ASVs and eDNA reads, including the Blacksmith *Chromis punctipinnis*, the Opaleye *Girella nigricans*, the Sheephead *Bodianus pulcher* or the Black perch *Embiotoca jacksoni*. In other cases, the eDNA data suggested the scuba surveys were severely underestimating the abundance of cryptic fish (Gobiidae) or some of the demersal fish (*Sebastes*), based on their high eDNA abundance. Other species that were not observed during scuba surveys but are known to be very abundant in the Channel Islands showed high abundances of eDNA reads and ASVs, including the Northern anchovy *Engraulis mordax* and the Pacific sardine *Sardinops sagax*. These species are known to be highly migratory pelagic species, so may not be present in kelp forests during visual surveys despite being highly abundant in the region.

Of the 34 fish taxa that we identified in the 12S analysis to the species level, thirteen matched with species observed to be caught in the California set gillnet fishery (Species common names marked with * in Table 3a) (NMFS 2017).

In general, shallow sites had consistently lower numbers of fish ASVs compared to the values observed at the deep sites (Fig. 7). Also, the total number of fish ASVs from shallow sites (9,322) was lower than the value observed from deep sites (12,028, Supplementary File 2). This suggests that a significant fraction of the fish diversity, both at the species level and genetic variation within species, is found exclusively in deep sites. Notably, only 625 ASVs (3%) were shared between shallow and deep sites, while the vast majority were exclusive from shallow (42%) or deep sites (55%). The analysis of 150 distinct fish taxa identified across all sites indicated higher diversity at shallow sites (110 fish taxa identified at various taxonomic levels) compared to deep sites (70 fish taxa, Supplementary File 2). Also, only 30 fish taxa (20%) were shared between shallow and deep samples, while again most were exclusive from shallow (90 fish taxa, or 53.3%) and exclusive from deep sites (26.6%). These patterns suggest that although more fish taxa were taxonomically assigned from shallow sites, a larger number of fish ASVs that are exclusive to deep sites remain to be assigned taxonomically. Complementing reference databases with genetic sequences from taxa inhabiting deep ecosystems could greatly improve the taxonomic resolution and power for ecological inference of the eDNA analyses.

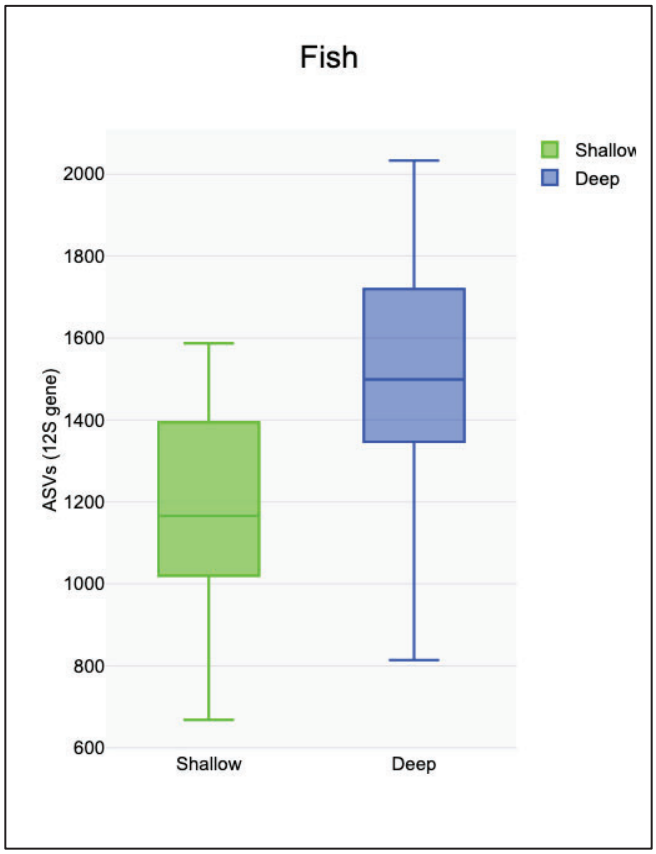

Figure 7. Boxplot showing the distribution of the number of fish ASVs observed within each of the nine shallow and nine deep sampled sites.

Table 4. List of 14 fish detected with visual scuba surveys that were simultaneous to water collection for eDNA at shallow sites, including common and scientific names and observed abundance. For comparison, we include which species were detected with the eDNA survey, their eDNA taxonomic assignment, the total number of ASVs and reads observed.

		SCUBA surveys		eDNA survey		
#	Common Name	Scientific Name	Abundance scuba survey	eDNA taxonomic assignment	ASVs	Total reads
1	Senorita	*Oxyjulis californica*	373	*Labridae*	439	24,015
2	Blacksmith	*Chromis punctipinnis*	277	*Chromis punctipinnis*	784	469,431
3	Opaleye	*Girella nigricans*	53	*Girella nigricans*	32	3,211
4	Sheephead	*Bodianus pulcher*	45	*Bodianus pulcher*	158	1,217
5	Garibaldi	*Hypsypops rubicundus*	39	*Hypsypops rubicundus*	5	323
6	Black perch	*Embiotoca jacksoni*	12	*Embiotoca jacksoni*	28	730
7, 8	Olive/yellowtail, Kelp rockfish	*Sebastes flavidus, Sebastes atrovirens*	14	*Sebastes sp.*	816	267,069
9	Goby (unid)	*Gobiidae*	6	*Gobiidae*	189	35,337
10	Kelp greenling	*Hexagrammos decagrammus*	4		0	0
11	Kelp Bass	*Paralabrax clathratus*	3	*Paralabrax*	96	17,242
12	Rock wrasse	*Halichoeres semicinctus*	3	*Halichoeres*	26	1,825
13	Painted Greenling	*Oxylebius pictus*	1		0	0
14	CA Halibut	*Paralichthys californicus*	1		0	0

Richness of Eukaryotic Operational Taxonomic Units (OTUs) and Fish Amplified Sequence Variants (ASVs) Per Site

The analyses that included the 18 different sampled sites (Table 5, Supplementary Files 1 and 2) indicated the eukaryotic diversity ranged between 1,451 OTUs/Species (Offshore Rat Rock, Anacapa Island) and 4,096 OTUs/Species (Offshore SE Santa Barbara Island, Fig. 8). Other sites showing the largest eukaryotic diversity ($\geq$ 3,570 OTUs/Species) included Flame Reef (Santa Cruz Island), Cat Rock and Offshore Cat Rock (Anacapa Island, Fig. 8). Most sites showed between 2,500 and 3,500 OTUs/Species (Table 5).

Table 5. Number of eukaryotic OTUs/species and fish unique sequences (ASVs) detected via eDNA in each of the 18 sampled sites.

#	Site	Eukaryotes OTUs	Fish ASVs	Fish Taxa
1	Yellowbanks	2,666	668	10
2	Offshore Yellowbanks	3,040	1,492	16
3	Flame Reef	3,612	1,450	20
4	Offshore Flame Reef	3,537	814	5
5	Cat Rock	3,796	1,056	29
6	Offshore Cat Rock	3,570	1,550	19
7	Cathedral Cove	2,787	1,587	22
8	Anacapa Passage	3,017	1,707	26
9	Three Sisters Rocks	2,890	1,358	30
10	Offshore Santa Barbara	4,096	1,379	12
11	Ranger Point	3,457	953	21
12	Offshore Ranger Point	2,614	1,249	9
13	Little Scorpion Anchorage	3,035	1,166	7
14	Offshore Scorpion	2,766	1,499	18
15	Heart Attack Reef	3,000	1,042	27
16	Offshore Heart Attack	2,952	1,758	22
17	Rat Rock	2,551	1,376	21
18	Offshore Rat Rock	1,451	2,033	5
	Sum All	**11,211**	**20,725**	**150**

Fish diversity in terms of ASVs ranged between 668 ASVs (Yellowbanks 1, Santa Cruz Island, Fig. 9) and 2,033 ASVs (Offshore Rat Rock, Anacapa Island). Other sites showing the largest ASV fish diversity ($\geq$ 1,587 ASVs) included Cathedral Cove (Anacapa Island), Anacapa Passage and Offshore Heart Attack (Santa Cruz Island, Fig. 9). Most sites showed between 1,000 and 1,500 fish ASVs (Table 5).

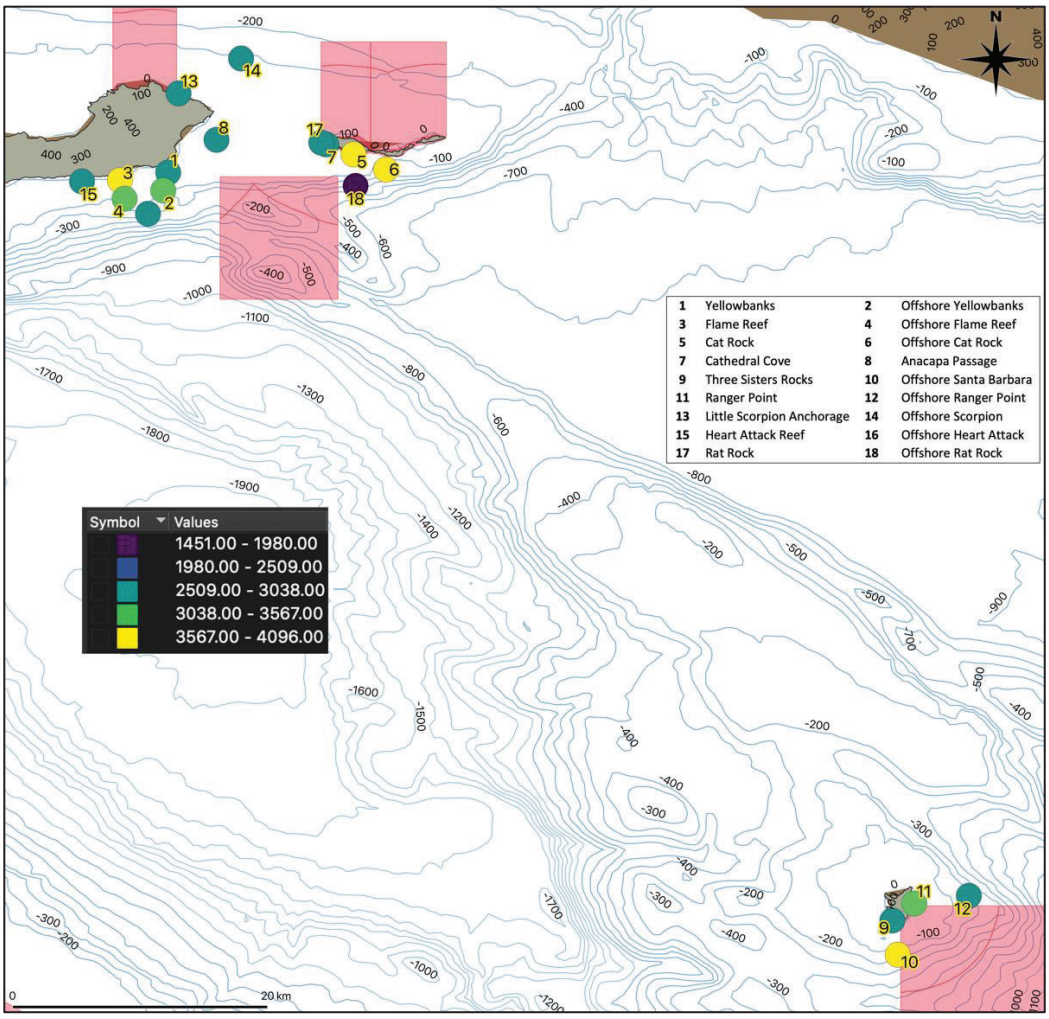

Figure 8. Spatial patterns of diversity of OTUs/Species of Eukaryotes detected via eDNA metabarcoding in the Northern Channel Islands. Numbers in yellow correspond to the site number shown in the right inset. Local bathymetry is represented by 100 m isobaths. Pink polygons represent Marine Protected Areas.

Fish diversity in terms of distinct fish taxa (species) ranged between 5 taxa (Flame Reef Offshore-Santa Cruz Island and Offshore Rat Rock-Anacapa Island Fig. 10) and 30 taxa (Three Sisters Rocks, Santa Barbara Island). Other sites showing the largest ASV fish diversity ($\geq$ 26 fish taxa) included Cat Rock (Anacapa Island), Heart Attack Reef (Santa Cruz Island) and Anacapa Passage, Fig. 10). Most sites showed between 7 and 22 fish taxa (Table 5).

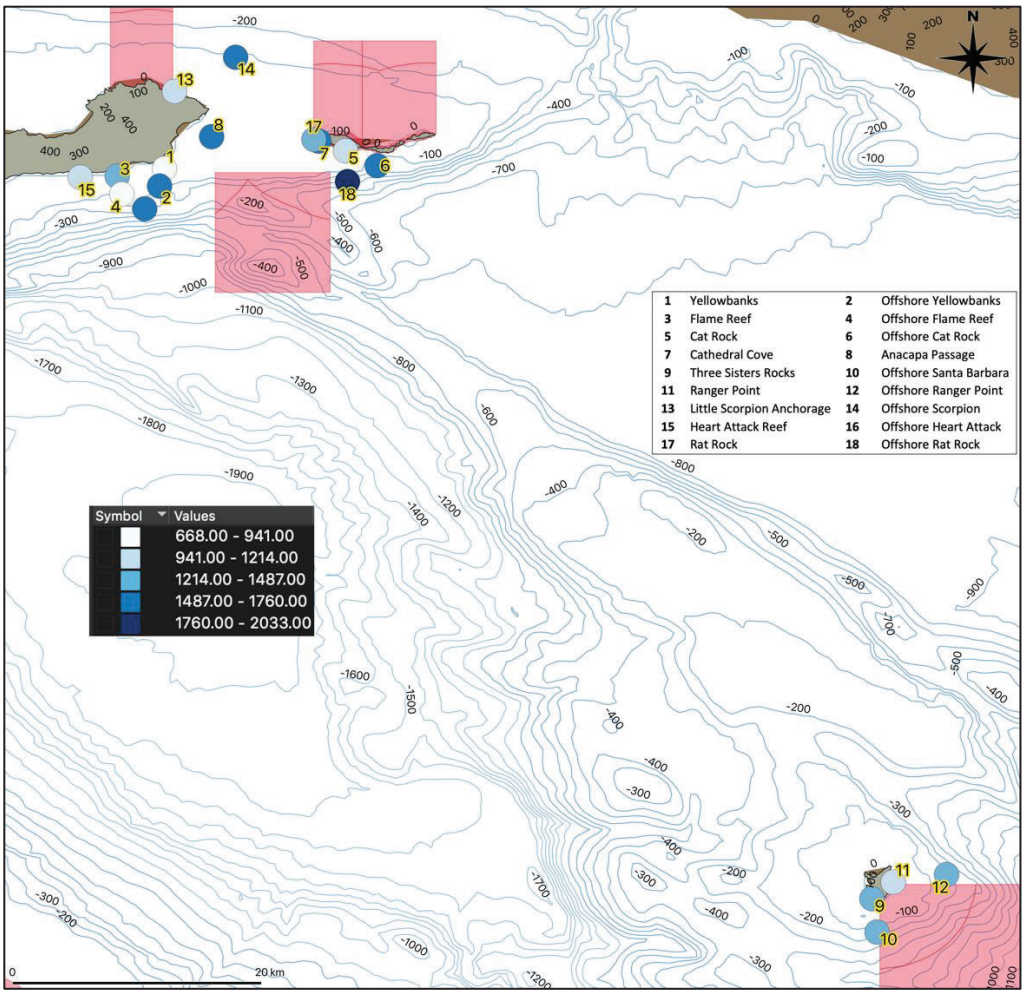

Figure 9. Spatial patterns of diversity of distinct sequences of fish (ASV diversity) detected via eDNA metabarcoding in the Northern Channel Islands. Numbers in yellow correspond to the site number shown in the right inset. Local bathymetry is represented by 100 m isobaths. Pink polygons represent Marine Protected Areas.

Some of the offshore sites sampled with the Niskin bottle at ~100 m deep (e.g., Offshore Cat Rock Anacapa Island and Offshore SE Santa Barbara Island) had the largest diversity of eukaryotes detected in the region (Fig. 8). The sites near the coast (sampled with scuba) generally had higher levels of richness of fish taxa (Fig. 10), while patterns of richness of fish ASV (Fig. 9) were not aligned with the number of fish taxa detected at each site.

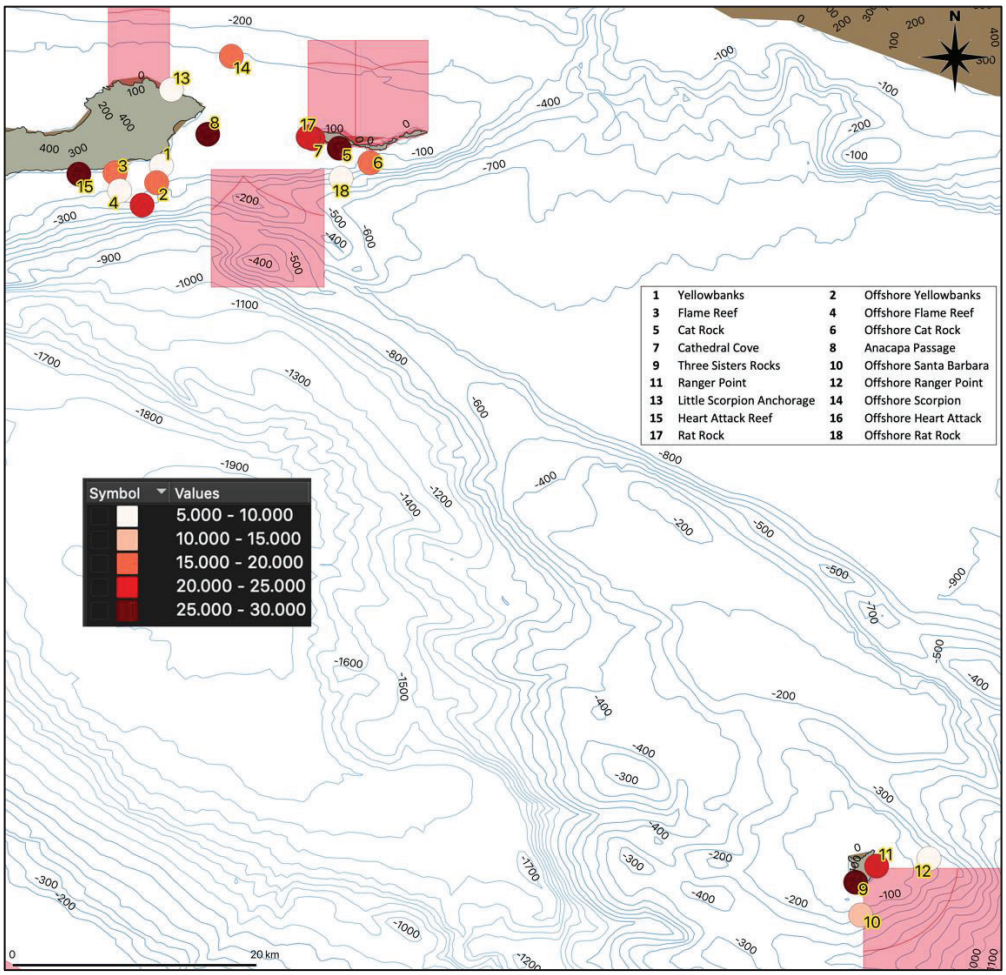

Figure 10. Spatial patterns of diversity of distinct fish taxa (species) detected via eDNA metabarcoding in the Northern Channel Islands. Numbers in yellow correspond to the site number shown in the right inset. Local bathymetry is represented by 100 m isobaths. Pink polygons represent Marine Protected Areas.

Conclusions

eDNA metabarcoding was used to characterize complex marine communities with thousands of species, highlighting the diversity of marine eukaryotes in the marine ecosystems surrounding the Channel Islands is at least an order of magnitude larger than what can be observed using other methods in this region. The application of eDNA methods to sample deeper ecosystems that are logistically challenging to study illustrates how useful this tool is. This analysis showed the deep oceanic areas around the islands are home to rich biological communities that compare to or sometimes surpass the diversity of shallow coastal reefs. We also found that the biological communities from the deep ecosystems contain very distinct sets of species compared to those from the shallow ecosystems, and that only 42% of the eukaryotes and 20% of the fish taxa were shared between both sampled depths. Describing, understanding and protecting the high levels of marine biodiversity at shallow and deep ecosystems is important because of the key ecosystem services they provide to sustain life. For example, half of the eukaryotes found are members of the SAR (Stramenopiles, Alveolata and Rhizaria) group of microeukaryotes that are hyperdiverse, ubiquitous and abundant, but also poorly described taxonomically and ecologically. These species may play critical roles in the cycle of nutrients in the ocean and in marine food webs.

Although eDNA metabarcoding excels at detecting the presence of sequences from diverse organisms in the environment, we are faced with the major challenge of lack of reference sequences from taxonomically identified samples for most of the taxa. In both the eukaryotic and fish libraries, 20 to 50% of the sequences could not be assigned confidently at all, and for the rest we observed a bias towards assignments at higher taxonomic ranks (Phyla, Class, Order, Family). The proportion of taxonomic assignments at the genus or species level was only 3.6% for the eukaryotes and 30% for the fish. Complementing reference databases with genetic sequences from taxa inhabiting deep ecosystems could greatly improve the taxonomic resolution and power for ecological inference of the eDNA analyses.

Since marine species closely track shifting isotherms due to climate change towards higher latitudes but also greater depths (Pinsky et al., 2020), techniques like eDNA metabarcoding will become more useful to monitor temperature-driven community restructuring in-situ at different ocean depths and across different seasons and years. Projected range shifts based on climate velocities are faster in the deep ocean compared to the surface, particularly for the mesopelagic zone between 200-1,000 m deep (Brito-Morales et al., 2020). Since deep reefs are important habitat for some commercial species, the redistribution of marine biodiversity may also have economic impacts to the fishing sector.

Some non-selective fishing gears that have raised alarm and conservation concerns due to impacts to sensitive species have been prohibited in areas to protect nearshore ecosystems. For example, the California set gillnet fishery is fished exclusively in the Southern California Bight but the area within one nautical mile of the Channel Islands and three nautical miles of the mainland is closed to this gear type, in addition to a network of marine protected areas. These results, however, highlight the high diversity of marine life in the deeper and offshore ecosystems of southern California still open to set gillnets, and the value of biodiversity protections across depth ranges. The lack of understanding of these deep communities – which species inhabitant them, their roles in functional and resilient ecosystems, and which species may be disappearing without monitoring due to human impacts – is a critical gap impeding conservation and management. By failing to recognize the interconnectedness of

shallow and deepwater habitats, we risk undermining the very biodiversity that we are trying to protect. These often-overlooked deep-sea communities harbor species that play pivotal roles in maintaining ecological balance and resilience. Without a better understanding of these ecosystems, we cannot fully assess which species are vulnerable to overfishing, climate change or habitat destruction, which inevitably leads to gaps in conservation and management strategies. In the absence of such knowledge and understanding, precautionary management and regulation of threats, including fishing, pollution, fossil fuels and plastics, should be employed. Studies such as these provide critical 21st century baselines of regional biodiversity for persistent monitoring as anthropogenic threats continue to impact all parts of our ocean ecosystems.

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