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Journal
ICES Journal of Marine Science, 78(9)

ISSN
1054-3139

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Publication Date
2021-11-25

DOI
10.1093/icesjms/fsab107

Peer reviewed

ICES Journal of
Marine Science

ICES Journal of Marine Science (2021), https://doi.org/10.1093/icesjms/fsab107

Recommendations for interpreting zooplankton metabarcoding and integrating molecular methods with morphological analyses

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Matthews, S. A., Goetze, E., and Ohman, M. D. Recommendations for interpreting zooplankton metabarcoding and integrating molecular methods with morphological analyses. – ICES Journal of Marine Science, 0: 1–10.

Received 14 December 2020; revised 6 May 2021; accepted 18 May 2021.

Metabarcoding of zooplankton communities is becoming more common, but molecular results must be interpreted carefully and validated with morphology-based analyses, where possible. To evaluate our metabarcoding approach within the *California Current Ecosystem*, we tested whether physical subsampling and PCR replication affects observed community composition; whether community composition resolved by metabarcoding is comparable to morphological analyses by digital imaging; and whether pH neutralization of ethanol with ammonium hydroxide affects molecular diversity. We found that (1) PCR replication was important to accurately resolve alpha diversity and that physical subsampling can decrease sensitivity to rare taxa; (2) there were significant correlations between relative read abundance and proportions of carbon biomass for most taxonomic groups analyzed, but such relationships showed better agreement for the more dominant taxonomic groups; and (3) ammonium hydroxide in ethanol had no effect on molecular diversity. Together, these results indicate that with appropriate replication, paired metabarcoding and morphological analyses can characterize zooplankton community structure and biomass, and that metabarcoding methods are to some extent indicative of relative community composition when absolute measures of abundance or biomass are not available.

Keywords: *California Current Ecosystem*, community structure, metabarcoding, zooplankton, ZooScan

Introduction

The field of zooplankton ecology is beginning to embrace metabarcoding as an approach for describing spatial patterns in diversity and community composition, but there are some notable challenges in data interpretation. It remains difficult to link molecular estimates of diversity and community structure with morphological richness and abundance (Laakmann *et al.*, 2020), and the lack of standardization in metabarcoding methods makes it difficult to compare molecular richness across studies (Santoferrara, 2019). A better understanding of the relationship between metabarcoding and morphological analyses can help optimize methodological choices for studying zooplankton ecology (Bucklin *et al.*, 2019; Brisbin *et al.*, 2020). Here, we provide recommendations for best practices regarding subsampling, replication, and ethanol preservation. In addition, we test whether metabarcoding read counts are a suitable representation of relative biomass within the zooplankton community.

One of the strengths of metabarcoding is the ability to rapidly characterize community richness. Estimates of zooplankton richness using molecular markers can be up to an order of magnitude greater than morphological species richness (Laakmann *et al.*, 2020). The increased richness captured by metabarcoding may be due to detection of morphologically cryptic species, increased sensitivity to rare species, detection of intraspecific genetic variability (Brown *et al.*, 2015), or presence of pseudogenes or PCR chimeras. In contrast, many zooplankton metabarcoding pipelines require subsampling prior to DNA extraction which can potentially decrease observed richness (Loos and Nijland, 2020). Quantitative subsampling of bulk-fixed samples is a common procedure for zooplankton analyses (Frolander, 1968; van Guelpen *et al.*, 1982), but the effects of subsampling on molecular diversity have not yet been tested.

A recurring challenge for metabarcoding is characterizing community structure from read counts (Laakmann *et al.*, 2020). While species richness is an important aspect of diversity, abundance and demographic structure are also important for understanding ecosystem dynamics (Elbrecht and Leese, 2015). PCR and sequencing biases can be normalized by including mock communities. This approach has been used for some studies of zooplankton communities (e.g. Hirai *et al.*, 2017) but remains relatively rare, and the effects of body composition, life history stage, cell count, copy number, and primer bias across diverse taxonomic groups are greater in metazoan than in microbial communities (Braukmann *et al.*, 2019). Despite these potential sources of bias, read counts have been positively correlated with morphologically identified abundance or biomass for some zooplankton taxa and in some ecosystems (Lindeque *et al.*, 2013; Hirai *et al.*, 2015; Harvey *et al.*, 2017; Bucklin *et al.*, 2019).

Preservation of field-collected zooplankton samples requires advance selection of a fixation protocol that is optimized for the target organisms and methods. The most common fixative for molecular analyses is 95–100% non-denatured ethanol (Loos and Nijland, 2020). However, ethanol is acidic and long-term storage of zooplankton tissue in ethanol can further increase acidity, resulting in dissolution of calcareous structures (Oakes *et al.*, 2019). This effect is particularly important for long-term preservation of calcifying organisms, when shell dissolution and changes in shell morphology may be interpreted as indicators of ocean acidification (e.g. Bednaršek *et al.*, 2017). Ammonium hydroxide has been used as a neutralizing agent for ethanol-preserved samples, successfully preserving pteropod shells for morphological analysis (Bednaršek and Ohman, 2015; Oakes *et al.*, 2019). However, the effect of ammonium hydroxide neuralization on molecular analyses is unknown.

In this study, we test the effects of subsampling, PCR replication, and preservation method on metabarcoding analysis of zooplankton community richness. We also directly compare paired metabarcoding and imaging analyses to test whether there is a relationship between read counts and morphology-based community composition at coarse taxonomic levels. We expected PCR replication to be a useful method for determining whether rare sequence variants are real or artifacts, and we expected minimal effects of physical subsampling on observed diversity. We also expected to find no relationship between normalized read counts and zooplankton abundance or biomass, given the many methodological steps at which biases could be introduced. We tested these questions using two molecular markers and ZooScan digital imaging of net-collected zooplankton samples from a range of environments across the California Current upwelling biome.

Methods

Zooplankton collection

Samples were collected between 29 April–8 May 2016 on cruise P1604 of the *California Current Ecosystem* LTER program using a depth-stratified 1 m^2 mouth area, 202 μm mesh MOCNESS with paired day and night tows (Table S1). Sampling locations (designated "Cycles"; see Ohman *et al.*, 2013) ranged from the high-biomass nearshore upwelling environment to the offshore mesotrophic California Current. Zooplankton were sampled in 25 or 50 m vertical strata between the surface and 400 m. Each net-collected sample was quantitatively split with a Folsom splitter, then 50% fixed in 95% non-denatured ethanol neutralized with 5 mM ammonium hydroxide and 50% fixed in 1.7% formaldehyde buffered with sodium tetraborate. A total of 64 sets of paired ethanol and formaldehyde fixed samples were included in this analysis.

ZooScan Imaging

Zooplankton samples preserved in formaldehyde were imaged using a ZooScan (Gorsky *et al.*, 2010; Ohman *et al.*, 2012). Samples were size fractioned into 0.2–1.0-, 1.0–5.0-, and > 5-mm fractions. Quantitative subsamples of each were manually dispersed on the scanning surface, then digitally scanned. Images were segmented into regions of interest (ROIs) in ImageJ, and ROIs classified using machine-learning algorithms trained on manually sorted images of preserved zooplankton from the CCE (Ellen *et al.*, 2015). In addition, 100% of taxonomic assignments of ROIs were validated manually. Carbon biomass for each ROI was calculated from feret diameter using taxon-specific relationships between length and carbon (Lavaniegos and Ohman, 2007). Taxon abundance and carbon biomass were standardized for aliquot volumes and the volume of seawater filtered for each sample.

DNA extraction and amplification

Ethanol-fixed zooplankton samples were quantitatively split to a subsample small enough for DNA extraction with OMEGA EZNA Blood and Tissue Maxi kits (<5 g, typically 1/8–1/16th of the sample). Three to six subsamples were analyzed for four high biomass samples (subsequently termed "subsample replicates"; see Table S1). Subsample replicates were treated independently throughout extraction and amplification. DNA extractions were modified as previously described (Sommer *et al.*, 2017). Extraction negative controls were performed on a blank Nitex filter and included in all PCR steps. Eluent DNA concentrations were normalized to 20 ng μl^{-1} prior to PCR amplification.

To more broadly resolve the zooplankton community, two marker regions were amplified and sequenced. A 313-bp region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified with jgLCO1490 and jgHCO2198 (Leray *et al.*, 2013), and a 400-bp fragment in the V4 region of 18S was amplified using Uni18S and Uni18SR (Zhan *et al.*, 2013). Each subsample was amplified and sequenced in triplicate (subsequently termed "PCR replicates"). We used two-step PCR amplification and library preparation with duplicate dual indexing to mitigate index hopping, with unique indexes for PCR replicates to test for index biases and stochastic variability (Table S1 and S2; Costello *et al.*, 2018). The first reaction for each PCR replicate was duplicated, then the two reactions pooled and diluted as template for the indexing PCR (Table S2). All PCRs used high-fidelity "MyFi" polymerase (Bioline). Amplified DNA was bead-cleaned and final DNA concentrations quantified by Qubit or PicoGreen. Negative controls were included in sequencing runs if DNA was detected in the bead-cleaned product. Samples were pooled in equimolar concentrations and sequenced on two Illumina MiSeq runs (300bp paired-end V3).

Bioinformatic processing

Sequences were demultiplexed using cutadapt, and only sequences with exact matches to all four indexes retained (Martin, 2011).

Subsequent bioinformatic filtering in QIIME2 v. 2020.6.0 (Bolyen *et al.*, 2018) used marker-specific criteria as follows. For 18S, forward and reverse reads were truncated at 290bp and 275bp. Amplicon sequence variants (ASVs) were denoised independently for each sequencing run using DADA2 within QIIME2 with consensus chimera detection and pseudo-pooling (Callahan *et al.*, 2016). ASVs were not clustered in operational taxonomic units (OTUs) for 18S, as this gene region is highly conserved and even 99% OTUs can fail to resolve species-level differences (Clarke *et al.*, 2017). ASVs were aligned using MAFFT and taxonomy assigned using the 99% SILVA132 database, and by determining the lowest common ancestor (LCA) of up to five top *blastn* hits from GenBank (Pedregosa *et al.*, 2011; Yilmaz *et al.*, 2014; Clark *et al.*, 2016).

Sequences from COI were analyzed similarly. Forward and reverse reads were truncated at 280 and 250 bp. Denoised ASVs from DADA2 from each sequencing run were merged and clustered into *de novo* OTUs at 97% similarity using VSEARCH in QIIME2 (Rognes *et al.*, 2016). The 97% clustering threshold was selected so as not to overestimate alpha diversity, as intraspecific dissimilarity at this region of COI can reach 3–9% (Leray *et al.*, 2013). Representative sequences for each OTU were assigned taxonomy using the MIDORI "unique" database, and by LCA of GenBank *blastn* matches (Pedregosa *et al.*, 2011; Clark *et al.*, 2016; Machida *et al.*, 2017). All subsequent analyses were carried out in R 4.0.0 (R Development Core Team, 2009).

Statistical analyses

Statistical tests in R used *"phyloseq," "vegan,"* and the *tidyverse* (Dixon, 2003; McMurdie and Holmes, 2013; Wickham *et al.*, 2019). Contaminant sequences were identified from negative controls with the combined method in "*decontam*," with independent detection for each marker and sequencing run (Davis *et al.*, 2018). Contaminants were removed from all samples and negative controls were excluded from subsequent analyses. Sequences identified as non-metazoans, fishes, or mammals also were removed.

Samples were rarefied to 20,000 reads and 25,000 reads for 18S and COI, respectively, with samples falling below these thresholds removed. The relationship between rarified observed richness, defined as OTU/ASV counts, and sequencing depth was tested with Spearman's rank-order correlation. Appearance in PCR replicates was tested with a Kruskal-Wallis 1-way ANOVA, including only samples retaining at least three PCR replicates. Observed richness was plotted against the fraction of the plankton sample analyzed, illustrated with the nonparametric LOESS best fit.

To test whether relative read counts were correlated with independent ZooScan-based measures of biomass and abundance, zooplankton sequences were compared to numerical abundance and carbon biomass for taxonomic groups resolved using both digital imaging and at least one metabarcoding marker. Taxonomic groups were combined as necessary so that comparison groups were the same for both methods, including collapsing the fine taxonomic resolution that is present in metabarcoding data. Cnidarians and ctenophores were combined into a single category. Malacostracan sequences were compared against ZooScan-identified euphausiids and shrimp-like decapods. Comparisons were made for all thaliaceans together, and for salps, doliolids, and pyrosomes where identifiable. Separate comparisons were made for eucalanid copepods and for calanoid copepods excluding eucalanids. All data were converted to proportions and arcsine square root transformed, and Pearson's product-moment correlations were computed for each comparison, with Bonferroni correction.

Ethanol neutralization

To test the effects of ethanol neutralization with ammonium hydroxide on molecular diversity, three samples were collected near La Jolla Canyon using a 1 m diameter, 333 μm mesh ring-net surface tow on 18 December 2017 and near the CCE2 mooring (http://mooring.ucsd.edu/dev/cce2/cce2_12/) using a 0.71 m diameter, 202 μm mesh Bongo net towed obliquely to 180 m on 13–14 March 2018. Each sample was split quantitatively, and 50% preserved in unamended 95% non-denatured ethanol (pH 6.5) and 50% in 95% non-denatured ethanol neutralized with 5-mM ammonium hydroxide (final pH 8.0). These samples were analyzed using metabarcoding as described above, except were sequenced only at the COI marker. These samples were processed in their entirety, using multiple DNA extractions as necessary. Amplified samples were sequenced on two Illumina MiSeq runs, with each sample included in both runs (300bp paired-end V3). Sequences from replicate extractions and MiSeq runs were combined within each sample, and samples were rarefied to the lowest sequencing depth. Observed richness in the paired samples was compared using a Wilcoxon Matched Pairs Signed Rank test. Non-rarefied data were converted to relative abundances, samples clustered using average neighbor clustering based on Bray–Curtis distance and clusters tested for significance with a SIMPROF test. A PERMANOVA was subsequently used to test whether sampling event or preservation method affected observed community composition (Oksanen *et al.*, 2009).

Results

Bioinformatics

On average, 95% of reads demultiplexed by dual 8-mer indexing had the appropriate 6-mer indexes. A total of 12,923,747 sequences were recovered at the 18S marker, 6,882,057 of which passed quality and chimera filtering and were denoised into ASVs. After removal of 16 ASVs identified as contaminants and 280 non-metazoan, mammal, and fish ASVs, 966 ASVs were recovered at 18S. Of the 13,014,170 total COI sequences, 8,751,265 passed quality and chimera filtering. Removal of 19 contaminants and 105 non-metazoan, mammal, and fish OTUs resulted in 1943 OTUs at COI. Observed taxa spanned 17 phyla and included some meroplanktonic and parasitic species (Table 1). OTU rarefaction curves begin to reach saturation at 25,000 reads at COI, and 20,000 reads at 18S (Figure 1a and b). Despite rarefaction curves approaching saturation, richness was positively correlated with sequencing depth at COI (Figure 1c, $p = 2.7\text{e-}09$, $\rho = 0.411$, two-sided Spearman's rank), and negatively correlated at 18S (Figure 1d, $p = 7.4\text{e-}12$, $\rho = -0.483$, two-sided Spearman's rank). Analyses with non-rarified data were similarly significant.

Sub-sampling and PCR replication

Each plankton subsample was analyzed with three independent PCR replicates with unique multiplexing indexes. There was no significant difference in community structure between PCR replicates, ($p \geq 0.05$, SIMPROF analysis of Bray-Curtis distance, not shown). However, there was some variability in the appearance of

Table 1. The number of OTUs or ASVs resolved by each marker for all taxa classified at least to phylum. OTU or ASV counts for each group are reported for both taxonomic assignment methods used and for both marker regions, such that a species can appear in each of the four columns independently. Taxa not classified to phylum are absent from this table.

	COI OTUs		18S ASVs	
	NCBI	MIDORI	NCBI	SILVA
Annelida	47	18	54	49
Arthropoda	995	742	478	502
Brachiopoda	–	–	5	2
Bryozoa	12	8	1	1
Chaetognatha	19	5	–	–
Chordata (Tunicata)	9	1	53	50
Cnidaria	80	64	117	112
Ctenophora	4	–	9	6
Echinodermata	39	25	20	20
Hemichordata	–	–	2	2
Mollusca	142	30	38	35
Nematoda	2	1	–	–
Nemertea	6	2	3	3
Phoronida	1	–	2	–
Platyhelminthes	9	2	2	2
Porifera	–	–	3	–
Sipuncula	1	–	5	5

taxa between PCR replicates. Taxa that did not appear in all three PCR replicates tended to be lower rank order OTUs and ASVs that were less abundant based on sequence counts, but more abundant taxa were also often absent from one or more of the PCR replicates (Figure 2, $p < 2.2e\text{-}16$, Kruskal–Wallis one-way ANOVA). Replicate analyses with non-rarefied data showed similar results.

Each zooplankton sample was subsampled to the maximum biomass possible for a single DNA extraction, resulting in different fractions of the parent sample being analyzed. Observed richness within subsamples increased with the fraction of parent sample analyzed, with richness appearing to saturate around 6.25%, or 1/16th of the initial bulk sample (Figure 3), for both markers. Richness was not correlated with the volume of seawater filtered (Figure 3, colour, Spearman's rank; COI: $p = 0.085$, $\rho = 0.124$; 18S: $p = 0.213$, $\rho = 0.094$). Replicate analyses with non-rarefied data found similar correlations and significance values.

Comparisons between imaging and sequencing

To test whether relative read abundance reflects community structure, read counts were compared with numerical abundance and carbon biomass from ZooScan analyses. Comparisons were made using proportions, as metabarcoding data are proportional (Lindeque *et al.*, 2013; Hirai *et al.*, 2015), and were possible for 16 taxonomic groups of zooplankton, 12 of which were resolved at both metabarcoding markers. Of the 16 taxa analyzed, 15 groups showed a significant correlation ($P \leq 0.05$) between proportion of biomass and proportion of sequences at either COI, 18S, or both (Table 2), although the strength of the correlation was variable. More abundant taxa showed a stronger relationship between carbon biomass and sequences (Table 2, Figure 4). At COI, the strength of the relationship was significantly correlated with average proportion of sequences, but this relationship was not significant at 18S (Figure 4). There was some variability between markers, with oithonids, do-

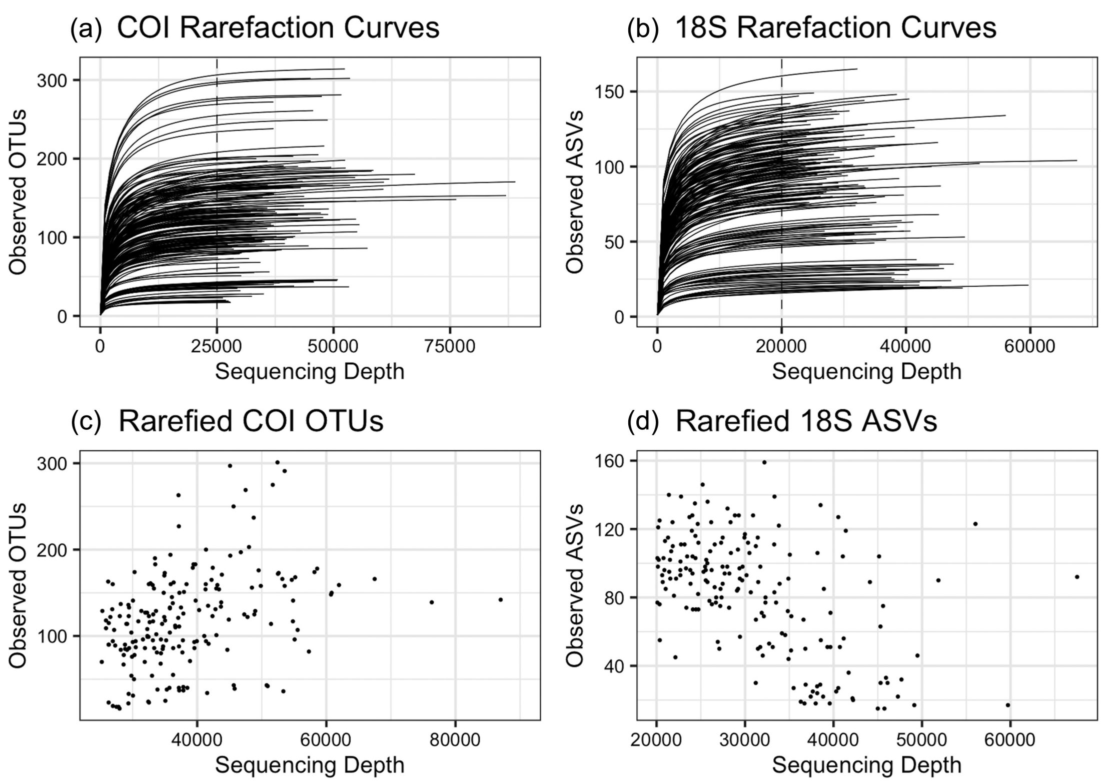

Figure 1. Rarefaction curves for (a) COI OTUs and (b) 18S ASVs, based on sequencing depth. Dashed lines represent the rarefaction depth for each marker. Richness, measured as observed OTUs/ASVs, increased with increasing sequencing depth at (c) COI, but not at (D) 18S.

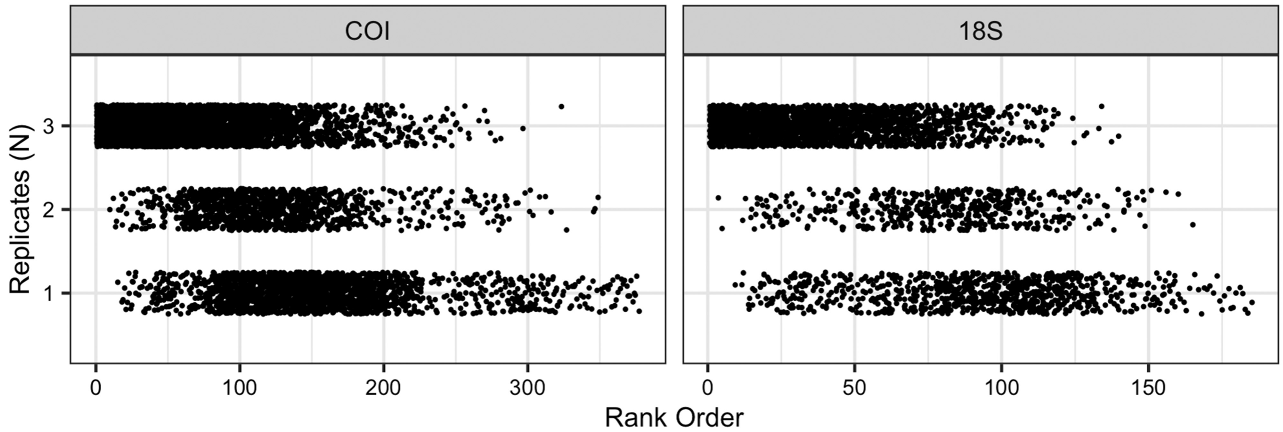

Figure 2. The number of PCR replicates in which each OTU/ASV occurs in relation to rank order of taxa. Rank order is based on summed abundance across PCR replicates. Higher rank order taxa (rarer) were less likely to occur in all three replicates, while low rank order taxa (dominants) tended to occur in more replicates. Data have been rarefied to 25000 and 20000 reads for COI and 18S, respectively.

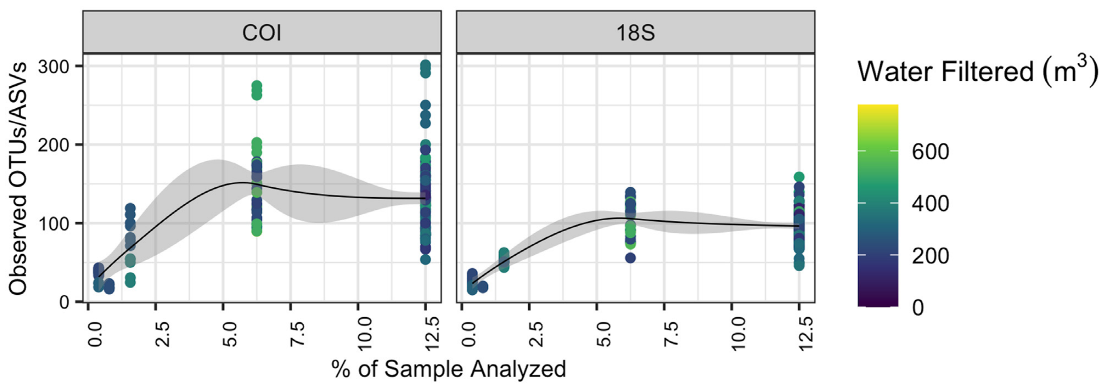

Figure 3. Observed richness at (left-hand panel) COI and (right-hand panel) 18S (in relation to the percentage of the zooplankton sample analyzed). For both markers, richness across all samples approached saturation at ~6.25% analyzed. Colour scale represents the total volume of seawater filtered at collection. Curves show the LOESS fit and shading represents 95% confidence intervals.

liolids, and pyrosomes only resolved at 18S and chaetognaths only resolved by COI. For most groups, the relationship between carbon biomass and sequence counts was equal to or stronger than the relationship between numerical abundance and sequence counts, with the exception of oithonids (Table 2, Figures S1 and S2). Chaetognath sequences did not show a significant relationship against either abundance or carbon biomass. Contrary to our expectation that crustaceans would show a better relationship than gelatinous organisms, there was not a close correspondence between tissue composition and strength of correlation between proportions of sequences and carbon biomass (Table 2).

A closer examination showed that there was wide scatter underlying even strong correlations. Figure 5 illustrates relationships for four representative taxa, showing two abundant copepod groups with stronger relationships and two other taxonomic groups (thaliaceans, ostracods) with weaker relationships and greater variability between markers, while Figures S1 and S2 include all taxa. Notably, there were some data points that were outliers in multiple correlations, such as those from Cycle 3, 250–400 m and Cycle 2, 200–250 and 350–400 m, which were found in the upper left quartile of the eucalanid correlation for both markers, and the lower right quartile for the calanoid correlation at COI (Figure 5). In addition to the taxa absent from one marker, there were taxa found at both markers that showed stronger relationships at one. Ostracods showed a stronger relationship between COI reads and biomass, while thaliaceans had a strong relationship only at 18S (Figure 5).

Ethanol neutralization

For our test of ammonium hydroxide as a neutralizing agent, 494 COI OTUs were found. Total richness and richness within taxonomic groups were not significantly different between neutralized and untreated samples ($p = 0.22$, Wilcoxon, Figure 6) and there was no taxonomic bias, which would appear as a directional shift within points of a given color in Figure 5. Clustering of samples identified two significant groups separating by collection location (Figure S3, SIMPROF alpha = 0.05). A PERMANOVA revealed that preservation method had no effect on community structure, but that collection event was a significant predictor of observed community structure ($R^2 = 0.79$, $p = 0.002$). Parallel analyses with non-rarefied OTU tables and presence–absence data showed similar results.

Discussion

Our results provide a framework for interpreting metabarcoding results of zooplankton community composition. We found that tech-

Table 2. Pearson's correlation coefficient (R) and the significance level for all comparisons between proportion of sequences and proportion of biomass or numerical abundance (ZooScan-based measures). All proportions have been arcsine square root transformed.

		Proportion carbon biomass vs. proportion sequences				Proportion abundance vs. proportion sequences			
	Taxon	COI R		18S R		COI R		18S R	
Crustaceans	**Eucalanids**	0.77	***	0.72	***	0.67	***	0.66	***
	Calanoids	0.81	***	0.74	***	0.72	***	0.69	***
	Oithonids	–	–	0.46	***	–	–	0.64	***
	Poecilostomatoids	0.37	***	0.11	***	0.11	NS	− 0.17	NS
	Malacostracans	0.58	***	0.38	***	0.64	***	0.29	***
	Ostracods	0.57	***	0.19	NS	0.58	***	0.36	***
Tunicates	**Doliolids**	–	–	0.66	***	–	–	0.65	***
	Pyrosomes	–	–	0.9	***	–	–	0.88	***
	Salps	0.05	NS	0.09	***	0.03	NS	0.09	NS
	Thaliaceans	− 0.03	NS	0.88	***	− 0.01	NS	0.42	***
	Appendicularians	0.22	NS	0.37	***	0.19	NS	0.27	NS
Other	**Cnidarians & Ctenophores**	0.48	***	0.36	***	0.48	***	0.18	NS
	Bryozoans	0.87	***	0.72	***	0.77	***	0.62	***
	Polychaetes	0.5	***	0.58	***	0.45	***	0.55	***
	Pteropods	0.39	***	0.46	***	0.62	***	0.0.49	***
	Chaetognaths	− 0.12	NS	–	–	0.03	NS	–	–

NS - not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; Bonferroni corrected significance levels.

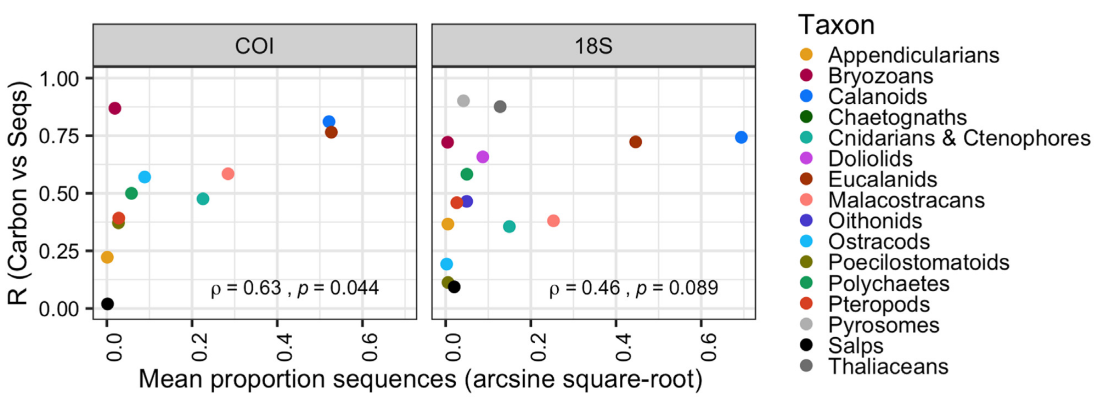

Figure 4. For each taxonomic group, Pearson's correlation coefficient (R) for the relationship between the proportion of sequences and proportion of carbon biomass is plotted against the average proportion of sequences across all samples. The strength of the correlation was stronger for more abundant groups at the COI marker, but the relationship at 18S was not significant ($p < 0.05$, Spearman's rank).

nical replication increases the detection of rare taxa and that sub-sampling has the potential to depress observed richness. In comparisons of relative read counts with morphological analyses, read abundance was positively correlated with the proportion of zooplankton biomass for all but one of the taxonomic groups analyzed, with considerable variability in the strength of the correlation among taxonomic groups. Thus, metabarcoding can be a viable approach for representing relative biomass of several major taxonomic groups but should be interpreted cautiously for less abundant taxa. In an important point for field fixation protocols, we found that neutralized ethanol provides unbiased measures of community composition.

Sub-sampling and PCR replication

We found a positive relationship between sequencing depth and richness at COI and a negative relationship at 18S. As sequencing depth is determined by sample pooling and library size (Herbold *et al.*, 2015), the observed relationships were primarily an artefact of library pooling. Parallel analyses with non-rarefied data were similar, indicating that the relationship was not an artefact of rarefaction (McMurdie and Holmes, 2014; Willis, 2019).

Good overall correspondence was observed between PCR replicates, although some taxa were absent from one or more PCR replicates. These taxa could be interpreted as spurious sequences arising from PCR errors, and some studies recommend removing OTUs

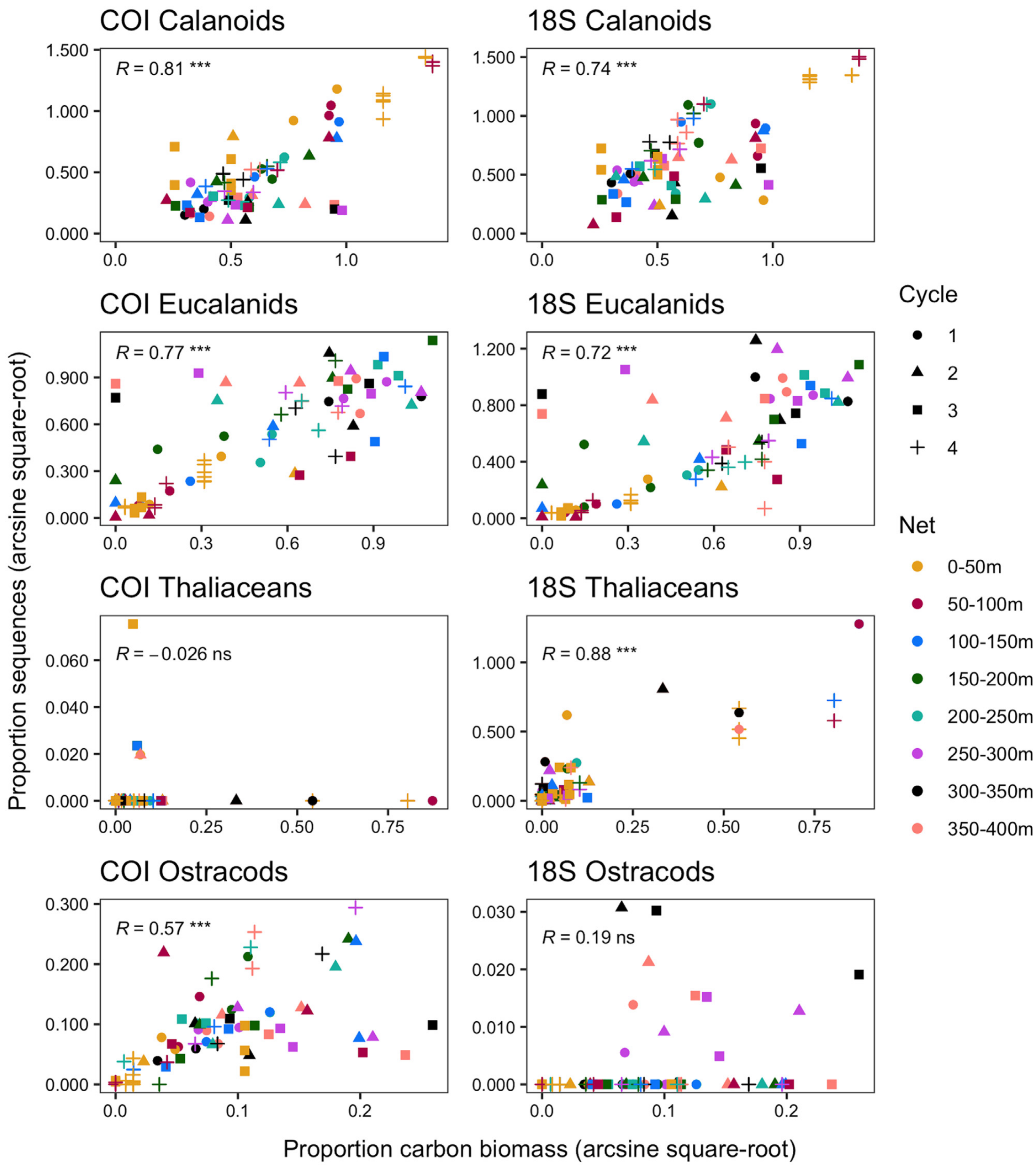

Figure 5. The proportion of metabarcoding sequences classified as calanoid, eucalanid, thaliacean, and ostracod in relation to the proportion of carbon biomass within the full zooplankton community, calculated from ZooScan ROIs. There is a strong positive relationship at both markers for the two copepod groups, and for thaliaceans at 18S and ostracods at COI. Note the different scales for each panel. All data have been arcsine square root transformed. Collection locations (Cycle, Net) are shown to enable comparison between taxa. Additional taxa and all abundance relationships are shown in Figures S1 and S2. (ns = $p > 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

that do not appear in all replicates (Loos and Nijland, 2020, but see also Lahoz-Monfort *et al.*, 2016). However, many of these taxa were found in higher abundance in nearby samples, indicating they represented rare individuals or fragments of animals. Multiple PCR replicates are widely recommended to minimize false negatives in environmental DNA studies (Ficetola *et al.*, 2016; Ruppert *et al.*, 2019). Stochastic variability in the detection of rare species has been observed in tissue-based metabarcoding analyses (Leray and Knowlton, 2017), and our results indicate that in a natural community this stochasticity can affect both rare and more abundant taxa. For our study site, at least three PCR replicates were necessary to fully capture the diversity of the zooplankton community, and we recommend this for other regions as well.

Richness increased as a function of the fraction of the sample analyzed and appeared to saturate at around 6.25% of the sample. This comparison was made across ocean environments from nearshore upwelling dominated by a few taxa to higher diversity, offshore mesotrophic waters, and at depths ranging from the surface to 400 m. Addition of replicate subsamples resulted in species accumulation curves closely matching that of the full sample set, indicating that the low richness is an artefact of subsampling. Our results indicate that in addition to technical replication, a minimum

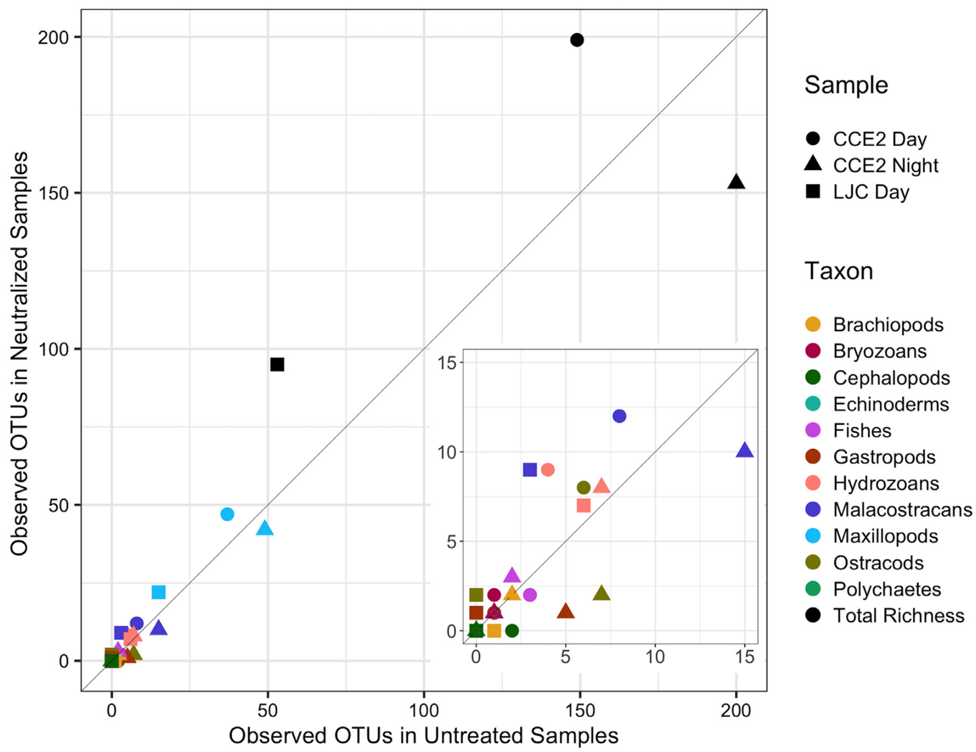

Figure 6. Comparison of number of COI OTUs from paired samples preserved in ethanol neutralized with ammonium hydroxide with those in unamended ethanol. The diagonal is the 1:1 line. Colours indicate zooplankton taxa; symbols indicate the three sets of samples compared. Inset shows OTUs at low counts. There was no difference in overall richness and no taxonomic bias between neutralized and untreated samples.

fraction of the initial bulk sample should be analyzed. For zooplankton assemblages in our region, this threshold was > 6.25% of the sample, but this value should be estimated for other ocean ecosystems with distinct rank abundance profiles.

The importance of replicate field samples, subsampling, PCR replication, and sequencing depth are likely to vary between ecosystems and study designs, with greater replication at all stages necessary for more diverse systems and increased field sampling effort in systems with high spatial variability. Metabarcoding analyses should be designed with careful consideration of the goals of the study and the types of variability present in the ecosystem of interest (Kelly *et al.*, 2019).

Comparisons between imaging and sequencing

The relationships between carbon biomass and read abundance were stronger for more abundant groups. We found strong relationships at one or both markers for eucalanid and calanoid copepods, malacostracans, ostracods, bryozoans, and polychaetes. Several previous analyses have reported positive relationships between read counts and abundance or biomass within the zooplankton community, for a range of taxonomic groups and marker regions (Harvey *et al.*, 2017; Bucklin *et al.*, 2019; Schroeder *et al.*, 2020). The strong positive relationships we observed for more abundant taxa corroborate these studies, and we similarly found that such relationships are not always significant.

The poor or non-significant relationships observed for some groups are also important, as they help inform the limitations of estimating biomass or abundance from read counts. Because metabarcoding data are proportional, biases introduced by variability in gene copy number, amplification efficiency or primer bias propagate to all taxa within a sample (McLaren *et al.*, 2019). In our analysis, some of these biases can be seen in the absence of oithonid copepods, doliolids, and salps at COI, of chaetognaths at 18S, and in the better relationships for ostracods and thaliaceans at COI and 18S, respectively. Approaches to account for bias in amplicon data sets, including the use of taxon: taxon proportions or estimation of amplification efficiency from known community composition, often require mock communities or independent data on community composition or primer bias which are less available for zooplankton than for microbial communities (Kelly *et al.*, 2019; McLaren *et al.*, 2019). Our analyses indicate that these normalization procedures could be particularly important for less abundant taxonomic groups.

There was a notable difference between the strengths of relationships observed at different taxonomic levels within a group. For thaliaceans as a whole, there was a strong correlation between proportion biomass and proportion sequences at 18S. However, this relationship was due to good correspondence for doliolids and pyrosomes, while that for salps was quite low. Chaetognath COI sequences and biomass or abundance were not correlated, but chaetognath sequences were only recovered from mesopelagic species while chaetognaths were identifiable from ZooScan images throughout the water column. Similarly, ostracods at 18S were only detected in mesopelagic samples, while ostracods were found in all samples using COI or using ZooScan imaging. These mismatches may be due to poor representation in the NCBI, MIDORI, and SILVA databases used to assign taxonomy, or due to variability in amplification efficiency between species present in different depth zones. Previous work has found that agreement between methods increases as species are grouped at coarser taxonomic levels (Leray and Knowlton, 2015; Harvey *et al.*, 2017). In contrast, our data indicate that relationships can be either strengthened or weakened at coarser taxonomic resolution, depending on the variance in marker performance between closely related taxa. We recommend that marker biases be accounted for and that estimates of relative abundance and comparisons between survey methods be limited to taxa that are well resolved.

Some outlying samples were clearly visible in the scatterplots of eucalanid and calanoid relationships (Figure 5). These samples were outliers for both marker regions, and incorrect classification with independent reference databases is unlikely. Further verifications of image classifications did not detect misidentifications. It is most likely that these samples reflect a true mismatch in the detection capabilities of the molecular and imaging methods compared. The outliers in question were collected from the upper mesopelagic (200–400 m) and likely included eucalanid copepodites that were not easily identifiable to genus by digital imaging (hence only assigned to calanoid copepods) but were classifiable as eucalanids by metabarcoding. Digital imaging lacks the morphological detail necessary to identify most species, especially for young developmental stages, hence comparisons must be made at more aggregated taxonomic levels. However, ZooScanning accurately reflects body sizes (Gorsky *et al.*, 2010) and numerical abundances (Whitmore *et al.*, 2019) when compared to other methods. Conversions from body length to carbon biomass introduce another source of uncertainty, but were optimized for the zooplankton taxa of our study region (Lavaniegos and Ohman, 2007) and are unlikely to bias the biomass proportions calculated here. These outlier samples illustrate the strengths of combining multiple observation methods. While metabarcoding lacks information such as body size, sex, or life history stage, it can increase detection sensitivity to rarer species or taxa that are undetected by visual surveys or other collection methods (Stat *et al.*, 2019). Because metabarcoding does not yield measurements of absolute abundance, we recommend that metabarcoding and morphological or imaging methods be used in combination.

Ethanol neutralization

For our comparison of paired samples preserved in either unamended ethanol or ethanol neutralized with ammonium hydroxide, we recovered fewer OTUs than were found in the full data set, likely due to limited sampling. However, the observed OTUs included a similar taxonomic range. We did not detect any effect of ammonium hydroxide on community structure, composition, or richness between the two sets of samples. These results indicate that ammonium hydroxide addition to ethanol (Bednaršek and Ohman, 2015) permits a single fixative to be used for both molecular and morphological analyses, including calcareous zooplankton. This result bears confirmation with other DNA extraction protocols.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Data availability

OTU tables, fasta files, and sample metadata are available through CCE-LTER DataZoo. Raw sequence are under NCBI SRA PRJNA679794. Code is available at < *https://github.com/samatthews/Matthews_etal_IJMS_2021*>

Acknowledgements

Thanks to the *California Current Ecosystem* LTER program, the R/V Sikuliaq, and P. Zerofski for sample collections. E. Tovar and H. Zheng assisted with laboratory work. The SIO Pelagic Invertebrate Collection provided access to zooplankton samples. We thank L. Hauser and two anonymous reviewers for constructive comments on this manuscript. Support was provided by a US NSF Graduate Research Fellowship to S. Matthews, NSF to the *California Current Ecosystem* LTER site, and by Gordon and Betty Moore Foundation support to MDO. This study is a contribution to the Scientific Committee on Oceanic Research (SCOR) working group 157 (MetaZooGene).

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Handling editor: Lorenz Hauser

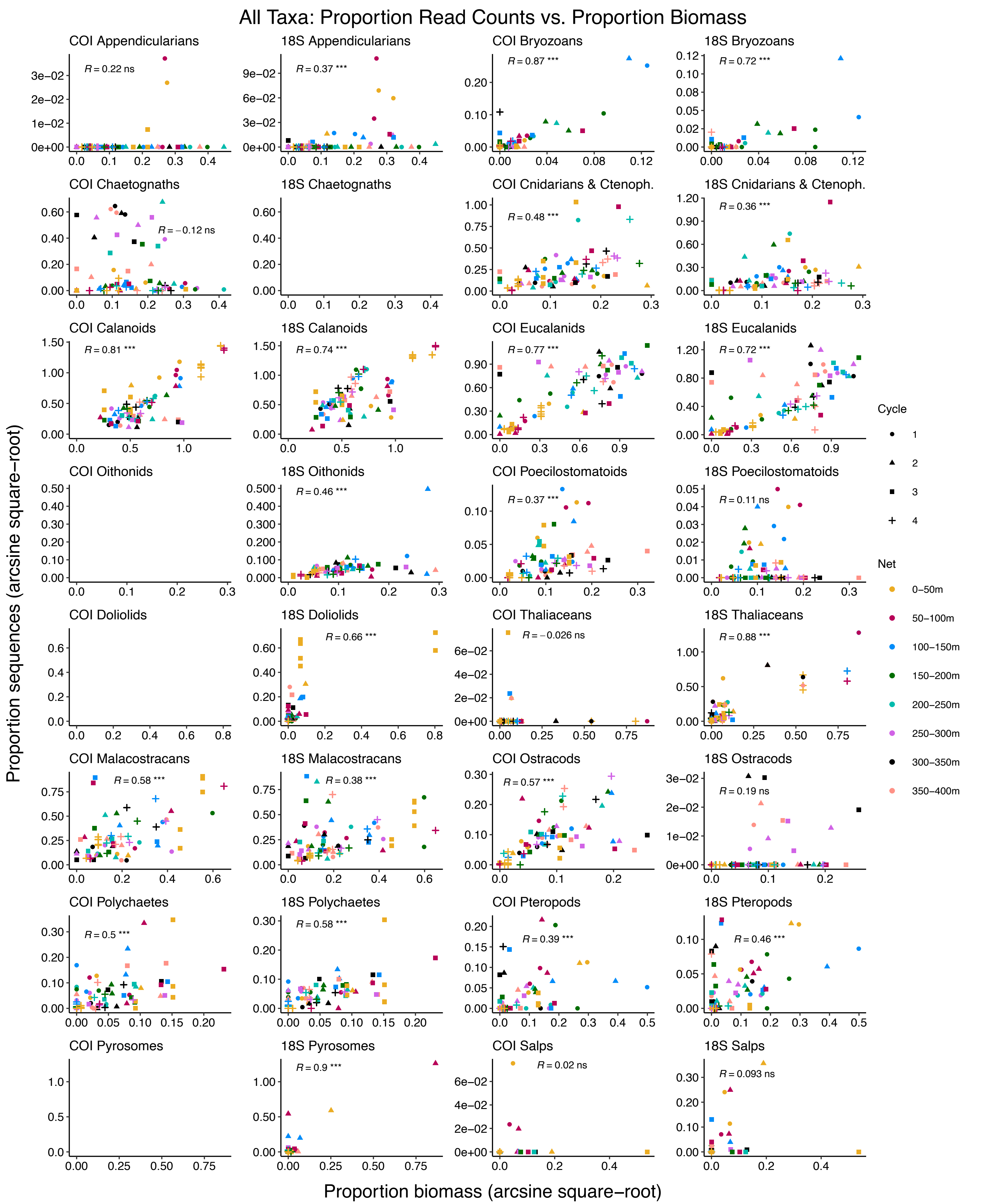

Figure S1. The proportion of metabarcoding sequences for each taxonomic group is plotted against the proportion of carbon biomass of the group within the full zooplankton community, calculated from Zooscan ROIs. Collection location (Cycle, Net) is shown to enable comparison between taxa. Blank panels are due to the absence of data at that marker. Note the different scales for each panel. (ns = p > 0.05, * p < 0.05, ** p < 0.01, ** p < 0.001).

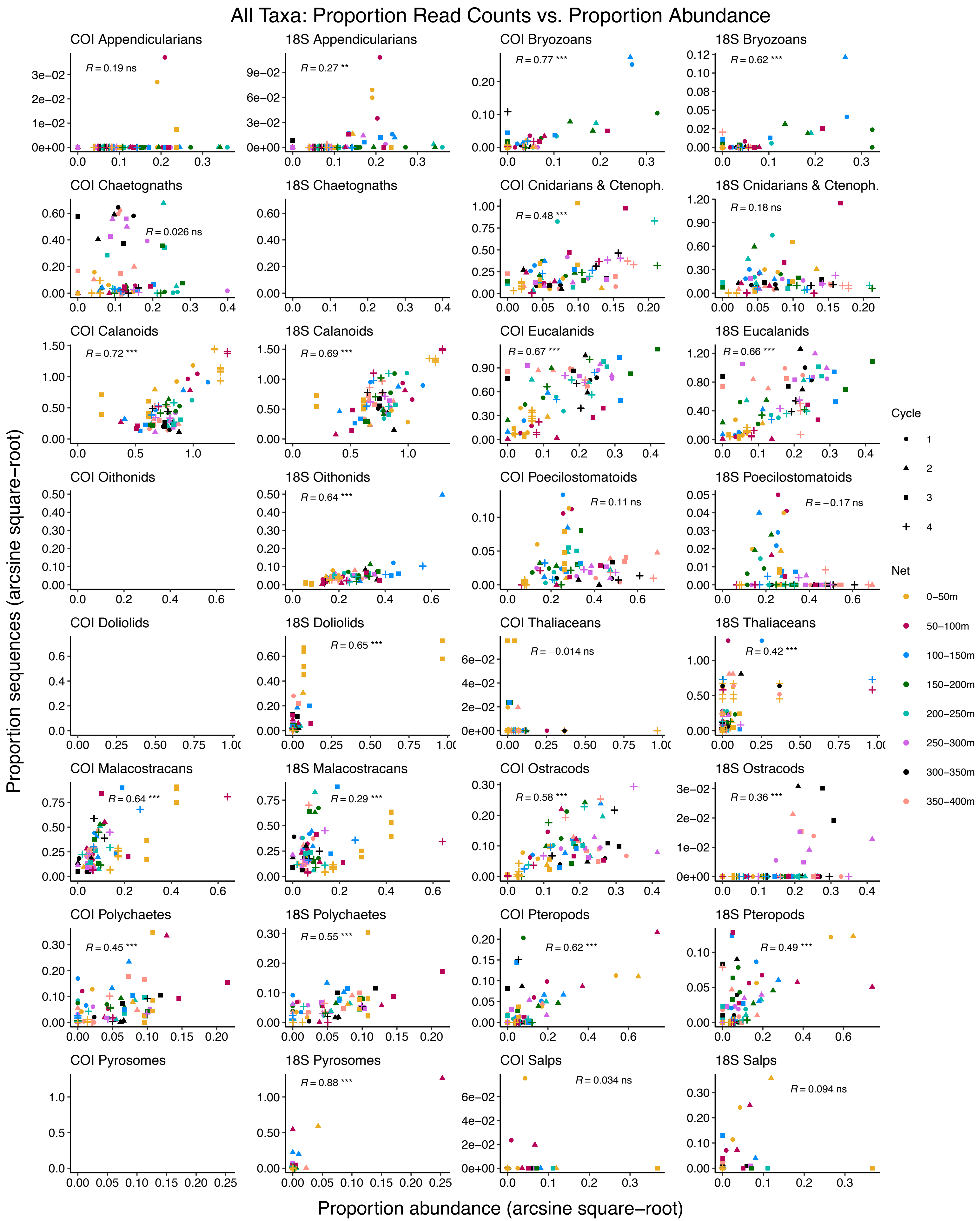

Figure S2. The proportion of metabarcoding sequences for each taxonomic group is plotted against the proportion of numerical abundance of the group within the full zooplankton community, calculated from Zooscan ROIs. Collection location (Cycle, Net) is shown to enable comparison between taxa. Blank panels are due to the absence of data at that marker. Note the different scales for each panel. (ns = p > 0.05, * p < 0.05, ** p < 0.01, ** p < 0.001).

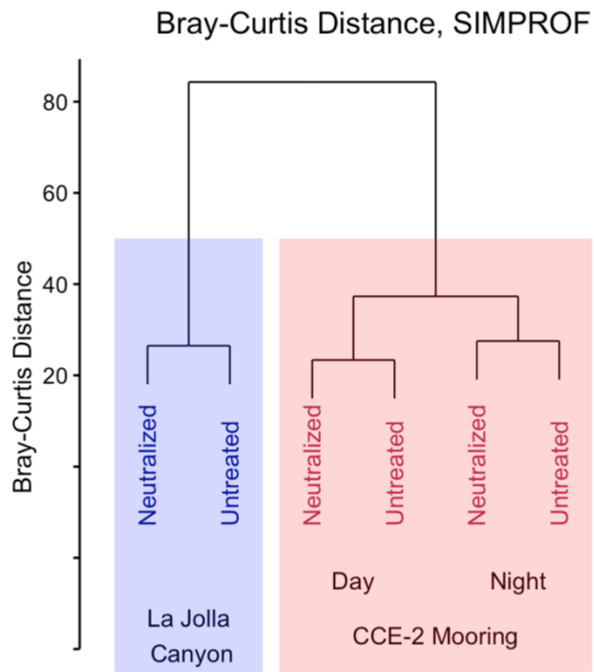

Figure S3. A SIMPROF test with average-neighbor clustering based on Bray Curtis distances. Clusters with non-significant structure are highlighted in blue and red boxes, respectively. Samples collected from two different locations were significantly different, but there was no difference between paired samples preserved in either neutralized or untreated ethanol.

Table S1. Metadata for all samples included in the metabarcoding analyses. Data fields are as follows: Sample ID: unique sample identifier; Diel: sample collection time; Latitude: latitude at beginning of MOCNESS tow (N); Longitude: longitude at beginning of MOCNESS tow (E); Date: local date of sample collection; Time: local time when MOCNESS net began sampling; % Processed: quantitative split of original net collected sample that was analyzed with metabarcoding; Biomass processed: estimated wet biomass of subsample processed; Cycle, Haul, and Tow: unique identifier for sampling event within the CCE P1604 cruise (for more information see http://cce.lternet.edu/data/cruises/cce-p1604); Net: MOCNESS net number; Depth: target depth stratum sampled; DNA Conc.: DNA concentration prior to equimolar pooling for sequencing, calculated by nanodrop or picogreen; Demultiplexed Reads: number of reads for each sample after demultiplexing with two sets of dual indexes; Denoised reads: number of reads per sample after quality and chimera filtering and denoising; Physical sample: unique identifier for each physical subsample (shared across replicates and markers); Replicate: replicate number within each sequencing run; Indexes: 6bp and 8bp indexes used for multiplexing samples within each MiSeq lane; Sequencing run: MiSeq lane.

Sample ID	Diel	Latitude	Longitude	Date	Time (PST)	Cycle	Haul	Tow 2	Net	Depth	% Processed	Biomass processed (g)	DNA Conc. (ug/mL)	Demultiplexed Reads	Denoised Reads	Physical Sample	Replicate	PCR1 F index	PCR1 R index	PCR2 F index	PCR2 R index	Sequencing Run
S124	night	33.04663	-122.90350	22-Apr-2016	2234	Cycle 1	Haul 2	Tow 2	Net 1	400-350	0.0625	1.428	4.36	67,912	29,426	Sample 1	1	F1_ATCACG	R1_GTGGCC	F9_CGTCTAAT	R6_CATGCCTA_	1
S125	night	33.04663	-122.90350	22-Apr-2016	2250	Cycle 1	Haul 2	Tow 2	Net 2	350-300	0.0625	2.285	7.40	49,744	21,268	Sample 2	1	F1_ATCACG	R2_GTTTCG	F10_TCTCTCCG	R6_CATGCCTA_	1
S126	night	33.04663	-122.90350	22-Apr-2016	2258	Cycle 1	Haul 2	Tow 2	Net 3	300-250	0.125	2.38	12.64	54,118	19,814	Sample 3	1	F1_ATCACG	R3_CGTACG	F11_TCGACTAG	R6_CATGCCTA_	1
S127	night	33.04663	-122.90350	22-Apr-2016	2313	Cycle 1	Haul 2	Tow 2	Net 4	250-200	0.125	2.264	11.60	60,020	27,630	Sample 4	1	F1_ATCACG	R4_GAGTGG	F12_TTCTAGCT	R6_CATGCCTA_	1
S128	night	33.04663	-122.90350	22-Apr-2016	2323	Cycle 1	Haul 2	Tow 2	Net 5	200-150	0.0625	3.083	14.20	51,242	22,147	Sample 5	1	F1_ATCACG	R5_ACTGAT	F13_CCTAGAGT	R6_CATGCCTA_	1
S129	night	33.04663	-122.90350	22-Apr-2016	2333	Cycle 1	Haul 2	Tow 2	Net 6	150-100	0.0625	3.984	5.84	48,537	20,422	Sample 6	1	F1_ATCACG	R6_ATTCCT	F14_CTATTAAG	R6_CATGCCTA_	1
S130	night	33.04663	-122.90350	22-Apr-2016	2344	Cycle 1	Haul 2	Tow 2	Net 7	100-50	0.0625	2.122	13.24	60,486	21,511	Sample 7	1	F1_ATCACG	R7_TTCCAC	F15_AAGGCTAT	R6_CATGCCTA_	1
S131	night	33.04663	-122.90350	22-Apr-2016	2354	Cycle 1	Haul 2	Tow 2	Net 8	50-0	0.125	1.598	11.04	69,839	26,255	Sample 8	1	F1_ATCACG	R8_TTGGCA	F16_GAGCCTTA	R6_CATGCCTA_	1
S132	night	33.5297	-122.0517	29-Apr-2016	2211	Cycle 2	Haul 4	Tow 2	Net 1	400-350	0.125	1.018	9.52	68,047	26,274	Sample 9	1	F2_CGATGT	R1_GTGGCC	F9_CGTCTAAT	R7_GTAGAGAG_	1
S133	night	33.5297	-122.0517	29-Apr-2016	2215	Cycle 2	Haul 4	Tow 2	Net 2	350-300	0.125	2.403	7.36	69,695	45,696	Sample 10	1	F2_CGATGT	R2_GTTTCG	F10_TCTCTCCG	R7_GTAGAGAG_	1
S134	night	33.5297	-122.0517	29-Apr-2016	2226	Cycle 2	Haul 4	Tow 2	Net 3	300-250	0.125	1.144	7.72	80,095	52,043	Sample 11	1	F2_CGATGT	R3_CGTACG	F11_TCGACTAG	R7_GTAGAGAG_	1
S135	night	33.5297	-122.0517	29-Apr-2016	2230	Cycle 2	Haul 4	Tow 2	Net 4	250-200	0.125	1.186	7.40	81,314	40,719	Sample 12	1	F2_CGATGT	R4_GAGTGG	F12_TTCTAGCT	R7_GTAGAGAG_	1
S136	night	33.5297	-122.0517	29-Apr-2016	2235	Cycle 2	Haul 4	Tow 2	Net 5	200-150	0.125	1.924	14.00	46,543	20,638	Sample 13	1	F2_CGATGT	R5_ACTGAT	F13_CCTAGAGT	R7_GTAGAGAG_	1
S137	night	33.5297	-122.0517	29-Apr-2016	2242	Cycle 2	Haul 4	Tow 2	Net 6	150-100	0.125	1.8	4.76	74,549	32,220	Sample 14	1	F2_CGATGT	R6_ATTCCT	F14_CTATTAAG	R7_GTAGAGAG_	1
S138	night	33.5297	-122.0517	29-Apr-2016	2248	Cycle 2	Haul 4	Tow 2	Net 7	100-50	0.0625	2.413	5.36	146,233	67,936	Sample 15	1	F2_CGATGT	R7_TTCCAC	F15_AAGGCTAT	R7_GTAGAGAG_	1
S139	night	33.5297	-122.0517	29-Apr-2016	2300	Cycle 2	Haul 4	Tow 2	Net 8	50-0	0.125	1.605	15.28	53,802	17,888	Sample 16	1	F2_CGATGT	R8_TTGGCA	F16_GAGCCTTA	R7_GTAGAGAG_	1
S140	night	34.68737	-121.2542	3-May-2016	2155	Cycle 3	Haul 9	Tow 2	Net 1	400-350	0.0625	2.19	12.40	64,232	29,812	Sample 17	1	F3_TTAGGC	R1_GTGGCC	F9_CGTCTAAT	R8_CCTCTCTG_	1
S141	night	34.68737	-121.2542	3-May-2016	2200	Cycle 3	Haul 9	Tow 2	Net 2	350-300	0.0625	1.424	13.24	58,139	24,778	Sample 18	1	F3_TTAGGC	R2_GTTTCG	F10_TCTCTCCG	R8_CCTCTCTG_	1
S142	night	34.68737	-121.2542	3-May-2016	2205	Cycle 3	Haul 9	Tow 2	Net 3	300-250	0.125	2.108	15.64	55,305	29,479	Sample 19	1	F3_TTAGGC	R3_CGTACG	F11_TCGACTAG	R8_CCTCTCTG_	1
S143	night	34.68737	-121.2542	3-May-2016	2209	Cycle 3	Haul 9	Tow 2	Net 4	250-200	0.125	1.23	13.84	52,684	25,418	Sample 20	1	F3_TTAGGC	R4_GAGTGG	F12_TTCTAGCT	R8_CCTCTCTG_	1
S144	night	34.68737	-121.2542	3-May-2016	2213	Cycle 3	Haul 9	Tow 2	Net 5	200-150	0.125	1.175	15.12	103,391	56,582	Sample 21	1	F3_TTAGGC	R5_ACTGAT	F13_CCTAGAGT	R8_CCTCTCTG_	1
S145	night	34.68737	-121.2542	3-May-2016	2218	Cycle 3	Haul 9	Tow 2	Net 6	150-100	0.0625	2.735	7.04	58,289	33,466	Sample 22	1	F3_TTAGGC	R6_ATTCCT	F14_CTATTAAG	R8_CCTCTCTG_	1
S146	night	34.68737	-121.2542	3-May-2016	2222	Cycle 3	Haul 9	Tow 2	Net 7	100-50	0.0625	2.204	16.16	55,325	24,674	Sample 23	1	F3_TTAGGC	R7_TTCCAC	F15_AAGGCTAT	R8_CCTCTCTG_	1
S147	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.825	15.04	64,196	32,070	Sample 24	1	F3_TTAGGC	R8_TTGGCA	F16_GAGCCTTA	R8_CCTCTCTG_	1
S148	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	2.005	13.96	70,856	38,776	Sample 25	1	F4_TGACCA	R1_GTGGCC	F9_CGTCTAAT	R9_AGCGTAGC_	1
S149	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.678	18.72	49,411	27,226	Sample 26	1	F4_TGACCA	R2_GTTTCG	F10_TCTCTCCG	R9_AGCGTAGC_	1
S150	night	34.5183	-120.7625	7-May-2016	2146	Cycle 4	Haul 13	Tow 2	Net 1	200-175	0.125	3.041	16.72	58,244	31,904	Sample 28	1	F4_TGACCA	R3_CGTACG	F11_TCGACTAG	R9_AGCGTAGC_	1
S151	night	34.5183	-120.7625	7-May-2016	2150	Cycle 4	Haul 13	Tow 2	Net 2	175-150	0.125	3.883	12.04	76,114	35,001	Sample 29	1	F4_TGACCA	R4_GAGTGG	F12_TTCTAGCT	R9_AGCGTAGC_	1
S152	night	34.5183	-120.7625	7-May-2016	2154	Cycle 4	Haul 13	Tow 2	Net 3	150-125	0.125	2.907	14.64	65,705	29,474	Sample 30	1	F4_TGACCA	R5_ACTGAT	F13_CCTAGAGT	R9_AGCGTAGC_	1
S153	night	34.5183	-120.7625	7-May-2016	2159	Cycle 4	Haul 13	Tow 2	Net 4	125-100	0.125	1.515	5.08	82,326	44,232	Sample 31	1	F4_TGACCA	R6_ATTCCT	F14_CTATTAAG	R9_AGCGTAGC_	1
S154	night	34.5183	-120.7625	7-May-2016	2203	Cycle 4	Haul 13	Tow 2	Net 5	100-75	0.125	3.278	15.52	63,655	31,759	Sample 32	1	F4_TGACCA	R7_TTCCAC	F15_AAGGCTAT	R9_AGCGTAGC_	1
S155	night	34.5183	-120.7625	7-May-2016	2208	Cycle 4	Haul 13	Tow 2	Net 6	75-50	0.0625	2.545	16.24	62,580	32,337	Sample 33	1	F4_TGACCA	R8_TTGGCA	F16_GAGCCTTA	R9_AGCGTAGC_	1
S156	night	34.5183	-120.7625	7-May-2016	2213	Cycle 4	Haul 13	Tow 2	Net 7	50-25	0.0625	5.319	14.44	65,196	41,219	Sample 34	1	F5_ACAGTG	R1_GTGGCC	F9_CGTCTAAT	R10_CAGCCTCG_	1
S157	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	5.566	13.36	60,461	46,225	Sample 35	1	F5_ACAGTG	R2_GTTTCG	F10_TCTCTCCG	R10_CAGCCTCG_	1
S158	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	3.838	13.32	62,249	45,453	Sample 36	1	F5_ACAGTG	R3_CGTACG	F11_TCGACTAG	R10_CAGCCTCG_	1
S159	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.685	11.44	62,090	46,006	Sample 37	1	F5_ACAGTG	R4_GAGTGG	F12_TTCTAGCT	R10_CAGCCTCG_	1
S160	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.686	12.72	54,683	40,322	Sample 38	1	F5_ACAGTG	R5_ACTGAT	F13_CCTAGAGT	R10_CAGCCTCG_	1
S161	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.547	5.56	47,169	37,761	Sample 39	1	F5_ACAGTG	R6_ATTCCT	F14_CTATTAAG	R10_CAGCCTCG_	1
S162	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.411	16.00	46,074	35,500	Sample 40	1	F5_ACAGTG	R7_TTCCAC	F15_AAGGCTAT	R10_CAGCCTCG_	1
S163	night					Ext. control	Ext. control	Ext. control	Ext. control	Ext. control			0.935	1,034	0	Sample 0	1	F5_ACAGTG	R8_TTGGCA	F16_GAGCCTTA	R10_CAGCCTCG_	1
S164	night																					
S165	night												0.01	15,435	0	Neg. Control		F11_GGCTAC	R1_GTGGCC	F10_TCTCTCCG	R11_TGCCTCTT_	1
S167	night	33.04663	-122.90350	22-Apr-2016	2234	Cycle 1	Haul 2	Tow 2	Net 1	400-350	0.0625	1.428	6.92	39,252	18,303	Sample 1	2	F6_GCCAAT	R1_GTGGCC	F17_GCGTAAGA_	R1_TCGCCTTA	1
S168	night	33.04663	-122.90350	22-Apr-2016	2250	Cycle 1	Haul 2	Tow 2	Net 2	350-300	0.0625	2.285	20.32	38,421	18,309	Sample 2	2	F6_GCCAAT	R2_GTTTCG	F18_TTATGCGA_	R1_TCGCCTTA	1
S169	night	33.04663	-122.90350	22-Apr-2016	2258	Cycle 1	Haul 2	Tow 2	Net 3	300-250	0.125	2.38	13.40	57,932	24,822	Sample 3	2	F6_GCCAAT	R3_CGTACG	F19_AGCGCTAG	R1_TCGCCTTA	1
S170	night	33.04663	-122.90350	22-Apr-2016	2313	Cycle 1	Haul 2	Tow 2	Net 4	250-200	0.125	2.264	10.88	63,551	29,918	Sample 4	2	F6_GCCAAT	R4_GAGTGG	F20_GATATCGA	R1_TCGCCTTA	1
S171	night	33.04663	-122.90350	22-Apr-2016	2323	Cycle 1	Haul 2	Tow 2	Net 5	200-150	0.0625	3.083	10.84	64,651	30,398	Sample 5	2	F6_GCCAAT	R5_ACTGAT	F21_CGCAGACG	R1_TCGCCTTA	1
S172	night	33.04663	-122.90350	22-Apr-2016	2333	Cycle 1	Haul 2	Tow 2	Net 6	150-100	0.0625	3.984	10.12	64,322	29,199	Sample 6	2	F6_GCCAAT	R6_ATTCCT	F22_TATGAGTA	R1_TCGCCTTA	1
S173	night	33.04663	-122.90350	22-Apr-2016	2344	Cycle 1	Haul 2	Tow 2	Net 7	100-50	0.0625	2.122	11.88	65,837	25,867	Sample 7	2	F6_GCCAAT	R7_TTCCAC	F23_AGGTGCGT	R1_TCGCCTTA	1
S174	night	33.04663	-122.90350	22-Apr-2016	2354	Cycle 1	Haul 2	Tow 2	Net 8	50-0	0.125	1.598	11.88	69,412	25,176	Sample 8	2	F6_GCCAAT	R8_TTGGCA	F24_GAACATAC	R1_TCGCCTTA	1
S175	night	33.5297	-122.0517	29-Apr-2016	2211	Cycle 2	Haul 4	Tow 2	Net 1	400-350	0.125	1.018	5.88	33,226	14,027	Sample 9	2	F7_CAGATC	R1_GTGGCC	F17_GCGTAAGA_	R2_CTAGTACG	1
S176	night	33.5297	-122.0517	29-Apr-2016	2215	Cycle 2	Haul 4	Tow 2	Net 2	350-300	0.125	2.403	5.72	55,491	39,714	Sample 10	2	F7_CAGATC	R2_GTTTCG	F18_TTATGCGA_	R2_CTAGTACG	1
S177	night	33.5297	-122.0517	29-Apr-2016	2226	Cycle 2	Haul 4	Tow 2	Net 3	300-250	0.125	1.144	17.52	47,846	30,773	Sample 11	2	F7_CAGATC	R3_CGTACG	F19_AGCGCTAG	R2_CTAGTACG	1
S178	night	33.5297	-122.0517	29-Apr-2016	2230	Cycle 2	Haul 4	Tow 2	Net 4	250-200	0.125	1.186	17.56	36,024	18,018	Sample 12	2	F7_CAGATC	R4_GAGTGG	F20_GATATCGA	R2_CTAGTACG	1
S179	night	33.5297	-122.0517	29-Apr-2016	2235	Cycle 2	Haul 4	Tow 2	Net 5	200-150	0.125	1.924	14.16	55,782	25,814	Sample 13	2	F7_CAGATC	R5_ACTGAT	F21_CGCAGACG	R2_CTAGTACG	1
S180	night	33.5297	-122.0517	29-Apr-2016	2242	Cycle 2	Haul 4	Tow 2	Net 6	150-100	0.125	1.8	12.80	61,635	25,633	Sample 14	2	F7_CAGATC	R6_ATTCCT	F22_TATGAGTA	R2_CTAGTACG	1
S181	night	33.5297	-122.0517	29-Apr-2016	2248	Cycle 2	Haul 4	Tow 2	Net 7	100-50	0.0625	2.413	15.44	51,181	24,811	Sample 15	2	F7_CAGATC	R7_TTCCAC	F23_AGGTGCGT	R2_CTAGTACG	1
S182	night	33.5297	-122.0517	29-Apr-2016	2300	Cycle 2	Haul 4	Tow 2	Net 8	50-0	0.125	1.605	14.52	63,955	21,912	Sample 16	2	F7_CAGATC	R8_TTGGCA	F24_GAACATAC	R2_CTAGTACG	1
S183	night	34.68737	-121.2542	3-May-2016	2155	Cycle 3	Haul 9	Tow 2	Net 1	400-350	0.0625	2.19	4.02	29,642	15,915	Sample 17	2	F8_ACTTGA	R1_GTGGCC	F17_GCGTAAGA_	R3_TTCTGCCT	1
S184	night	34.68737	-121.2542	3-May-2016	2200	Cycle 3	Haul 9	Tow 2	Net 2	350-300	0.0625	1.424	7.68	71,086	31,545	Sample 18	2	F8_ACTTGA	R2_GTTTCG	F18_TTATGCGA_	R3_TTCTGCCT	1
S185	night	34.68737	-121.2542	3-May-2016	2205	Cycle 3	Haul 9	Tow 2	Net 3	300-250	0.125	2.108	11.96	43,018	23,496	Sample 19	2	F8_ACTTGA	R3_CGTACG	F19_AGCGCTAG	R3_TTCTGCCT	1
S186	night	34.68737	-121.2542	3-May-2016	2209	Cycle 3	Haul 9	Tow 2	Net 4	250-200	0.125	1.23	9.92	50,822	25,708	Sample 20	2	F8_ACTTGA	R4_GAGTGG	F20_GATATCGA	R3_TTCTGCCT	1
S187	night	34.68737	-121.2542	3-May-2016	2213	Cycle 3	Haul 9	Tow 2	Net 5	200-150	0.125	1.175	12.00	50,655	26,256	Sample 21	2	F8_ACTTGA	R5_ACTGAT	F21_CGCAGACG	R3_TTCTGCCT	1
S188	night	34.68737	-121.2542	3-May-2016	2218	Cycle 3	Haul 9	Tow 2	Net 6	150-100	0.0625	2.735	10.24	63,776	38,708	Sample 22	2	F8_ACTTGA	R6_ATTCCT	F22_TATGAGTA	R3_TTCTGCCT	1
S189	night	34.68737	-121.2542	3-May-2016	2222	Cycle 3	Haul 9	Tow 2	Net 7	100-50	0.0625	2.204	9.76	55,204	28,603	Sample 23	2	F8_ACTTGA	R7_TTCCAC	F23_AGGTGCGT	R3_TTCTGCCT	1
S190	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.825	12.92	74,229	37,377	Sample 24	2	F8_ACTTGA	R8_TTGGCA	F24_GAACATAC	R3_TTCTGCCT	1
S191	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	2.005	4.80	59,950	34,541	Sample 25	2	F9_GATCAG	R1_GTGGCC	F17_GCGTAAGA_	R4_GCTCAGGA	1
S192	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.678	15.08	69,485	39,151	Sample 26	2	F9_GATCAG	R2_GTTTCG	F18_TTATGCGA_	R4_GCTCAGGA	1
S193	night	34.5183	-120.7625	7-May-2016	2146	Cycle 4	Haul 13	Tow 2	Net 1	200-175	0.125	3.041	17.52	59,955	35,271	Sample 28	2	F9_GATCAG	R3_CGTACG	F19_AGCGCTAG	R4_GCTCAGGA	1
S194	night	34.5183	-120.7625	7-May-2016	2150	Cycle 4	Haul 13	Tow 2	Net 2	175-150	0.125	3.883	12.88	53,825	28,370	Sample 29	2	F9_GATCAG	R4_GAGTGG	F20_GATATCGA	R4_GCTCAGGA	1
S195	night	34.5183	-120.7625	7-May-2016	2154	Cycle 4	Haul 13	Tow 2	Net 3	150-125	0.125	2.907	10.08	91,084	45,235	Sample 30	2	F9_GATCAG	R5_ACTGAT	F21_CGCAGACG	R4_GCTCAGGA	1
S196	night	34.5183	-120.7625	7-May-2016	2159	Cycle 4	Haul 13	Tow 2	Net 4	125-100	0.125	1.515	12.56	60,175	29,149	Sample 31	2	F9_GATCAG	R6_ATTCCT	F22_TATGAGTA	R4_GCTCAGGA	1
S197	night	34.5183	-120.7625	7-May-2016	2203	Cycle 4	Haul 13	Tow 2	Net 5	100-75	0.125	3.278	14.48	45,439	24,076	Sample 32	2	F9_GATCAG	R7_TTCCAC	F23_AGGTGCGT	R4_GCTCAGGA	1
S198	night	34.5183	-120.7625	7-May-2016	2208	Cycle 4	Haul 13	Tow 2	Net 6	75-50	0.0625	2.545	14.72	62,513	33,357	Sample 33	2	F9_GATCAG	R8_TTGGCA	F24_GAACATAC	R4_GCTCAGGA	1
S199	night	34.5183	-120.7625	7-May-2016	2213	Cycle 4	Haul 13	Tow 2	Net 7	50-25	0.0625	5.319	2.50	24,643	17,918	Sample 34	2	F10 TAGCTT	R1_GTGGCC	F17_GCGTAAGA_	R5_AGGAGTCC	1
S200	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	5.566	10.36	58,495	47,754	Sample 35	2	F10 TAGCTT	R2_GTTTCG	F18_TTATGCGA_	R5_AGGAGTCC	1
S201	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	3.838	12.84	63,299	47,443	Sample 36	2	F10 TAGCTT	R3_CGTACG	F19_AGCGCTAG	R5_AGGAGTCC	1
S202	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.685	11.64	49,705	38,301	Sample 37	2	F10 TAGCTT	R4_GAGTGG	F20_GATATCGA	R5_AGGAGTCC	1
S203	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.686	12.88	52,858	40,615	Sample 38	2	F10 TAGCTT	R5_ACTGAT	F21_CGCAGACG	R5_AGGAGTCC	1
S204	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.547	11.96	48,770	38,859	Sample 39	2	F10 TAGCTT	R6_ATTCCT	F22_TATGAGTA	R5_AGGAGTCC	1
S205	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.411	12.20	49,180	38,520	Sample 40	2	F10 TAGCTT	R7_TTCCAC	F23_AGGTGCGT	R5_AGGAGTCC	1
S208	night	33.04663	-122.90350	22-Apr-2016	2250	Cycle 1	Haul 2	Tow 2	Net 2	350-300	0.0625	2.285	10.64	54,371	23,351	Sample 2	3	F1_ATCACG	R10 GTACAC	F18_TTATGCGA_	R6_CATGCCTA_	1
S209	night	33.04663	-122.90350	22-Apr-2016	2258	Cycle 1	Haul 2	Tow 2	Net 3	300-250	0.125	2.38	8.96	58,380	21,111	Sample 3	3	F1_ATCACG	R11_GATGGA	F19_AGCGCTAG	R6_CATGCCTA_	1
S210	night	33.04663	-122.90350	22-Apr-2016	2313	Cycle 1	Haul 2	Tow 2	Net 4	250-200	0.125	2.264	5.96	30,358	13,538	Sample 4	3	F1_ATCACG	R12_CTACCG	F20_GATATCGA	R6_CATGCCTA_	1
S212	night	33.04663	-122.90350	22-Apr-2016	2333	Cycle 1	Haul 2	Tow 2	Net 6	150-100	0.0625	3.984	9.92	63,554	24,489	Sample 6	3	F2_CGATGT	R10 GTACAC	F22_TATGAGTA	R6_CATGCCTA_	1
S213	night	33.04663	-122.90350	22-Apr-2016	2344	Cycle 1	Haul 2	Tow 2	Net 7	100-50	0.0625	2.122	10.72	46,449	17,142	Sample 7	3	F2_CGATGT	R11_GATGGA	F23_AGGTGCGT	R6_CATGCCTA_	1
S214	night	33.04663	-122.90350	22-Apr-2016	2354	Cycle 1	Haul 2	Tow 2	Net 8	50-0	0.125	1.598	6.96	93,624	36,649	Sample 8	3	F2_CGATGT	R12_CTACCG	F24_GAACATAC	R6_CATGCCTA_	1
S216	night	33.5297	-122.0517	29-Apr-2016	2215	Cycle 2	Haul 4	Tow 2	Net 2	350-300	0.125	2.403	12.56	58,307	38,982	Sample 10	3	F3_TTAGGC	R10 GTACAC	F18_TTATGCGA_	R7_GTAGAGAG_	1
S217	night	33.5297	-122.0517	29-Apr-2016	2226	Cycle 2	Haul 4	Tow 2	Net 3	300-250	0.125	1.144	13.36	53,490	33,979	Sample 11	3	F3_TTAGGC	R11_GATGGA	F19_AGCGCTAG	R7_GTAGAGAG_	1
S218	night	33.5297	-122.0517	29-Apr-2016	2230	Cycle 2	Haul 4	Tow 2	Net 4	250-200	0.125	1.186	14.72	48,007	24,480	Sample 12	3	F3_TTAGGC	R12_CTACCG	F20_GATATCGA	R7_GTAGAGAG_	1
S220	night	33.5297	-122.0517	29-Apr-2016	2242	Cycle 2	Haul 4	Tow 2	Net 6	150-100	0.125	1.8	15.12	47,931	18,796	Sample 14	3	F4_TGACCA	R10 GTACAC	F22_TATGAGTA	R7_GTAGAGAG_	1
S221	night	33.5297	-122.0517	29-Apr-2016	2248	Cycle 2	Haul 4	Tow 2	Net 7	100-50	0.0625	2.413	19.60	42,221	19,996	Sample 15	3	F4_TGACCA	R11_GATGGA	F23_AGGTGCGT	R7_GTAGAGAG_	1
S222	night	33.5297	-122.0517	29-Apr-2016	2300	Cycle 2	Haul 4	Tow 2	Net 8	50-0	0.125	1.605	16.12	50,268	17,881	Sample 16	3	F4_TGACCA	R12_CTACCG	F24_GAACATAC	R7_GTAGAGAG_	1
S224	night	34.68737	-121.2542	3-May-2016	2200	Cycle 3	Haul 9	Tow 2	Net 2	350-300	0.0625	1.424	13.56	56,302	26,237	Sample 18	3	F5_ACAGTG	R10 GTACAC	F18_TTATGCGA_	R8_CCTCTCTG_	1
S225	night	34.68737	-121.2542	3-May-2016	2205	Cycle 3	Haul 9	Tow 2	Net 3	300-250	0.125	2.108	16.12	57,631	30,598	Sample 19	3	F5_ACAGTG	R11_GATGGA	F19_AGCGCTAG	R8_CCTCTCTG_	1
S226	night	34.68737	-121.2542	3-May-2016	2209	Cycle 3	Haul 9	Tow 2	Net 4	250-200	0.125	1.23	14.04	41,267	20,708	Sample 20	3	F5_ACAGTG	R12_CTACCG	F20_GATATCGA	R8_CCTCTCTG_	1
S228	night	34.68737	-121.2542	3-May-2016	2218	Cycle 3	Haul 9	Tow 2	Net 6	150-100	0.0625	2.735	16.04	41,252	21,896	Sample 22	3	F6_GCCAAT	R10 GTACAC	F22_TATGAGTA	R8_CCTCTCTG_	1
S229	night	34.68737	-121.2542	3-May-2016	2222	Cycle 3	Haul 9	Tow 2	Net 7	100-50	0.0625	2.204	16.44	45,350	20,659	Sample 23	3	F6_GCCAAT	R11_GATGGA	F23_AGGTGCGT	R8_CCTCTCTG_	1
S230	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.825	13.72	47,483	25,880	Sample 24	3	F6_GCCAAT	R12_CTACCG	F24_GAACATAC	R8_CCTCTCTG_	1
S232	night	34.68737	-121.2542	3-May-2016	2227	Cycle 3	Haul 9	Tow 2	Net 8	50-0	0.01563	1.678	7.40	55,550	33,131	Sample 26	3	F7_CAGATC	R10 GTACAC	F18_TTATGCGA_	R9_AGCGTAGC_	1
S233	night	34.5183	-120.7625	7-May-2016	2146	Cycle 4	Haul 13	Tow 2	Net 1	200-175	0.125	3.041	15.00	64,202	34,863	Sample 28	3	F7_CAGATC	R11_GATGGA	F19_AGCGCTAG	R9_AGCGTAGC_	1
S234	night	34.5183	-120.7625	7-May-2016	2150	Cycle 4	Haul 13	Tow 2	Net 2	175-150	0.125	3.883	14.52	43,030	19,615	Sample 29	3	F7_CAGATC	R12_CTACCG	F20_GATATCGA	R9_AGCGTAGC_	1
S236	night	34.5183	-120.7625	7-May-2016	2159	Cycle 4	Haul 13	Tow 2	Net 4	125-100	0.125	1.515	14.44	55,057	27,738	Sample 31	3	F8_ACTTGA	R10 GTACAC	F22_TATGAGTA	R9_AGCGTAGC_	1
S237	night	34.5183	-120.7625	7-May-2016	2203	Cycle 4	Haul 13	Tow 2	Net 5	100-75	0.125	3.278	12.72	53,040	26,589	Sample 32	3	F8_ACTTGA	R11_GATGGA	F23_AGGTGCGT	R9_AGCGTAGC_	1
S238	night	34.5183	-120.7625	7-May-2016	2208	Cycle 4	Haul 13	Tow 2	Net 6	75-50	0.0625	2.545	15.76	61,373	32,343	Sample 33	3	F8_ACTTGA	R12_CTACCG	F24_GAACATAC	R9_AGCGTAGC_	1
S240	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	5.566	15.16	53,619	41,764	Sample 35	3	F9_GATCAG	R10 GTACAC	F18_TTATGCGA_	R10_CAGCCTCG_	1
S241	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	3.838	16.64	50,890	36,874	Sample 36	3	F9_GATCAG	R11_GATGGA	F19_AGCGCTAG	R10_CAGCCTCG_	1
S242	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.685	14.72	42,536	31,252	Sample 37	3	F9_GATCAG	R12_CTACCG	F20_GATATCGA	R10_CAGCCTCG_	1
S244	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.547	13.36	50,032	38,210	Sample 39	3	F10 TAGCTT	R10 GTACAC	F22_TATGAGTA	R10_CAGCCTCG_	1
S245	night	34.5183	-120.7625	7-May-2016	2217	Cycle 4	Haul 13	Tow 2	Net 8	25-0	0.00391	4.411	14.76	49,651	37,446	Sample 40	3	F10 TAGCTT	R11_GATGGA	F23_AGGTGCGT	R10_CAGCCTCG_	1
S248	night												0.71	0	0	Neg control		F11_GGCTAC	R9_TCGCAA	F18_TTATGCGA_	R11_TGCCTCTT_	1
S371	day	33.03124	-122.93350	22-Apr-2016	1509	Cycle 1	Haul 1	Tow 1	Net 1	400-350	0.125	1.1738	11.49	34,922	15,530	Sample 43	1	F1_ATCACG	R1_GTGGCC	F9_CGTCTAAT	R6_CATGCCTA_	2
S372	day	33.03124	-122.93350	22-Apr-2016	1515	Cycle 1	Haul 1	Tow 1	Net 2	350-300	0.125	2.7917	15.02	43,680	18,283	Sample 44	1	F1_ATCACG	R2_GTTTCG	F10_TCTCTCCG	R6_CATGCCTA_	2
S373	day	33.03124	-122.93350	22-Apr-2016	1521	Cycle 1	Haul 1	Tow 1	Net 3	300-250	0.125	2.8615	13.36	42,852	18,150	Sample 45	1	F1_ATCACG	R3_CGTACG	F11_TCGACTAG	R6_CATGCCTA_	2
S374	day	33.03124	-122.93350	22-Apr-2016	1528	Cycle 1	Haul 1	Tow 1	Net 4	250-200	0.125	2.032	13.20	56,712	26,280	Sample 46	1	F1_ATCACG	R4_GAGTGG	F12_TTCTAGCT	R6_CATGCCTA_	2
S375	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 1	Tow 1	Net 5	200-150	0.125	1.7606	16.36	47,436	25,808	Sample 47	1	F1_ATCACG	R5_ACTGAT	F13_CCTAGAGT	R6_CATGCCTA_	2
S376	day	33.03124	-122.93350	22-Apr-2016	1544	Cycle 1	Haul 1	Tow 1	Net 6	150-100	0.125	1.3003	15.50	54,090	29,683	Sample 48	1	F1_ATCACG	R6_ATTCCT	F14_CTATTAAG	R6_CATGCCTA_	2
S377	day	33.03124	-122.93350	22-Apr-2016	1552	Cycle 1	Haul 1	Tow 1	Net 7	100-50	0.125	2.154	17.44	50,747	23,740	Sample 49	1	F1_ATCACG	R7_TTCCAC	F15_AAGGCTAT	R6_CATGCCTA_	2
S378	day	33.03124	-122.93350	22-Apr-2016	1601	Cycle 1	Haul 1	Tow 1	Net 8	50-0	0.125	3.5241	16.76	49,798	23,906	Sample 50	1	F1_ATCACG	R8_TTGGCA	F16_GAGCCTTA	R6_CATGCCTA_	2
S379	day	33.5584	-122.1327	29-Apr-2016	1248	Cycle 2	Haul 3	Tow 1	Net 1	400-350	0.125	0.5675	9.59	52,104	20,992	Sample 51	1	F2_CGATGT	R1_GTGGCC	F9_CGTCTAAT	R7_GTAGAGAG_	2
S380	day	33.5584	-122.1327	29-Apr-2016	1255	Cycle 2	Haul 3	Tow 1	Net 2	350-300	0.125	2.1215	13.07	66,880	30,262	Sample 52	1	F2_CGATGT	R2_GTTTCG	F10_TCTCTCCG	R7_GTAGAGAG_	2
S381	day	33.5584	-122.1327	29-Apr-2016	1306	Cycle 2	Haul 3	Tow 1	Net 3	300-250	0.125	2.5957	16.35	42,257	20,321	Sample 53	1	F2_CGATGT	R3_CGTACG	F11_TCGACTAG	R7_GTAGAGAG_	2
S382	day	33.5584	-122.1327	29-Apr-2016	1314	Cycle 2	Haul 3	Tow 1	Net 4	250-200	0.125	1.367	16.51	57,865	27,690	Sample 54	1	F2_CGATGT	R4_GAGTGG	F12_TTCTAGCT	R7_GTAGAGAG_	2
S383	day	33.5584	-122.1327	29-Apr-2016	1320	Cycle 2	Haul 3	Tow 1	Net 5	200-150	0.125	0.3816	16.69	52,168	24,721	Sample 55	1	F2_CGATGT	R5_ACTGAT	F13_CCTAGAGT	R7_GTAGAGAG_	2
S384	day	33.5584	-122.1327	29-Apr-2016	1324	Cycle 2	Haul 3	Tow 1	Net 6	150-100	0.125	0.2137	13.80	58,804	27,439	Sample 56	1	F2_CGATGT	R6_ATTCCT	F14_CTATTAAG	R7_GTAGAGAG_	2
S385	day	33.5584	-122.1327	29-Apr-2016	1328	Cycle 2	Haul 3	Tow 1	Net 7	100-50	0.125	5.2092	16.45	54,315	39,254	Sample 57	1	F2_CGATGT	R7_TTCCAC	F15_AAGGCTAT	R7_GTAGAGAG_	2
S386	day	33.5584	-122.1327	29-Apr-2016	1335	Cycle 2	Haul 3	Tow 1	Net 8	50-0	0.125	1.2503	16.24	54,277	20,696	Sample 58	1	F2_CGATGT	R8_TTGGCA	F16_GAGCCTTA	R7_GTAGAGAG_	2
S387	day	34.73994	-121.2671	3-May-2016	1235	Cycle 3	Haul 8	Tow 1	Net 1	400-350	0.125	1.7395	8.59	56,492	24,410	Sample 61	1	F3_TTAGGC	R1_GTGGCC	F9_CGTCTAAT	R8_CCTCTCTG_	2
S388	day	34.73994	-121.2671	3-May-2016	1242	Cycle 3	Haul 8	Tow 1	Net 2	350-300	0.125	3.0102	12.13	50,210	21,048	Sample 62	1	F3_TTAGGC	R2_GTTTCG	F10_TCTCTCCG	R8_CCTCTCTG_	2
S389	day	34.73994	-121.2671	3-May-2016	1251	Cycle 3	Haul 8	Tow 1	Net 3	300-250	0.125	2.3474	13.75	45,850	21,148	Sample 63	1	F3_TTAGGC	R3_CGTACG	F11_TCGACTAG	R8_CCTCTCTG_	2
S390	day	34.73994	-121.2671	3-May-2016	1256	Cycle 3	Haul 8	Tow 1	Net 4	250-200	0.125	1.4034	15.25	57,374	28,006	Sample 64	1	F3_TTAGGC	R4_GAGTGG	F12_TTCTAGCT	R8_CCTCTCTG_	2
S391	day	34.73994	-121.2671	3-May-2016	1300	Cycle 3	Haul 8	Tow 1	Net 5	200-150	0.125	1.3523	14.40	43,651	25,691	Sample 65	1	F3_TTAGGC	R5_ACTGAT	F13_CCTAGAGT	R8_CCTCTCTG_	2
S392	day	34.73994	-121.2671	3-May-2016	1304	Cycle 3	Haul 8	Tow 1	Net 6	150-100	0.125	0.9717	16.17	59,667	34,278	Sample 66	1	F3_TTAGGC	R6_ATTCCT	F14_CTATTAAG	R8_CCTCTCTG_	2
S393	day	34.73994	-121.2671	3-May-2016	1306	Cycle 3	Haul 8	Tow 1	Net 7	100-50	0.125	2.529	16.81	57,235	39,249	Sample 67	1	F3_TTAGGC	R7_TTCCAC	F15_AAGGCTAT	R8_CCTCTCTG_	2
S394	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	5.9138	19.43	56,880	34,490	Sample 68	1	F3_TTAGGC	R8_TTGGCA	F16_GAGCCTTA	R8_CCTCTCTG_	2
S395	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	6.9153	11.20	55,921	34,943	Sample 69	1	F4_TGACCA	R1_GTGGCC	F9_CGTCTAAT	R9_AGCGTAGC_	2
S396	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 12	Tow 1	Net 1	200-175	0.125	4.3321	13.34	59,357	26,811	Sample 70	1	F4_TGACCA	R2_GTTTCG	F10_TCTCTCCG	R9_AGCGTAGC_	2
S397	day	34.49348	-120.7343	7-May-2016	1234	Cycle 4	Haul 12	Tow 1	Net 2	175-150	0.125	4.2514	14.76	53,035	26,095	Sample 71	1	F4_TGACCA	R3_CGTACG	F11_TCGACTAG	R9_AGCGTAGC_	2
S398	day	34.49348	-120.7343	7-May-2016	1240	Cycle 4	Haul 12	Tow 1	Net 3	150-125	0.125	2.9025	13.83	63,940	29,392	Sample 72	1	F4_TGACCA	R4_GAGTGG	F12_TTCTAGCT	R9_AGCGTAGC_	2
S399	day	34.49348	-120.7343	7-May-2016	1244	Cycle 4	Haul 12	Tow 1	Net 4	125-100	0.125	2.5853	15.66	53,818	32,214	Sample 73	1	F4_TGACCA	R5_ACTGAT	F13_CCTAGAGT	R9_AGCGTAGC_	2
S400	day	34.49348	-120.7343	7-May-2016	1247	Cycle 4	Haul 12	Tow 1	Net 5	100-75	0.125	5.9409	15.23	59,452	36,791	Sample 74	1	F4_TGACCA	R6_ATTCCT	F14_CTATTAAG	R9_AGCGTAGC_	2
S401	day	34.49348	-120.7343	7-May-2016	1253	Cycle 4	Haul 12	Tow 1	Net 6	75-50	0.125	3.8322	14.03	61,699	33,631	Sample 75	1	F4_TGACCA	R7_TTCCAC	F15_AAGGCTAT	R9_AGCGTAGC_	2
S402	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	2.6284	17.40	57,496	49,836	Sample 76	1	F4_TGACCA	R8_TTGGCA	F16_GAGCCTTA	R9_AGCGTAGC_	2
S403	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	4.0717	10.14	53,935	45,116	Sample 77	1	F5_ACAGTG	R1_GTGGCC	F9_CGTCTAAT	R10_CAGCCTCG_	2
S404	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.9767	16.01	48,421	36,391	Sample 78	1	F5_ACAGTG	R2_GTTTCG	F10_TCTCTCCG	R10_CAGCCTCG_	2
S405	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.8912	12.12	48,890	36,771	Sample 79	1	F5_ACAGTG	R3_CGTACG	F11_TCGACTAG	R10_CAGCCTCG_	2
S406	day					Ext. Control	Ext. Control	Ext. Control	Ext. Control	Ext. Control	0	0	1.29	33,139	29,322	Sample 80	1	F5_ACAGTG	R4_GAGTGG	F12_TTCTAGCT	R10_CAGCCTCG_	2
S407	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 2	Tow 1	Net 5	200-150	0.0625	3.083	15.58	50,919	23,929	Sample 5	1	F5_ACAGTG	R5_ACTGAT	F13_CCTAGAGT	R10_CAGCCTCG_	2
S408	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 9	Tow 1	Net 8	50-0	0.01563	1.678	13.69	67,605	41,093	Sample 26	1	F5_ACAGTG	R6_ATTCCT	F14_CTATTAAG	R10_CAGCCTCG_	2
S409	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 13	Tow 1	Net 1	200-175	0.125	3.883	14.79	59,533	31,571	Sample 29	1	F5_ACAGTG	R7_TTCCAC	F15_AAGGCTAT	R10_CAGCCTCG_	2
S410	day												0.03	137	0	Neg. Control		F5_ACAGTG	R8_TTGGCA	F16_GAGCCTTA	R10_CAGCCTCG_	2
S411	day	33.03124	-122.93350	22-Apr-2016	1509	Cycle 1	Haul 1	Tow 1	Net 1	400-350	0.125	1.1738	4.95	45,124	20,879	Sample 43	2	F6_GCCAAT	R1_GTGGCC	F17_GCGTAAGA_	R1_TCGCCTTA	2
S412	day	33.03124	-122.93350	22-Apr-2016	1515	Cycle 1	Haul 1	Tow 1	Net 2	350-300	0.125	2.7917	6.57	49,593	20,910	Sample 44	2	F6_GCCAAT	R2_GTTTCG	F18_TTATGCGA_	R1_TCGCCTTA	2
S413	day	33.03124	-122.93350	22-Apr-2016	1521	Cycle 1	Haul 1	Tow 1	Net 3	300-250	0.125	2.8615	9.46	35,699	16,116	Sample 45	2	F6_GCCAAT	R3_CGTACG	F19_AGCGCTAG	R1_TCGCCTTA	2
S414	day	33.03124	-122.93350	22-Apr-2016	1528	Cycle 1	Haul 1	Tow 1	Net 4	250-200	0.125	2.032	11.80	35,110	17,585	Sample 46	2	F6_GCCAAT	R4_GAGTGG	F20_GATATCGA	R1_TCGCCTTA	2
S415	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 1	Tow 1	Net 5	200-150	0.125	1.7606	9.97	55,603	31,574	Sample 47	2	F6_GCCAAT	R5_ACTGAT	F21_CGCAGACG	R1_TCGCCTTA	2
S416	day	33.03124	-122.93350	22-Apr-2016	1544	Cycle 1	Haul 1	Tow 1	Net 6	150-100	0.125	1.3003	14.34	51,743	29,276	Sample 48	2	F6_GCCAAT	R6_ATTCCT	F22_TATGAGTA	R1_TCGCCTTA	2
S417	day	33.03124	-122.93350	22-Apr-2016	1552	Cycle 1	Haul 1	Tow 1	Net 7	100-50	0.125	2.154	14.98	50,262	24,419	Sample 49	2	F6_GCCAAT	R7_TTCCAC	F23_AGGTGCGT	R1_TCGCCTTA	2
S418	day	33.03124	-122.93350	22-Apr-2016	1601	Cycle 1	Haul 1	Tow 1	Net 8	50-0	0.125	3.5241	11.47	31,084	15,263	Sample 50	2	F6_GCCAAT	R8_TTGGCA	F24_GAACATAC	R1_TCGCCTTA	2
S419	day	33.5584	-122.1327	29-Apr-2016	1248	Cycle 2	Haul 3	Tow 1	Net 1	400-350	0.125	0.5675	10.82	54,802	21,046	Sample 51	2	F7_CAGATC	R1_GTGGCC	F17_GCGTAAGA_	R2_CTAGTACG	2
S420	day	33.5584	-122.1327	29-Apr-2016	1255	Cycle 2	Haul 3	Tow 1	Net 2	350-300	0.125	2.1215	9.45	34,933	15,713	Sample 52	2	F7_CAGATC	R2_GTTTCG	F18_TTATGCGA_	R2_CTAGTACG	2
S421	day	33.5584	-122.1327	29-Apr-2016	1306	Cycle 2	Haul 3	Tow 1	Net 3	300-250	0.125	2.5957	6.00	48,153	22,743	Sample 53	2	F7_CAGATC	R3_CGTACG	F19_AGCGCTAG	R2_CTAGTACG	2
S422	day	33.5584	-122.1327	29-Apr-2016	1314	Cycle 2	Haul 3	Tow 1	Net 4	250-200	0.125	1.367	13.04	28,975	13,966	Sample 54	2	F7_CAGATC	R4_GAGTGG	F20_GATATCGA	R2_CTAGTACG	2
S423	day	33.5584	-122.1327	29-Apr-2016	1320	Cycle 2	Haul 3	Tow 1	Net 5	200-150	0.125	0.3816	11.78	49,352	23,949	Sample 55	2	F7_CAGATC	R5_ACTGAT	F21_CGCAGACG	R2_CTAGTACG	2
S424	day	33.5584	-122.1327	29-Apr-2016	1324	Cycle 2	Haul 3	Tow 1	Net 6	150-100	0.125	0.2137	13.41	36,457	16,975	Sample 56	2	F7_CAGATC	R6_ATTCCT	F22_TATGAGTA	R2_CTAGTACG	2
S425	day	33.5584	-122.1327	29-Apr-2016	1328	Cycle 2	Haul 3	Tow 1	Net 7	100-50	0.125	5.2092	15.35	52,354	37,302	Sample 57	2	F7_CAGATC	R7_TTCCAC	F23_AGGTGCGT	R2_CTAGTACG	2
S426	day	33.5584	-122.1327	29-Apr-2016	1335	Cycle 2	Haul 3	Tow 1	Net 8	50-0	0.125	1.2503	13.95	55,457	20,576	Sample 58	2	F7_CAGATC	R8_TTGGCA	F24_GAACATAC	R2_CTAGTACG	2
S427	day	34.73994	-121.2671	3-May-2016	1235	Cycle 3	Haul 8	Tow 1	Net 1	400-350	0.125	1.7395	11.71	64,700	26,225	Sample 61	2	F8_ACTTGA	R1_GTGGCC	F17_GCGTAAGA_	R3_TTCTGCCT	2
S428	day	34.73994	-121.2671	3-May-2016	1242	Cycle 3	Haul 8	Tow 1	Net 2	350-300	0.125	3.0102	9.53	43,801	17,923	Sample 62	2	F8_ACTTGA	R2_GTTTCG	F18_TTATGCGA_	R3_TTCTGCCT	2
S429	day	34.73994	-121.2671	3-May-2016	1251	Cycle 3	Haul 8	Tow 1	Net 3	300-250	0.125	2.3474	12.56	50,363	22,923	Sample 63	2	F8_ACTTGA	R3_CGTACG	F19_AGCGCTAG	R3_TTCTGCCT	2
S430	day	34.73994	-121.2671	3-May-2016	1256	Cycle 3	Haul 8	Tow 1	Net 4	250-200	0.125	1.4034	7.01	45,285	22,218	Sample 64	2	F8_ACTTGA	R4_GAGTGG	F20_GATATCGA	R3_TTCTGCCT	2
S431	day	34.73994	-121.2671	3-May-2016	1300	Cycle 3	Haul 8	Tow 1	Net 5	200-150	0.125	1.3523	9.82	29,626	17,878	Sample 65	2	F8_ACTTGA	R5_ACTGAT	F21_CGCAGACG	R3_TTCTGCCT	2
S432	day	34.73994	-121.2671	3-May-2016	1304	Cycle 3	Haul 8	Tow 1	Net 6	150-100	0.125	0.9717	12.90	73,229	41,969	Sample 66	2	F8_ACTTGA	R6_ATTCCT	F22_TATGAGTA	R3_TTCTGCCT	2
S433	day	34.73994	-121.2671	3-May-2016	1306	Cycle 3	Haul 8	Tow 1	Net 7	100-50	0.125	2.529	14.61	38,117	26,450	Sample 67	2	F8_ACTTGA	R7_TTCCAC	F23_AGGTGCGT	R3_TTCTGCCT	2
S434	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	5.9138	14.47	56,593	34,848	Sample 68	2	F8_ACTTGA	R8_TTGGCA	F24_GAACATAC	R3_TTCTGCCT	2
S435	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	6.9153	9.81	80,131	49,518	Sample 69	2	F9_GATCAG	R1_GTGGCC	F17_GCGTAAGA_	R4_GCTCAGGA	2
S436	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 12	Tow 1	Net 1	200-175	0.125	4.3321	9.17	52,702	23,453	Sample 70	2	F9_GATCAG	R2_GTTTCG	F18_TTATGCGA_	R4_GCTCAGGA	2
S437	day	34.49348	-120.7343	7-May-2016	1234	Cycle 4	Haul 12	Tow 1	Net 2	175-150	0.125	4.2514	16.08	41,924	20,172	Sample 71	2	F9_GATCAG	R3_CGTACG	F19_AGCGCTAG	R4_GCTCAGGA	2
S438	day	34.49348	-120.7343	7-May-2016	1240	Cycle 4	Haul 12	Tow 1	Net 3	150-125	0.125	2.9025	10.12	32,025	14,334	Sample 72	2	F9_GATCAG	R4_GAGTGG	F20_GATATCGA	R4_GCTCAGGA	2
S439	day	34.49348	-120.7343	7-May-2016	1244	Cycle 4	Haul 12	Tow 1	Net 4	125-100	0.125	2.5853	13.59	41,341	24,171	Sample 73	2	F9_GATCAG	R5_ACTGAT	F21_CGCAGACG	R4_GCTCAGGA	2
S440	day	34.49348	-120.7343	7-May-2016	1247	Cycle 4	Haul 12	Tow 1	Net 5	100-75	0.125	5.9409	15.26	33,917	20,462	Sample 74	2	F9_GATCAG	R6_ATTCCT	F22_TATGAGTA	R4_GCTCAGGA	2
S441	day	34.49348	-120.7343	7-May-2016	1253	Cycle 4	Haul 12	Tow 1	Net 6	75-50	0.125	3.8322	14.48	50,737	27,421	Sample 75	2	F9_GATCAG	R7_TTCCAC	F23_AGGTGCGT	R4_GCTCAGGA	2
S442	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	2.6284	14.18	50,723	42,770	Sample 76	2	F9_GATCAG	R8_TTGGCA	F24_GAACATAC	R4_GCTCAGGA	2
S443	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	4.0717	9.36	53,664	45,852	Sample 77	2	F10 TAGCTT	R1_GTGGCC	F17_GCGTAAGA_	R5_AGGAGTCC	2
S444	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.9767	8.23	52,375	39,605	Sample 78	2	F10 TAGCTT	R2_GTTTCG	F18_TTATGCGA_	R5_AGGAGTCC	2
S445	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.8912	8.83	19,954	15,108	Sample 79	2	F10 TAGCTT	R3_CGTACG	F19_AGCGCTAG	R5_AGGAGTCC	2
S446	day					Ext. Control	Ext. Control	Ext. Control	Ext. Control	Ext. Control	0	0	2.17	34,756	30,535	Sample 80	2	F10 TAGCTT	R4_GAGTGG	F20_GATATCGA	R5_AGGAGTCC	2
S447	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 2	Tow 1	Net 5	200-150	0.0625	3.083	12.25	41,375	19,536	Sample 5	2	F10 TAGCTT	R5_ACTGAT	F21_CGCAGACG	R5_AGGAGTCC	2
S448	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 9	Tow 1	Net 8	50-0	0.01563	1.678	12.19	52,688	31,776	Sample 26	2	F10 TAGCTT	R6_ATTCCT	F22_TATGAGTA	R5_AGGAGTCC	2
S449	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 13	Tow 1	Net 1	200-175	0.125	3.883	15.84	64,264	33,635	Sample 29	2	F10 TAGCTT	R7_TTCCAC	F23_AGGTGCGT	R5_AGGAGTCC	2
S450	day												0.79	269	48	Neg. Control		F10 TAGCTT	R8_TTGGCA	F24_GAACATAC	R5_AGGAGTCC	2
S451	day	33.03124	-122.93350	22-Apr-2016	1509	Cycle 1	Haul 1	Tow 1	Net 1	400-350	0.125	1.1738	11.14	32,664	15,389	Sample 43	3	F11_GGCTAC	R1_GTGGCC	F17_GCGTAAGA_	R6_CATGCCTA_	2
S452	day	33.03124	-122.93350	22-Apr-2016	1515	Cycle 1	Haul 1	Tow 1	Net 2	350-300	0.125	2.7917	10.33	57,066	24,177	Sample 44	3	F1_ATCACG	R10 GTACAC	F18_TTATGCGA_	R6_CATGCCTA_	2
S453	day	33.03124	-122.93350	22-Apr-2016	1521	Cycle 1	Haul 1	Tow 1	Net 3	300-250	0.125	2.8615	11.10	23,439	10,219	Sample 45	3	F1_ATCACG	R11_GATGGA	F19_AGCGCTAG	R6_CATGCCTA_	2
S454	day	33.03124	-122.93350	22-Apr-2016	1528	Cycle 1	Haul 1	Tow 1	Net 4	250-200	0.125	2.032	10.57	54,918	27,205	Sample 46	3	F1_ATCACG	R12_CTACCG	F20_GATATCGA	R6_CATGCCTA_	2
S455	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 1	Tow 1	Net 5	200-150	0.125	1.7606	14.24	47,829	27,512	Sample 47	3	F11_GGCTAC	R6_ATTCCT	F21_CGCAGACG	R6_CATGCCTA_	2
S456	day	33.03124	-122.93350	22-Apr-2016	1544	Cycle 1	Haul 1	Tow 1	Net 6	150-100	0.125	1.3003	13.00	24,080	13,801	Sample 48	3	F2_CGATGT	R10 GTACAC	F22_TATGAGTA	R6_CATGCCTA_	2
S457	day	33.03124	-122.93350	22-Apr-2016	1552	Cycle 1	Haul 1	Tow 1	Net 7	100-50	0.125	2.154	14.44	25,314	12,807	Sample 49	3	F2_CGATGT	R11_GATGGA	F23_AGGTGCGT	R6_CATGCCTA_	2
S458	day	33.03124	-122.93350	22-Apr-2016	1601	Cycle 1	Haul 1	Tow 1	Net 8	50-0	0.125	3.5241	16.59	48,026	25,384	Sample 50	3	F2_CGATGT	R12_CTACCG	F24_GAACATAC	R6_CATGCCTA_	2
S459	day	33.5584	-122.1327	29-Apr-2016	1248	Cycle 2	Haul 3	Tow 1	Net 1	400-350	0.125	0.5675	9.76	57,590	23,974	Sample 51	3	F11_GGCTAC	R2_GTTTCG	F17_GCGTAAGA_	R7_GTAGAGAG_	2
S460	day	33.5584	-122.1327	29-Apr-2016	1255	Cycle 2	Haul 3	Tow 1	Net 2	350-300	0.125	2.1215	9.98	60,568	27,766	Sample 52	3	F3_TTAGGC	R10 GTACAC	F18_TTATGCGA_	R7_GTAGAGAG_	2
S461	day	33.5584	-122.1327	29-Apr-2016	1306	Cycle 2	Haul 3	Tow 1	Net 3	300-250	0.125	2.5957	11.93	61,276	29,302	Sample 53	3	F3_TTAGGC	R11_GATGGA	F19_AGCGCTAG	R7_GTAGAGAG_	2
S462	day	33.5584	-122.1327	29-Apr-2016	1314	Cycle 2	Haul 3	Tow 1	Net 4	250-200	0.125	1.367	13.86	43,983	21,846	Sample 54	3	F3_TTAGGC	R12_CTACCG	F20_GATATCGA	R7_GTAGAGAG_	2
S463	day	33.5584	-122.1327	29-Apr-2016	1320	Cycle 2	Haul 3	Tow 1	Net 5	200-150	0.125	0.3816	14.31	65,890	31,352	Sample 55	3	F11_GGCTAC	R7_TTCCAC	F21_CGCAGACG	R7_GTAGAGAG_	2
S464	day	33.5584	-122.1327	29-Apr-2016	1324	Cycle 2	Haul 3	Tow 1	Net 6	150-100	0.125	0.2137	13.36	60,330	27,330	Sample 56	3	F4_TGACCA	R10 GTACAC	F22_TATGAGTA	R7_GTAGAGAG_	2
S465	day	33.5584	-122.1327	29-Apr-2016	1328	Cycle 2	Haul 3	Tow 1	Net 7	100-50	0.125	5.2092	15.77	48,628	33,864	Sample 57	3	F4_TGACCA	R11_GATGGA	F23_AGGTGCGT	R7_GTAGAGAG_	2
S466	day	33.5584	-122.1327	29-Apr-2016	1335	Cycle 2	Haul 3	Tow 1	Net 8	50-0	0.125	1.2503	15.74	51,672	21,910	Sample 58	3	F4_TGACCA	R12_CTACCG	F24_GAACATAC	R7_GTAGAGAG_	2
S467	day	34.73994	-121.2671	3-May-2016	1235	Cycle 3	Haul 8	Tow 1	Net 1	400-350	0.125	1.7395	9.23	66,128	30,028	Sample 61	3	F11_GGCTAC	R3_CGTACG	F17_GCGTAAGA_	R8_CCTCTCTG_	2
S468	day	34.73994	-121.2671	3-May-2016	1242	Cycle 3	Haul 8	Tow 1	Net 2	350-300	0.125	3.0102	12.18	55,723	23,094	Sample 62	3	F5_ACAGTG	R10 GTACAC	F18_TTATGCGA_	R8_CCTCTCTG_	2
S469	day	34.73994	-121.2671	3-May-2016	1251	Cycle 3	Haul 8	Tow 1	Net 3	300-250	0.125	2.3474	14.01	56,804	25,795	Sample 63	3	F5_ACAGTG	R11_GATGGA	F19_AGCGCTAG	R8_CCTCTCTG_	2
S470	day	34.73994	-121.2671	3-May-2016	1256	Cycle 3	Haul 8	Tow 1	Net 4	250-200	0.125	1.4034	13.36	61,681	30,177	Sample 64	3	F5_ACAGTG	R12_CTACCG	F20_GATATCGA	R8_CCTCTCTG_	2
S471	day	34.73994	-121.2671	3-May-2016	1300	Cycle 3	Haul 8	Tow 1	Net 5	200-150	0.125	1.3523	12.41	67,892	41,124	Sample 65	3	F11_GGCTAC	R8_TTGGCA	F21_CGCAGACG	R8_CCTCTCTG_	2
S472	day	34.73994	-121.2671	3-May-2016	1304	Cycle 3	Haul 8	Tow 1	Net 6	150-100	0.125	0.9717	14.83	47,730	26,889	Sample 66	3	F6_GCCAAT	R10 GTACAC	F22_TATGAGTA	R8_CCTCTCTG_	2
S473	day	34.73994	-121.2671	3-May-2016	1306	Cycle 3	Haul 8	Tow 1	Net 7	100-50	0.125	2.529	18.98	51,927	35,818	Sample 67	3	F6_GCCAAT	R11_GATGGA	F23_AGGTGCGT	R8_CCTCTCTG_	2
S474	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	5.9138	14.13	70,404	45,364	Sample 68	3	F6_GCCAAT	R12_CTACCG	F24_GAACATAC	R8_CCTCTCTG_	2
S475	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 8	Tow 1	Net 8	50-0	0.01563	6.9153	11.70	60,916	39,666	Sample 69	3	F11_GGCTAC	R4_GAGTGG	F17_GCGTAAGA_	R9_AGCGTAGC_	2
S476	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 12	Tow 1	Net 1	200-175	0.125	4.3321	14.71	60,779	27,327	Sample 70	3	F7_CAGATC	R10 GTACAC	F18_TTATGCGA_	R9_AGCGTAGC_	2
S477	day	34.49348	-120.7343	7-May-2016	1234	Cycle 4	Haul 12	Tow 1	Net 2	175-150	0.125	4.2514	15.21	50,614	24,551	Sample 71	3	F7_CAGATC	R11_GATGGA	F19_AGCGCTAG	R9_AGCGTAGC_	2
S478	day	34.49348	-120.7343	7-May-2016	1240	Cycle 4	Haul 12	Tow 1	Net 3	150-125	0.125	2.9025	16.61	49,249	22,086	Sample 72	3	F7_CAGATC	R12_CTACCG	F20_GATATCGA	R9_AGCGTAGC_	2
S479	day	34.49348	-120.7343	7-May-2016	1244	Cycle 4	Haul 12	Tow 1	Net 4	125-100	0.125	2.5853	15.89	45,189	26,850	Sample 73	3	F11_GGCTAC	R10 GTACAC	F21_CGCAGACG	R9_AGCGTAGC_	2
S480	day	34.49348	-120.7343	7-May-2016	1247	Cycle 4	Haul 12	Tow 1	Net 5	100-75	0.125	5.9409	14.12	50,736	31,394	Sample 74	3	F8_ACTTGA	R10 GTACAC	F22_TATGAGTA	R9_AGCGTAGC_	2
S481	day	34.49348	-120.7343	7-May-2016	1253	Cycle 4	Haul 12	Tow 1	Net 6	75-50	0.125	3.8322	15.37	51,030	28,278	Sample 75	3	F8_ACTTGA	R11_GATGGA	F23_AGGTGCGT	R9_AGCGTAGC_	2
S482	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	2.6284	13.96	43,001	37,992	Sample 76	3	F8_ACTTGA	R12_CTACCG	F24_GAACATAC	R9_AGCGTAGC_	2
S483	day	34.49348	-120.7343	7-May-2016	1259	Cycle 4	Haul 12	Tow 1	Net 7	100-50	0.003906	4.0717	10.72	67,977	59,821	Sample 77	3	F11_GGCTAC	R5_ACTGAT	F17_GCGTAAGA_	R10_CAGCCTCG_	2
S484	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.9767	13.36	15,565	11,608	Sample 78	3	F9_GATCAG	R10 GTACAC	F18_TTATGCGA_	R10_CAGCCTCG_	2
S485	day	34.49348	-120.7343	7-May-2016	1305	Cycle 4	Haul 12	Tow 1	Net 8	25-0	0.0078125	3.8912	14.17	56,760	42,243	Sample 79	3	F9_GATCAG	R11_GATGGA	F19_AGCGCTAG	R10_CAGCCTCG_	2
S486	day					Ext. Control	Ext. Control	Ext. Control	Ext. Control	Ext. Control	0	0	3.37	74,811	65,512	Sample 80	3	F9_GATCAG	R12_CTACCG	F20_GATATCGA	R10_CAGCCTCG_	2
S487	day	33.03124	-122.93350	22-Apr-2016	1536	Cycle 1	Haul 2	Tow 1	Net 5	200-150	0.0625	3.083	15.06	55,242	26,294	Sample 5	3	F11_GGCTAC	R11_GATGGA	F21_CGCAGACG	R10_CAGCCTCG_	2
S488	day	34.73994	-121.2671	3-May-2016	1312	Cycle 3	Haul 9	Tow 1	Net 8	50-0	0.01563	1.678	15.81	66,066	39,730	Sample 26	3	F10 TAGCTT	R10 GTACAC	F22_TATGAGTA	R10_CAGCCTCG_	2
S489	day	34.49348	-120.7343	7-May-2016	1227	Cycle 4	Haul 13	Tow 1	Net 1	200-175	0.125	3.883	14.79	14,325	7,963	Sample 29	3	F10 TAGCTT	R11_GATGGA	F23_AGGTGCGT	R10_CAGCCTCG_	2

Table S2. Primer sequences and PCR protocols for both PCR steps for 18S and COI. Red Xs represent variable indexing region, with per-sample indexes found in Table S1.

18S PCR 1

	Primer name	Primer Seqence
Primers	Uni18S	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXXXXXAGGGCAAKYCTGGTGCCAGC
	Uni18SR	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGXXXXXXGRCGGTATCTRATCGYCTT

	Reagent	Volume
Reaction Volumes	MiFi mix	10 µl
	BSA	0.4 µl
	dH2O	6.6 µl
	DNA template	1 µl
	Primer 1	1 µl
	Primer 2	1 µl

	Temperature (°)	Time (MM:SS)	Cycles
PCR Cycling	95	3:00	
	95	0:15	28x
	52	0:30	
	72	0:15	
	72	5:00	

18S PCR 2

	Primer name	Primer Seqence
Primers	Tag_F	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTCAGATGTG
	Tag_R	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGGAGATGTGTA

	Reagent	Volume
Reaction Volumes	MiFi mix	7.5 µl
	BSA	0.3 µl
	dH2O	4.2 µl
	DNA template	1 µl
	Primer 1	1 µl
	Primer 2	1 µl

	Temperature (°)	Time (MM:SS)	Cycles
PCR Cycling	95	3:00	
	95	0:30	10x
	55	0:30	
	72	0:45	
	72	5:00	

COI PCR 1

	Primer name	Primer Seqence
Primers	jgLCO1490	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGXXXXXXGGWACWGGWTGAACWGTWTAYCCYCC
	jgHCO2198	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGXXXXXXTAIACYTCIGGRTGICCRAARAAYCA

	Reagent	Volume
Reaction Volumes	MiFi mix	10 µl
	BSA	0.4 µl
	dH2O	6.6 µl
	DNA template	1 µl
	Primer 1	1 µl
	Primer 2	1 µl

	Temperature (°)	Time (MM:SS)	Cycles
PCR Cycling	95	3:00	
	95	0:15	28x
	48	0:30	
	72	0:15	
	72	5:00	

COI PCR2

	Primer name	Primer Seqence
Primers	Tag_F	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXTCGTCGGCAGCGTCAGATGTG
	Tag_R	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTCGTGGGCTCGGAGATGTGTA

	Reagent	Volume
Reaction Volumes	MiFi mix	7.5 µl
	BSA	0.3 µl
	dH2O	4.2 µl
	DNA template	1 µl
	Primer 1	1 µl
	Primer 2	1 µl

	Temperature (°)	Time (MM:SS)	Cycles
PCR Cycling	95	3:00	
	95	0:30	10x
	55	0:30	
	72	0:45	
	72	5:00	