# Time-series metabarcoding analysis of zooplankton diversity of the NW Atlantic continental shelf

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## Abstract

Biodiversity of zooplankton is central to the functioning of ocean ecosystems, yet morphological taxonomic analysis requires teams of experts and detailed examination of many samples. Metabarcoding (DNA sequencing of short amplified regions of one or a few genes from environmental samples) is a powerful tool for analysis of the composition and diversity of natural communities. Metabarcoding of the marine zooplankton assemblage may allow rapid detection of ecosystem reorganizations and regime shifts. The 18S rRNA V9 hypervariable region was sequenced for 26 zooplankton samples collected from the Gulf of Maine, Georges Bank, and Mid-Atlantic Bight during Ecosystem Monitoring Surveys by the US Northeast Fisheries Science Center during 2002-2012. A total of 7,648,033 sequences and 22,072 operational taxonomic units (OTUs) were identified and classified into 28 taxonomic groups of plankton. Comparative analysis of molecular (V9 sequence numbers) and morphological (abundance counts) focused on 7 taxonomic groups and revealed similar patterns of variation among years and regions. Sequence numbers and abundance counts showed positive correlation for all groups, with significant correlations ($p<0.05$) for Calanoida, Gastropoda, and Chaetognatha. Shannon diversity index values calculated using sequence numbers and abundance counts showed highly significant correlation ($r=0.625$; $p=0.0008$) across all regions during 2002-2012. This study demonstrates the potential of metabarcoding for time-series analysis of zooplankton biodiversity, ocean ecosystem assessment, and fisheries management.

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## Introduction

*Time-series monitoring of the NW Atlantic continental shelf*

The NW Atlantic continental shelf has been the focus of decades-long time-series monitoring by the US National Marine Fisheries Service (NMFS) Northeast Fisheries Science Center (NEFSC; Kane, 2007; Hare and Kane, 2012) and intensive studies of population dynamics processes during the US Global Ocean Ecosystem Dynamics (GLOBEC) Georges Bank Study (Wiebe et al., 2006). From 2001 to the present, samples have been collected and preserved for genetic analysis at 20 stations randomly-selected among the 120 stations sampled during each quarterly survey of the the NEFSC Ecosystem Monitoring (EcoMon) program Northwest Atlantic continental shelf. These efforts have allowed examination of temporal variation on a range of scales (seasonal, interannual, decadal) in zooplankton diversity, distribution, and abundance (O'Brien et al., 2013).

As a whole, the continental shelf ecosystem of the NW Atlantic is influenced by water flowing towards the equator from the Arctic via the Labrador Sea/Shelf and Scotian Shelf (Loder et al., 1998). The ecosystem can be separated into distinct regions based on physical and biological oceanographic features (Figure 1A), and the changing composition of the water along the shelf is reflected in changes in the zooplankton species composition, with boreal species most abundant in the north and temperate species more prevalent in the south (Cox and Wiebe, 1979; Head and Sameoto, 2007). The Gulf of Maine / Georges Bank region represents a southern boundary for many boreal species, as well as a northern limit for some temperate and subtropical coastal species. However, this pattern is changing with the warming trend that is becoming evident in the area.

The zooplankton community of the NW Atlantic continental shelf has undergone periodic regime shifts or large, persistent changes in the structure and function of the ecosystem, with marked changes in pelagic community structure (Pershing et al., 2005; Kane, 2007), zooplankton diversity (Record et al., 2010; Johnson et al., 2011), and biomass (O'Brien et al., 2013), as well as a suite of ecosystem services (Biggs et al., 2009). On Georges Bank, a shift in community structure and biodiversity level occurred in ~1990, evidenced by increased zooplankton displacement volume (Kane, 2007; O'Brien et al., 2013). Another regime shift was evident in ~2000, when some - but not all - of the earlier changes on Georges Bank were partially reversed. In the Gulf of Maine, levels of zooplankton diversity increased markedly during the early 1990s and decreased rapidly about 2000 (Record et al., 2010; Johnson et al., 2011).

Based on time-series analyses in several regions of the NW Atlantic continental shelf, it appears that zooplankton diversity may serve as a "leading indicator" of environmental change (Johnson et al., 2011). Questions remain about whether and how total biodiversity levels (including all taxonomic groups of zooplankton, discrimination of cryptic species, and detection of rare species) may change over time and in association with the observed regime shifts. It is also unclear to what extent temporal changes, including regime shifts, may vary among the four regions of the NW Atlantic continental shelf (see Figure 1A). Regular, standardized, and sustained monitoring, with time-series analysis of diverse environmental and ecosystem parameters, may provide early-warning indicators of ecosystem regime shifts (Borja, 2014; Stern et al., 2018).

*Metabarcoding analysis of zooplankton diversity*

Metabarcoding (i.e., high throughput DNA sequencing of complex environmental samples for one or more barcode gene regions) shows considerable promise as a novel approach

for detailed and accurate biodiversity assessments of marine communities (Fonseca et al. 2010; Bourlat et al., 2013; Ji et al., 2013; Mohrbeck et al., 2015). The taxonomic complexity of marine zooplankton assemblages makes such approaches particularly useful, since metabarcoding may detect the hidden diversity of zooplankton assemblages (Lindeque et al., 2013) and allow accurate, high-resolution, rapid characterization of temporal and spatial patterns of variation. A number of different gene regions have been used to characterize zooplankton diversity across a range of systematic levels, including several hypervariable regions of the nuclear 18S ribosomal RNA (rRNA) gene: V1-V2 (Lindeque et al., 2013); V4 (Sun et al., 2015); and V9 (Pearman et al., 2014; De Vargas et al., 2015; Pearman and Irigoien, 2015; Albaina et al., 2016). Among these, the V9 region is shortest and usually most conserved, with the possible advantage of improved detection across the taxonomic spectrum, but generally lower levels of taxonomic resolution (with the possible exception of Copepoda; see Wu et al., 2015) and consequent underestimation of the true diversity in a community (Tang et al., 2012). A number of metabarcoding studies of zooplankton have used target regions of the mitochondrial cytochrome oxidase I (COI) gene, which allows detailed assessment of biodiversity at the species level (Leray et al., 2013). Several studies (Djurhuus et al., 2018; Steffani et al., 2018; Zhang et al., 2018) have compared metabarcoding results using 18S rRNA and COI gene regions, allowing detailed investigation of the impacts of marker choice on biodiversity estimates for zooplankton

There are a number of remaining challenges for interpretation of metabarcoding data to provide accurate and reliable estimates of biodiversity of zooplankton assemblages. Some challenges are a consequence of the molecular protocols, including variation in PCR and sequencing primer efficiencies and match/mis-match differences among taxonomic groups and among barcode gene regions; other challenges are due to impacts of bioinformatics parameters

and protocols on calculated biodiversity levels (Bucklin et al., 2016). A critically important approach to groundtruthing and evaluating accuracy of metabarcoding analysis is direct comparison with results of morphological taxonomic analysis of the same samples.

Another challenge is to examine the potential for quantitative interpretation (abundance and/or biomass) of metabarcoding results. Several studies have compared molecular and morphological quantitative analyses (Lindeque et al., 2013), including use of 'mock' samples constructed with known numbers or biomass of target taxa (Sun et al., 2015; Hirai et al., 2017b). Such direct comparisons between metabarcoding and traditional morphological taxonomic analysis are critically needed to evaluate the potential power of molecular approaches for describing variation across time and space in ocean ecosystems and detecting impacts of climate change on the zooplankton assemblage (Kelly, 2016).

*Integrative molecular – morphological taxonomic analysis of zooplankton biodiversity*

The EcoMon Survey has yielded invaluable archives of environmental and biological samples that have allowed characterization of patterns of temporal and spatial variation over several decades, including detection of responses to climate variability and ecosystem regime shifts (Kane, 2007; O'Brien et al., 2013). Detailed examination and analysis of these archives, including morphological taxonomic examination of zooplankton samples, have resulted in a comprehensive understanding of zooplankton diversity of the NW Atlantic continental shelf. This study analyzed archived time-series samples collected during EcoMon survey cruises during 2002-2012 to directly compare molecular (metabarcoding using the 18S rRNA V9 hypervariable region) and morphological taxonomic (abundance counts) analysis of zooplankton diversity. Bioinformatic and statistical analysis was designed to address important issues and challenges for use of metabarcoding for characterization of zooplankton diversity, including

taxonomic resolution and coverage of the V9 metabarcode region, quantitative analysis of taxa, relationship of biodiversity to environmental conditions, and characterization of temporal and spatial patterns of variation. Our goal is to evaluate prospects for zooplankton metabarcoding for applications for ocean monitoring and assessment, including detection of ecosystem shifts and impacts of climate change.

## Materials and Methods

### *Collection of samples*

Zooplankton samples were collected by the NOAA Northeast Fisheries Science Center (NEFSC) Oceanography Branch during surveys by the Ecosystem Monitoring Program (EcoMon) of the NW Atlantic continental shelf (Kane, 2007, 2011; Hare and Kane, 2012). Surveys are designed to allow sampling from all four regions of the shelf ecosystem (Figure 1A). Samples for morphological taxonomic analysis were collected following a standard protocol (Richardson et al., 2010), with both day and night sampling using a 61-cm bongo net fitted with a 333-μm mesh net; oblique tows were a minimum of 5-min in duration and sampled from the surface to within 5 m of the seabed or to a maximum depth of 200 m. A mechanical flowmeter was fitted in the mouth of each net to record the volume sampled. Samples were preserved in 5% formalin and archived at the NEFSC.

Zooplankton samples for genetic analysis were collected during EcoMon survey cruises at 5 randomly-selected locations in each region. Sampling was done using a 20-cm bongo net with 165-μm mesh nets, which was attached to the same cable and deployed with the 61-cm bongo nets. Samples were preserved immediately in 95% undenatured ethanol; ethanol was changed 24 hr after collection. Samples were transported to and archived at the University of Connecticut.

*Morphological taxonomic analysis*

Sample sorting and identification was done at the Morski Instytut Rybacki (Szczecin, Poland). Zooplankton samples were split to an aliquot containing approximately 500 specimens; individuals were sorted, counted, and identified to the lowest possible taxon (Kane, 2007, 2011). Data recorded include abundance measured by area (Conc/10$m^2$) and volume (Conc/100$m^3$) for each taxon. The data file (EcoMon_Plankton_Data_v3_1.xlsx) was downloaded from: ftp://ftp.nefsc.noaa.gov/pub/hydro/zooplankton_data/. Statistical analysis focused on 7 ecologically-important taxonomic groups of zooplankton for which data were available for both metabarcoding (sequence numbers categorized in relation to the SILVA database; Quast et al., 2013) and morphological abundance counts (from the NEFSC taxonomic database).

*Metabarcoding analysis*

A total of 26 samples was selected for metabarcoding analysis, including one sample collected in each of three regions, Georges Bank (GB), Gulf of Maine (GoM), and Mid-Atlantic Bight (MAB), during May/June of 2002 – 2012 (Figure 1B; Table 1). There were a number of sampling gaps due to cancelled cruises, bad weather, and other causes: no samples were analyzed from MAB during 2003 or GoM in 2006; no samples were analysed for 2008; and only a single GB sample was analysed for 2012 (Table 1). One sample, AL0605-53 #13; Table 1) was collected from the Southern New England (SNE) region, but was grouped with GB samples for analysis.

Extraction and quantification of genomic DNA: Samples were quantitatively sub-divided using a box splitter to reduce zooplankton volume to ~25 mL. The sample was then washed with distilled water; inserted into a 50 mL Falcon tube above 35 µm Nitex mesh, which served to suspend the material and dry the pellet; and centrifuged at 3500 g for 4 min. The pellet was

moved to a new 50 mL Falcon tube, and SDS buffer (Tris-HCl, 10 mM; EDTA, pH 8.0, 100mM; NaCl, 200mM; SDS 1%) 3 mL or equal to pellet volume, whichever was smaller) was added. The sample was homogenized using a hand-held homogenizer (D1000, Thomas Scientific, New Jersey, USA) with saw tooth blade for 4 min at level 5. Proteinase K (MP Biomedicals) was added (0.2 mg/mL of sample) and tubes were incubated overnight in a water bath at 55-56 ˚C. After centrifugation (3500 g for 15 min), 400 uL of the supernatant was transferred to individual sterile 2 mL Eppendorf tubes for storage as necessary at -20 or -80 ˚C. Total genomic DNA was extracted using the E.Z.N.A Mollusc DNA kit (Omega Bio-tek) following manufacturer instructions. Total genomic DNA was quantified on a Thermo-Fisher NanoDrop 2000 and normalized to a final concentration of 5 ng/ul.

PCR amplification: The PCR master mix (per sample) consisted of: 2.5 µl genomic DNA (5ng/µl); 5 µl forward PCR primer (1 µM); 5 µl reverse PCR primer (1 µM); 12.5 µl 2x KAPA HiFi HotStart ReadyMix; for a total volume per sample of 25 µl. The PCR primers used were 1380F and 1510R from Amaral-Zettler et al. (2009), with added Illumina adapter sequences at the 5' end (shown in bold):

- 1380F_EU 5' -**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCCTGCCHTTTGTACACAC- 3'
- 1510R_EU 5' -**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCTTCYGCAGGTTCACCTAC- 3'

The PCR protocol was: 98 °C for 30 s; 10 cycles of: 98 °C for 10 s, 56 °C for 30 s, 72 °C for 15 s; 15 cycles of: 98 °C for 10 s, 62 °C for 30 s 72 °C for 15 s; and 1 cycle of 72 °C for 7 min. Amplification was verified using an Agilent 2200 TapeStation automated electrophoresis system. The PCR products were purified using AMPure XP beads (Agencourt), with an elution volume of 50 ul.

Library preparation: Index primers were added in a second PCR amplification of the purified amplicon (PCR product), with a master mix composed of (per sample): 5.0 µl purified

PCR product; 5 µl Nextera XT Index 1 Primer; 5 µl Nextera XT Index 2 Primer; 25 µl 2x KAPA HiFi HotStart ReadyMix; 10 µl PCR-grade water; for a total volume of 50 µl. The PCR protocol was: 95 °C for 3 min; 8 cycles of: 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and 1 cycle of 72 °C for 5 min. The indexed PCR product was purified using AMPure XP beads, with a final elution volume of 25 ul. Successful library attachment was verified using an Agilent 2200 TapeStation automated electrophoresis system. Libraries were quantified using a Qubit 3.0 fluorometer, normalized according to amplicon size, pooled, and denatured with 0.2 N NaOH. Samples were spiked with a minimum of 5% PhiX (Illumina).

High-throughput DNA sequencing: Sequencing was done on an Illumina MiSeq NextGen platform using a MiSeq Reagent Nano Kit (Ver. 2) with 500 cycles. Paired-end sequencing was done to produce bi-directional reads, with determination of consensus sequences (contigs).

*Bioinformatics and statistical analysis*

All bioinformatics steps were conducted on the Xanadu cluster of the University of Connecticut Health Center using a custom script for the Mothur (Ver. 1.39.5; Schloss et al., 2009). From the bi-directional sequences, contigs with a minimum length of 112 bp and no ambiguities were retained for analysis. Unique sequences were identified and aligned to the V9 region of a reference database customized from the SILVA database (Release 132; Quast et al., 2013; https://www.arb-silva.de/documentation/release-132/) by inclusion of additional sequences for eukaryotic marine organisms obtained from the NCBI GenBank. Following alignment to the reference database, reads with homopolymers longer than 8 bp were removed from the analysis. Chimeras were identified and removed using the UCHIME function (Edgar et al., 2011; http://drive5.com/usearch/manual/uchime_algo.html). Uncorrected pairwise distances were calculated between aligned DNA sequences, and distances up to 0.016 dissimilarity (equivalent

to 1 bp for the analyzed length) were used for clustering sequences. Clustering was performed using the Opti-clust method (Westcott and Schloss, 2017). . Taxonomic assignment of sequences and OTUs was done using a reference database customized from SILVA Release 132 (Quast et al., 2013). Sequences with bootstrap values of at least 80% were classified.

The reference sequences for 600 of the most frequent OTUs from the DE1105-25 sample were aligned using Clustal-W and analyzed by the Neighbor Joining algorithm using the Molecular Evolutionary Genetics Analysis software (MEGA, Ver. 6; Tamura et al., 2013). Statistical analysis of the gene tree was by bootstrapping with 1,000 subreplicates.

Multivariate statistical analysis focused on molecular and morphological results for 7 taxonomic groups defined in the NEFSC EcoMon Survey database for which there were non-zero total numbers for both V9 sequences and abundance counts. The 7 groups are: Eucarida, Calanoida, Cyclopoida, Chaetognatha, Gastropoda, Hydrozoa, and Peracarida. No abundance counts were available for one sample from the Gulf of Maine (DE0305-38). Numbers of sequences and abundance counts for each group for all 25 samples was examined by functional regression analysis (Ricker, 1973). Data were transformed by $\log_{10}$ (value +1) prior to analysis. Further statistical analysis of sequence numbers and abundance counts for the 7 groups across all samples was done using a paired t-test of the means carried out in MatLab (Ver. 2015B).

Patterns of variation across time and space were statistically evaluated for numbers of sequences and abundance counts in relation to environmental conditions (temperature and salinity at the sample collection sites) in MatLab (Ver. 2015B). All environmental data used were based on sampling at the same stations where samples used for metabarcoding were collected during each of the EcoMon Surveys from 2002 to 2012 (Table 1; data available at

ftp://ftp.nefsc.noaa.gov/pub/hydro/). For the taxonomic data, the distance measure used was Bray-Curtis dissimilarity coefficient (Ricker, 1973). Differentiation among the 3 regions over 11 years was evaluated by Non-Metric Multidimensional Scaling (NMDS) using the FATHOM Toolbox for MatLab (Jones, 2015; http://www.marine.usf.edu/user/djones/matlab/matlab.html). Patterns of latitudinal variation for numbers of sequences and abundance counts for each group were examined by functional regression analysis (Ricker, 1973), with associated significance levels (p values). The Shannon and Simpson Diversity Indices (H and D; Pielou, 1977) were calculated using numbers of sequences and morphological taxonomic abundance counts for the 7 groups.

**Results**

Molecular and morphological taxonomic analysis of zooplankton diversity was carried out for samples collected from three regions of the NW Atlantic continental shelf (GB, GoM, and MAB) over 11 years from 2002 to 2012 (Figure 1B). A total of 26 samples was analyzed, with some sampling gaps (Table 1). Metabarcoding using the V9 hypervariable region of 18S rRNA yielded a total of 7,648,033 sequences (Suppl. Table 1) and 22,072 Operational Taxonomic Units (OTUs; Suppl. Table 2) that were identified and classified into 28 taxonomic groups of plankton based on the SILVA database (Quast et al., 2013; Release 132). Considering all samples together, numbers of sequences and OTUs were highly significantly correlated across all 28 taxonomic groups considered together ($r=0.967$, $p=0.000$), as well as for each of the groups considered individually. Definitive analysis focused on sequence numbers.

The impact of environmental conditions, including temperature and salinity measurements at the sample collection sites (Table 1), on patterns of metabarcode variability, measured as both sequence and OTU numbers per taxonomic group per sample, was evaluated

by functional regression analysis, which revealed no significant relationship ($p<0.05$) between the metabarcode data and any environmental parameter (results not shown).

Initial analysis of metabarcoding results included 28 taxonomic groups of plankton to which sequences (Suppl. Table 1) and OTUs (Suppl. Table 2) were assigned. Morphological taxonomic analysis of samples from EcoMon Surveys used 18 of these same 28 groups for identification and categorization of zooplankton, which are reported as counts per $10m^2$ (Suppl. Table 3). Some of these groups (Annelida, Anthozoa, Bivalvia, Echinoidea, Teleostei and Thecostraca) were excluded from detailed analysis, since they include few – or no – representatives of the holozooplankton. Several additional groups were observed either rarely or never in samples collected at the same sites as those analyzed for metabarcoding; these included Scyphozoa and Bryozoa (0 stations); Ctenophora (1), Ostracoda (2); and Salpida (4).

The 18S rRNA V9 hypervariable region showed good resolution of ecologically-important groups of marine plankton (Figure 2). Comparative statistical analysis of molecular (metabarcode) and morphological (abundance counts) data focused on 7 taxonomic groups of holozooplankton for which counts were available from the NEFSC EcoMon Database. Numbers of sequences and abundance counts based on morphological taxonomic identifications showed significant correlations for the 7 taxonomic groups considered together ($r=0.551$; $p<0.001$; Figure 3). Functional regression lines for all of the groups analyzed separately revealed positive correlation between sequence numbers and abundance counts, with significant correlation ($p < 0.05$) for 3 groups: Calanoida, Chaetognatha, and Gastropoda (Figure 3).

Comparison of sequence numbers and abundance counts by group for all 25 samples revealed overall similarities of temporal and spatial patterns (Figure 4). Statistical analysis across all samples and regions by paired t-test of the means revealed significant differences ($p < 0.05$)

between sequence numbers and abundance counts for several groups, including Calanoida, Cyclopoida, and Peracarida(Table 2). Comparisons by region yielded significant differences for samples from Georges Bank for Calanoida and Peracarida; from the Gulf of Maine for Eucarida, Calanoida, and Gastropoda; and from the Mid-Atlantic Bight for the Peracarida (see Table 2 for t-test results).

Both the V9 metabarcode data and the morphological abundance counts for the 7 groups revealed evidence of variation among the 3 regions based on two-dimensional NMDS analysis. Abundance counts showed a clear pattern (stress value = 0.164; Figure 5A), but requires cautious interpretation. Sequence numbers for the 7 groups revealed a useful pattern of regional variation (stress value = 0.143), with distinctiveness of the MAB region (Figure 5B). As stated in the analytical software guidelines provided by Jones (2015), NMDS stress values <0.1 indicate a good ordination, whereas values <0.2 indicate a useful picture, but caution is needed to avoid putting too much emphasis on the details of the plot.

Correlation analysis of sequence numbers and total abundance counts by group (with zero values removed) with latitude revealed similarities in the slopes of the regression lines for some groups: both positive for Eucarida and Calanoida and both negative for Cyclopoida, Chaetognatha and Gastropoda (Figure 6). The correlations for between abundance counts and latitude were statistically significant ($p < 0.05$) for Eucarida and Gastropoda (Figure 6A); and between sequence numbers and latitude for Calanoida, Cyclopoida, and Gastropoda (Figure 6B).

Biodiversity of EcoMon samples collected from 2002-2012 based on sequence numbers and abundance counts for the 7 taxonomic groups showed statistically significant correlation based on both the Shannon Diversity Index (H; $r=0.620$ $p<0.001$; Figure 7) and the Simpson Diversity Index (D; $r=0.613$ $p= 0.001$; data not shown).

**Discussion**

Comparative and integrative molecular and morphological taxonomic approaches to characterizing marine zooplankton diversity are based in the emergent science of integrative taxonomy, which seeks to promote use of diverse data types to understand complex biological communities and diverse assemblages (Dayrat, 2005). DNA barcoding has been widely used for discriminating and identifying marine zooplankton species (Bucklin et al., 2010a, 2010b, 2011). More recently, advances in high throughput DNA sequencing have allowed metabarcoding analysis of unsorted zooplankton samples from a variety of marine environments (Pearman and Irigoien, 2015; Bucklin et al., 2016; Hirai et al., 2017a; Sommer et al., 2017). Direct comparison of metabarcoding results and morphological taxonomic analysis has been carried out by analysis of the same samples or sample aliquots (Lindeque et al., 2013; Hirai et al., 2017b; Yang et al., 2017) and by use of constructed samples with known abundance counts (Hirai and Tsuda, 2015).

A number of studies have specifically addressed the application of metabarcoding for use in ocean ecosystem monitoring and assessment (Kelly et al., 2014; Casas et al., 2017; Deagle et al., 2017; Goodwin et al., 2017; Aylagas et al., 2018; Zhang et al, 2018). In particular, time-series analysis of integrative morphological and molecular taxonomic results of zooplankton diversity can provide rapid detection of complex ecosystem responses to environmental variation and climate change (Abad et al., 2016; Stern et al., 2018).

This study seeks to explore and demonstrate the usefulness of metabarcoding of zooplankton biodiversity for time-series monitoring of ocean ecosystems. Regular sampling as part of the decades-long time-series monitoring of the NW Atlantic continental shelf by NMFS NEFSC has allowed detailed examination of patterns of variation across time and space in the zooplankton assemblage (Kane, 2007; Hare and Kane, 2012; O'Brien et al., 2013). Direct

comparison with metabarcoding results is possible by analysis of archived samples collected and preserved for genetic analysis at 20 randomly-selected stations during surveys of the NEFSC Ecosystem Monitoring (EcoMon) program since 2001. Statistical comparison of temporal and spatial patterns of variation based on molecular metabarcoding and morphological taxonomic analysis is an important step toward integration and application of metabarcoding results for ocean ecosystem assessment and management.

Metabarcoding results have been shown to depend markedly on a number of factors, including molecular protocols, statistical analysis, and bioinformatics approaches. As described above, a key issue is the choice of barcode gene marker and metabarcoding studies of marine zooplankton have employed and evaluated results based on one or more of the usual barcode gene regions, including regions of the 18S rRNA gene and / or mitochondrial COI (Leray et al., 2013; Lindeque et al., 2013; Clarke et al., 2017; Corell and Rodriquez-Ezpeleta, 2014; de Vargas et al., 2015; Elbrecht and Leese, 2015; Steffani et al., 2018; Zhang et al., 2018). The V9 hypervariable region of the nuclear 18S rRNA gene has been used for assessment of diversity across many domains of life (Amaral-Zettler et al., 2009; de Vargas et al., 2015). Due to the exceptional taxonomic complexity of the marine zooplankton assemblage, the conservative nature of this barcode region may help maximize the likelihood of consistent detection of all represented groups (Gonzalez et al., 2012; de Vargas et al., 2015; Abad et al., 2016, 2017; Albaina etal., 2016). At the molecular level, protocols using V9 as the target gene region may exhibit improved success in amplification and sequencing, lower variation in priming efficiencies, and fewer "missed" taxa due to primer incompatibilities compared to more variable gene marker regions (Amaral-Zettler et al., 2009). The broad and reliable taxonomic coverage resulting from the conserved nature of the gene (and especially priming sites), may also result in

lower taxonomic resolution for identification and classification of sequences and OTUs (Tang et al., 2012; Zhan et al., 2014), albeit with some exceptions, including perhaps the Copepoda (Wu et al., 2015).

Of the 28 groups for which sequence and OTUs numbers were summarized (Suppl. Tables 1, 2), 18 groups were also used for morphological taxonomic analysis of the EcoMon zooplankton samples (Suppl. Table 3). Detailed statistical analysis of the morphological and molecular results for 7 groups has been described above, but there are several noteworthy differences resulting from comparison of morphological taxonomic counts and metabarcoding analysis of the other 11 taxonomic groups. As has been observed in previous studies (Hirai et al., 2017a; Stefanni et al., 2018), metabarcoding can improve detection of tiny and difficult-to-identify taxa. One example is the Ostracoda, an ecologically important and diverse group of holozooplankton that has routinely been overlooked by taxonomists (Nigro et al., 2016). V9 sequences classified as Ostracoda occurred at 4 stations, compared to 2 stations with Ostracoda found from the morphological analysis. Another advantage of metabarcoding is the detection of fragile species that are destroyed during collection by nets. In this study, the gelatinous zooplankton groups, Ctenophora and Scyphozoa, were found at all but 2 stations using metabarcoding, but reported at 0 and 2 stations, respectively, based on morphological counts. Another fragile gelatinous group, Salpida, was reported by morphological taxonomic counts at 4 stations versus 18 for metabarcoding. However, interpretation of metabarcoding data for analysis of biodiversity of the holozooplankton assemblage requires some caution and further consideration. Among the 26 samples analyzed, several meroplanktonic groups (Anthozoa, Bivalvia, Bryozoa, Echinoidea, and Theostraca) were detected in many – usually nearly all – samples based on metabarcode data, but were observed in either none or a few samples based on

morphological counts. This discrepancy may be best explained by the abundance of meroplanktonic larvae over the continental shelf regions. The larvae may have been overlooked by morphological taxonomists during the sample counts, but an important consideration is that the mesh size of nets (165 μm) used to collect samples for metabarcoding analysis was smaller than nets used to collect samples for morphological taxonomic counts (333 μm). This difference was a consequence of time constraints on ancillary sampling during the NOAA NEFSC EcoMon Surveys, but the differences in net mesh size very likely explain some of the differences between the metabarcoding results and morphological taxonomic counts. Net mesh size is known to impact both biomass and species composition of zooplankton samples: Skjoldal et al. (2013) reported a consistent relationship between retention or escapement and size of the organisms, with 50% of organisms escaping through nets of mesh size equal to their widths. The under-representation in morphological counts of taxonomic groups that are predominantly meroplanktonic may be best explained by the escapement of the larval forms through the larger mesh nets used for collection of these samples.

Evaluation of spatial patterns of variation of zooplankton biodiversity revealed by both metabarcoding and morphological counts variation focused on comparisons among 3 regions, as defined by the NOAA NEFSC EcoMon Program. Comparisons based on functional regression (Figures 4, 6), NMDS (Figure 5), and the Shannon Diversity Index (Figure 7) all revealed variation among the regions, consistent with expectations from previous time-series analyses (Hare and Kane, 2012; O'Brien et al., 2013). In general, the variation among regions was clear – and in many instances statistically significant – but the marked variation within regions based on these same analyses suggested caution in the definition and discrimination among the regions. The challenge of quantification of taxa (either abundance or biomass) based on metabarcoding is

of central importance for applications for ocean monitoring and assessment (Elbrecht and Leese, 2015). There are numerous factors that may influence sequence numbers resulting from metabarcoding analysis, many of which are exacerbated by the exceptional phylogenetic diversity of the zooplankton assemblage (Bucklin et al., 2016). Sequence numbers are impacted by the widely-varying sizes of organisms, variation in PCR amplification efficiency or primer bias, and differences in copy number of the target rRNA genes, among others (Elbrecht and Leese, 2015; Deagle et al., 2017). A number of studies have sought to further explain the determinants of sequence numbers and have explored analytical approaches to approximating abundance and / or biomass of invertebrate taxa from metabarcoding data (Lindeque et al., 2013; Thomas et al., 2016; Bista et al., 2018; Deagle et al., 2018; Lamb et al., 2018).

This study did not examine the underlying causes of variation in sequence numbers or the reasons for differences in observed relationships among taxonomic groups. Our analyses revealed highly significant positive correlation between total abundance counts from morphological taxonomic identification and metabarcoding sequence numbers across all 7 taxonomic groups; 3 of 7 group-specific comparisons yielded significant correlations (Figure 3). These findings indicate the power of metabarcoding using the V9 18S rRNA region for quantitative analysis of these ecologically-important taxonomic groups of zooplankton, and the promise of this approach for applications for fisheries management and ecosystem assessment. Estimation of biodiversity based on metabarcoding is both enormously promising and filled with remaining challenges, including accurate estimation of species-level diversity and questions of whether “cryptic” diversity is real (Bucklin et al., 2016; Creer et al., 2016). The analyses are markedly sensitive to variation among bioinformatics pipelines and parameters, and careful examination of detailed methodologies is needed to evaluate potential sources of error, including

quality controls on sequence data, clustering thresholds for OTU classification, minimum number of replicate sequences, among others (Brown et al., 2015; Edgar and Flyvbjerg, 2015; Xiong and Zhan, 2018). One useful approach is direct comparison of molecular metabarcoding and morphological taxonomic analysis of biodiversity for well-studied ocean regions (e.g., Abad et al., 2016; Steffani et al., 2018). Such comparisons also allow consideration of errors associated with sample handling (e.g., inaccurate subsampling of samples) and morphological taxonomic analysis (e.g., misidentifying species and overlooking rare or cryptic taxa).

This study used bioinformatics parameters and approaches for taxonomic assignment and classification of OTUs that are similar and comparable to those used for previous metabarcoding studies of zooplankton diversity (see review by Bucklin et al., 2016). Our direct comparison of metabarcoding data and morphological counts for 18 taxonomic groups of plankton in samples collected over 11 years and 3 regions allow us to state conclusively that OTU numbers – as calculated by us for the V9 18S rRNA region – cannot provide an accurate assessment of species diversity. The total of 22,072 OTUs clustered in 28 taxonomic groups included 273 OTUs classifed as fish and 2,481 classified into protistan groups (Ciliates, Dinoflagellates, Diatoms, and others), with all other OTUs classified as metazoan invertebrates, including 6,812 OTUs classified as calanoid copepods (Suppl. Table 2). Johnson et al. (2011) summarized zooplankton diversity of the Gulf of Maine based on database records from morphological taxonomic analysis of samples from time-series monitoring efforts; they reported 533 metazoan species, including 195 species of calanoid copepods and 247 species of fish, and noted that 47% of all species were not found among 2,246 species listed in the Gulf of Maine Register of Marine Species (GoMRMS). Clearly, cryptic, rare and unknown species occur in zooplankton assemblages of well-studied marine ecosystems, such as the NW Atlantic continental shelf (Sherman et al.,

2002; Pershing et al., 2005). However, it is not justifiable based on these results to conclude that numbers of OTUs reported in this study are equivalent to species or that actual levels of species diversity are 10-times – or more – than currently known.

Standardization of metabarcoding pipelines (Tedersoo et al., 2015) for determination of marine zooplankton diversity may be an ideal solution for applications of ocean ecosystem assessment and monitoring, but this approach may be unrealistic for the near future. Instead, further study is critically needed to understand the impacts of many variables on metabarcoding results, including different molecular methods (e.g., barcode gene markers, primers, protocols, sequencing platforms), and statistical and bioinformatics approaches (e.g., pipelines, parameters).

**Conclusions**

The primary goal of this study was to examine the feasibility and value of adding metabarcoding analysis to ongoing time-series monitoring of ocean ecosystems, with the specific example of the NOAA NEFSC Ecosystem Monitoring (EcoMon) Program surveys of the NW Atlantic continental shelf. The design of the study was also guided by the broader goal of contributing to the climate-related information that is the basis of fisheries and ecosystem management in the region (see Link et al., 2015; Hare et al., 2016). Metabarcoding analysis of zooplankton diversity was carried out for samples collected during EcoMon Surveys over 11 years (2002-2012). Patterns of temporal and spatial variation were analyzed based on sequence numbers for the V9 hypervariable region of 18S rRNA gene for 7 taxonomic groups for which counts from morphological taxonomic analysis were also available. Sequence numbers and abundance counts showed similar patterns of variation across all samples and among 3 sampled regions (Georges Bank, Gulf of Maine, and Mid-Atlantic Bight) based on diverse multivariate

statistical analyses. Numbers of sequences and abundance counts showed significant correlation for the 7 taxonomic groups considered together, and positive correlations for all groups individually. Shannon Diversity Index based on sequence numbers and abundance counts showed highly significant correlation. The overall similarities in patterns of variation across time and space of zooplankton biodiversity based on molecular (V9 sequence numbers) and morphological (abundance counts) for 7 taxonomic groups provide evidence of the potential usefulness of metabarcoding for accurate characterization of biodiversity of the pelagic assemblage and rapid detection of ecosystem reorganizations, with applications for monitoring, assessment, and management of marine ecosystems.

**Data Accessibility**

The V9 18S rRNA metabarcode sequence data have been deposited in the NCBI GenBank Short Read Archive (see https://www.ncbi.nlm.nih.gov/sra/). Sequence data files in FASTQ format are available for all 26 samples analyzed from EcoMon Program suveys and can be accessed using SRA BioProject ID PRJNA513188.

**Supplementary material**

The following supplementary material is available at ICESJMS online:

Supplementary Table 1. Numbers of sequences for samples from each EcoMon station for the 28 taxonomic groups resolved by 18S rRNA V9 metabarcodes.

Supplementary Table 2. Numbers of operational taxonomic units (OTUs) for samples from each EcoMon station for 28 taxonomic groups resolved by V9 metabarcodes.

Supplementary Table 3. Abundance counts for 18 taxonomic groups for which data are available from both molecular and morphological taxonomic analysis.

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*Time-series metabarcoding analysis of zooplankton diversity of the NW Atlantic continental shelf*

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**Tables and Figures with Legends (Rev. January 14, 2019)**

**Table 1.** Sampling locations and environmental data for EcoMon samples used for metabarcoding analysis. Regions are indicated by number: Middle Atlantic Bight (1); Georges Bank (2), and Gulf of Maine (3). See Figure 1 for maps of area and station locations.

| Sequential Stn No. | Cruise | Station | Region | Latitude (N) | Longitude (W) | Collection Date | Time (UTC) | Temp Surface (°C) | Salinity Surface (ppt) | Temp max depth (°C) | Salinity max depth (ppt) | Water column depth (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AL0206 | 11 | 1 | 37.582 | -74.498 | 24-MAY-2002 | 03:20 | 15.14 | 33.79 | 11.19 | 34.37 | 69 |
| 2 | AL0206 | 75 | 2 | 41.023 | -67.373 | 31-MAY-2002 | 08:33 | 12.02 | 32.64 | 7.76 | 32.68 | 68 |
| 3 | AL0206 | 108 | 3 | 43.015 | -67.382 | 04-JUN-2002 | 01:00 | 9.47 | 32.33 | 8.66 | 34.69 | 224 |
| 4 | DE0305 | 15 | 2 | 41.008 | -67.023 | 26-MAY-2003 | 03:18 | 7.65 | 32.71 | 9.01 | 33.45 | 69 |
| 5 | DE0305 | 38 | 3 | 43.700 | -67.425 | 29-MAY-2003 | 02:00 | 7.07 | 32.50 | 7.36 | 34.09 | 210 |
| 6 | AL0405 | 14 | 1 | 36.987 | -75.107 | 26-MAY-2004 | 02:00 | 21.32 | 29.41 | 6.69 | 33.37 | 39 |
| 7 | AL0405 | 53 | 2 | 41.602 | -68.808 | 03-JUN-2004 | 01:38 | 8.63 | 32.89 | 5.95 | 33.27 | 150 |
| 8 | AL0405 | 117 | 3 | 43.067 | -70.110 | 07-JUN-2004 | 12:42 | 10.90 | 31.52 | 3.11 | 32.76 | 133 |
| 9 | AL0505 | 24 | 1 | 37.857 | -74.582 | 26-MAY-2005 | 10:22 | 10.21 | 31.88 | 8.99 | 31.97 | 52 |
| 10 | AL0505 | 70 | 2 | 41.853 | -68.347 | 02-JUN-2005 | 10:22 | 9.39 | 32.01 | 6.21 | 32.27 | 205 |
| 11 | AL0505 | 111 | 3 | 43.937 | -67.382 | 06-JUN-2005 | 12:34 | 8.54 | 31.81 | 6.90 | 33.95 | 218 |
| 12 | AL0605 | 14 | 1 | 39.017 | -73.572 | 02-JUN-2006 | 10:51 | 15.59 | 31.92 | 13.34 | 34.60 | 51 |
| 13 | AL0605 | 53 | 2 | 40.100 | -69.790 | 06-JUN-2006 | 11:50 | 15.57 | 32.49 | 13.65 | 35.68 | 106 |
| 14 | DE0706 | 15 | 1 | 37.857 | -74.645 | 24-MAY-2007 | 01:00 | 13.90 | 33.30 | 9.90 | 33.46 | 44 |
| 15 | DE0706 | 76 | 2 | 41.193 | -67.575 | 30-MAY-2007 | 10:04 | 10.28 | 33.13 | 9.61 | 33.13 | 41 |
| 16 | DE0706 | 112 | 3 | 43.650 | -67.683 | 03-JUN-2007 | 10:43 | 9.71 | 32.36 | 7.04 | 34.20 | 232 |
| 17 | DE0905 | 35 | 1 | 37.727 | -74.913 | 31-MAY-2009 | 09:06 | 18.30 | 32.38 | 9.80 | 33.08 | 32 |
| 18 | DE0905 | 73 | 2 | 41.437 | -67.678 | 05-JUN-2009 | 02:47 | 10.20 | 32.68 | 10.20 | 32.76 | 36 |
| 19 | DE0905 | 112 | 3 | 43.187 | -68.343 | 09-JUN-2009 | 08:38 | 10.50 | 31.84 | 6.60 | 33.99 | 192 |
| 20 | DE1004 | 25 | 1 | 41.102 | -70.577 | 26-MAY-2010 | 09:48 | 15.39 | 31.24 | 7.16 | 32.35 | 43 |
| 21 | DE1004 | 73 | 2 | 40.895 | -68.443 | 03-JUN-2010 | 02:50 | 10.71 | 32.45 | 10.71 | 32.45 | 54 |
| 22 | DE1004 | 129 | 3 | 42.688 | -68.330 | 08-JUN-2010 | 05:51 | 12.98 | 31.83 | 9.12 | 34.57 | 204 |
| 23 | DE1105 | 25 | 1 | 37.563 | -74.997 | 05-JUN-2011 | 07:23 | 23.14 | 27.33 | 9.00 | 32.65 | 31 |
| 24 | DE1105 | 83 | 2 | 40.688 | -67.745 | 10-JUN-2011 | 08:22 | 13.97 | 32.21 | 7.65 | 32.77 | 78 |
| 25 | DE1105 | 127 | 3 | 43.848 | -67.315 | 14-JUN-2011 | 05:44 | 8.46 | 31.79 | 8.29 | 34.21 | 197 |
| 26 | HB1202 | 71 | 2 | 40.933 | -67.550 | 11-JUN-2012 | 11:10 | 12.00 | 32.90 | 9.20 | 33.00 | 73 |

**Table 2.** Results of statistical comparison between numbers of sequences and morphological counts for the 7 taxonomic groups in samples for each of the 3 regions (Mid-Atlantic Bight, Georges Bank, Gulf of Maine) and for all samples considered together. All data were $\log_{10}$ transformed before analysis. Abbreviations are t-statistic value (t-stat); significance level (p).

| | **Eucarida** | **Calanoida** | **Cyclopoida** | **Chaetgnatha** | **Gastropoda** | **Hydrozoa** | **Peracarida** |
|---|---|---|---|---|---|---|---|
| ***Mid-Atlantic Bight*** | | | | | | | |
| t-stat | 0.158 | 0.768 | -1.513 | -0.448 | 0.314 | -1.989 | **9.054** |
| p | 0.877 | 0.455 | 0.153 | 0.661 | 0.758 | 0.067 | 0.000 |
| ***Georges Bank*** | | | | | | | |
| t-stat | 2.047 | **2.624** | -1.446 | 1.497 | -0.270 | -0.181 | **4.367** |
| p | 0.056 | 0.017 | 0.165 | 0.152 | 0.790 | 0.859 | 0.000 |
| ***Gulf of Maine*** | | | | | | | |
| t-stat | **-2.669** | **3.659** | -1.195 | -1.069 | **-3.895** | -0.325 | 1.248 |
| p | 0.021 | 0.003 | 0.255 | 0.306 | 0.002 | 0.751 | 0.236 |
| ***All Areas*** | | | | | | | |
| t-stat | -0.755 | **2.639** | **-2.200** | -0.277 | -1.071 | -1.198 | **5.811** |
| p | 0.454 | 0.011 | 0.033 | 0.783 | 0.290 | 0.237 | 0.000 |

**Figure 1. (A)** Regions of the NW Atlantic continental shelf sampled during quarterly surveys of the NOAA Northeast Fisheries Science Center (NEFSC) Ecosystem Monitoring Program (EcoMon). **(B)** Locations of samples collected during EcoMon Surveys from 2002 to 2012 analyzed for this study. See Table 1 for collection metadata for each sample; stations are indicated by sequential numbering.

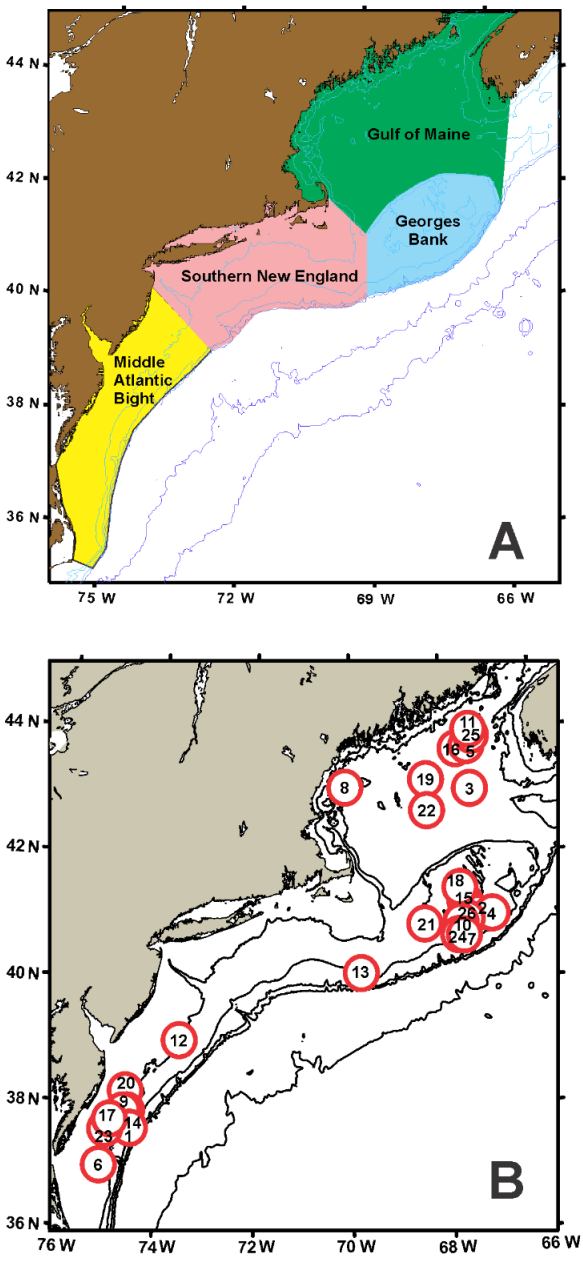


**Figure 2.** Neighbor Joining tree analysis of representative sequences for the 600 most abundant Operational Taxonomic Units (OTUs) resolved using the V9 hypervariable region 18S rRNA for one EcoMon sample, DE1105-25. Numbers are percentages of 1,000 bootstrap subreplicates supporting the cluster for each taxonomic group shown.

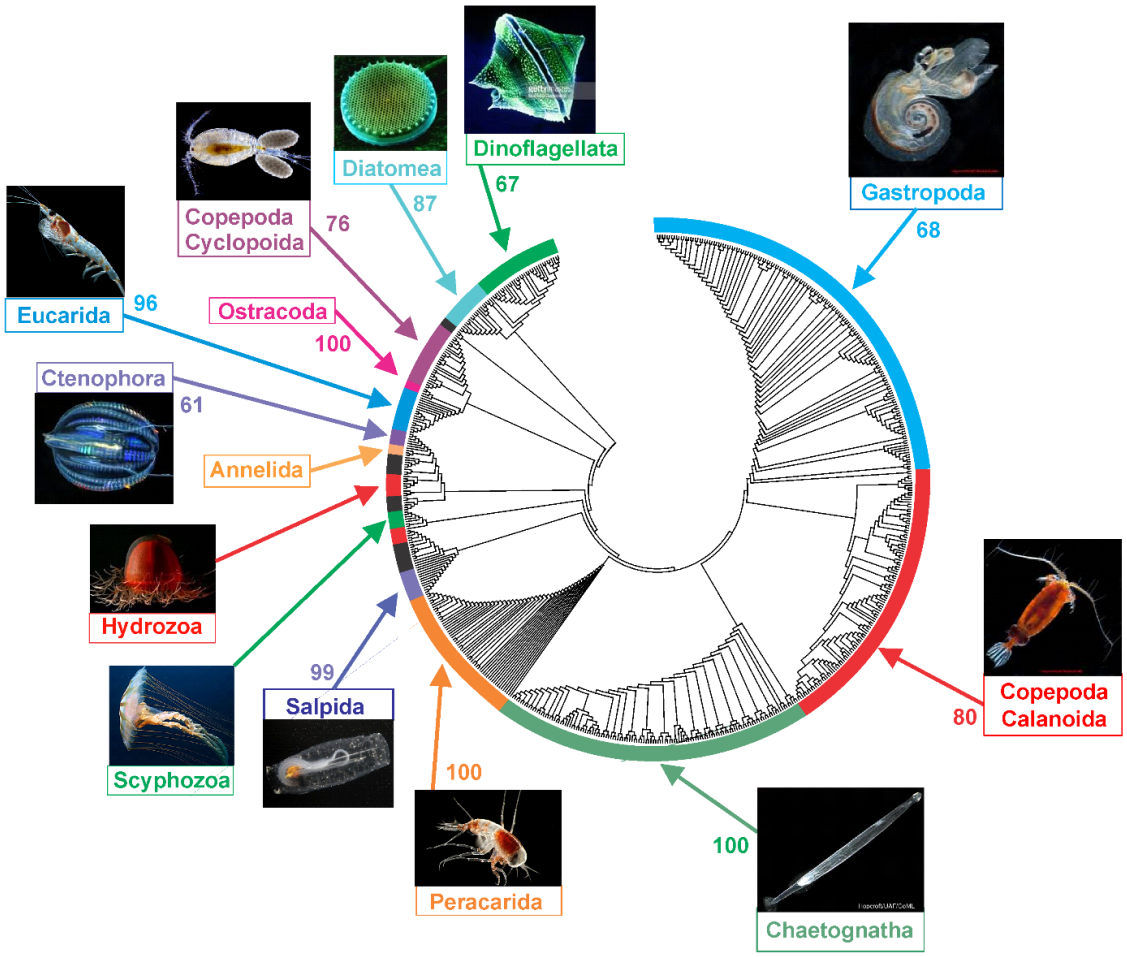


**Figure 3.** Functional regression lines for total abundances (Log10 transformed, numbers per 10m2) versus numbers of clustered sequences (Log10 transformed) for 7 taxonomic groups. Species counts are based on EcoMon taxonomic data. Zero values for species counts per sample for any taxonomic group were removed from the analysis.

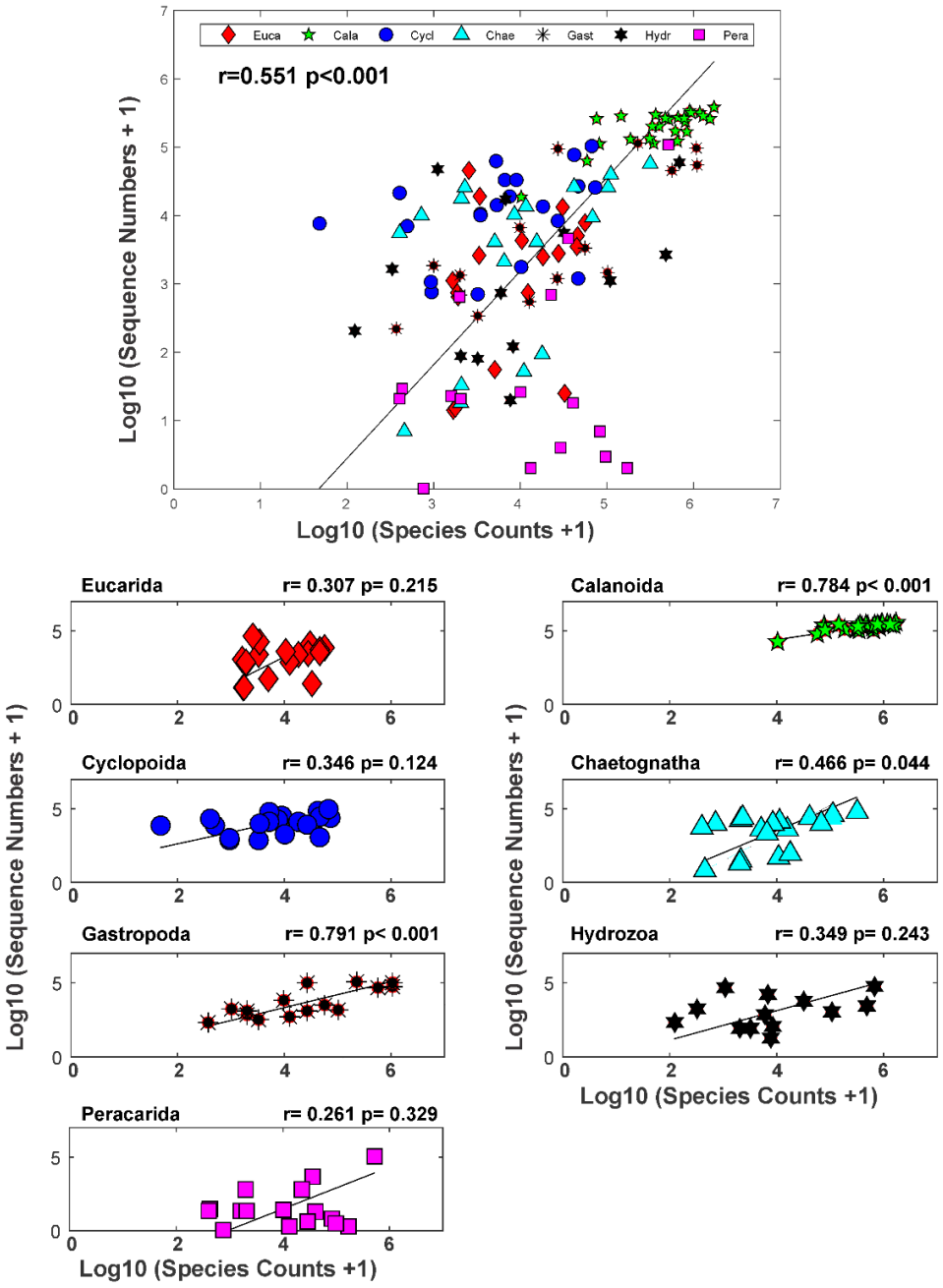


**Figure 4**. Stacked bar graphs for each of the 3 sampled EcoMon regions showing total abundance counts (A) and total numbers of sequences (B) summed across the 7 taxonomic groups for each sample from 2002 - 2012. Abundance counts and sequence numbers were $Log_{10}$ transformed after adding 1 (to allow inclusion of zeros).

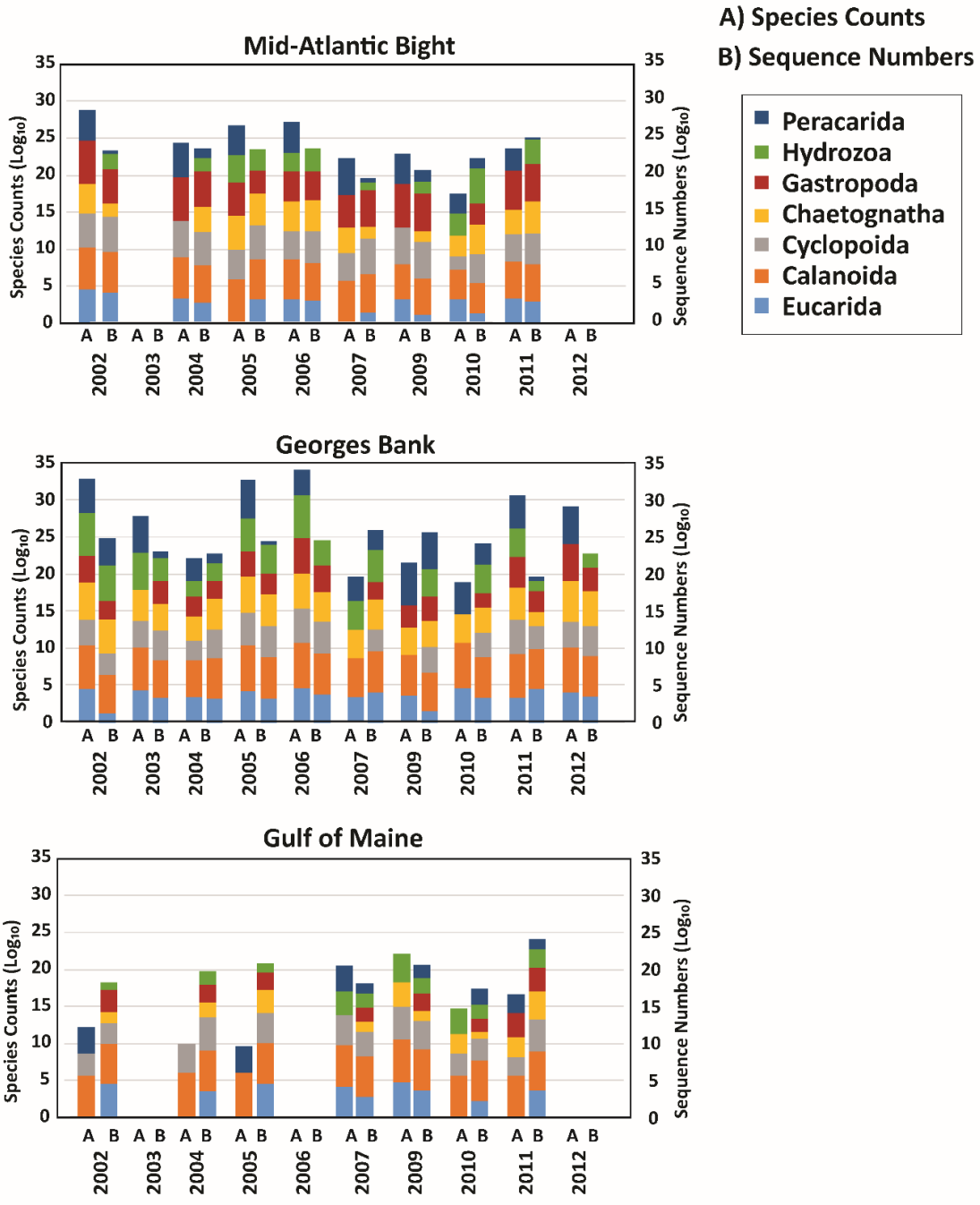


**Figure 5.** Two-dimensional non-metric multi-dimensional scaling (NMDS) analysis of regional variation for 7 taxonomic groups using Bray-Curtis dissimilarity coefficients based on total species abundances from morphological taxonomic abundance counts (A) and numbers of sequences from metabarcoding analysis (B). Two-dimensional resolution of variation for samples by year and region: MAB (red), GB (green), GoM (blue). NMDS stress values are: (A) stress = 0.164; (B) stress = 0.143. See Table 1 for collection metadata.

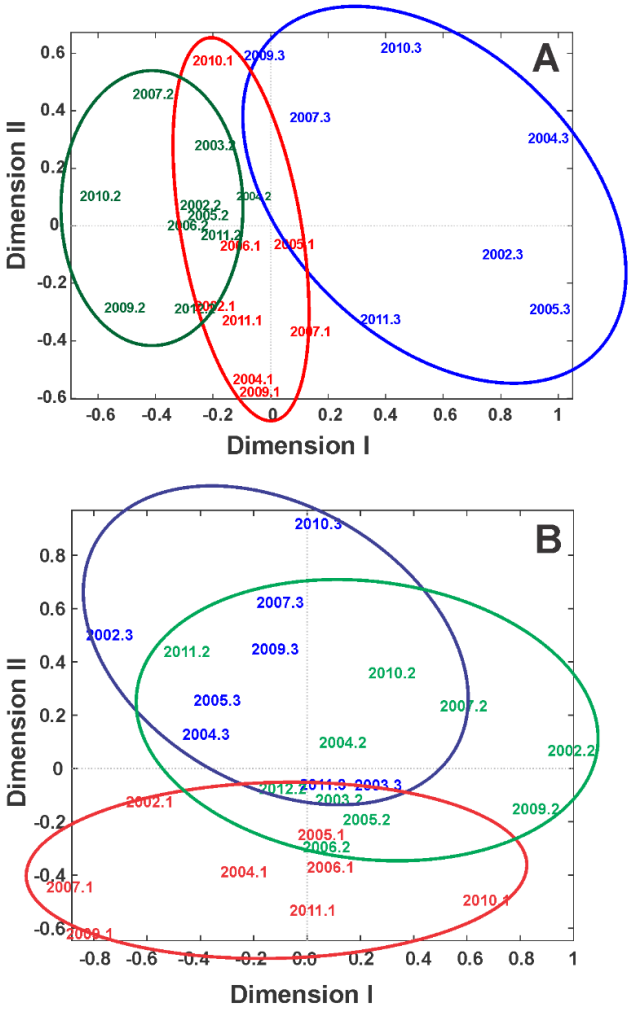


**Figure 6**. Functional regression analysis for 7 taxonomic groups for: A) morphological taxonomic abundance counts ($log_{10}$ / $10m^2$) versus latitude; and B) numbers of sequences ($log_{10}$) versus latitude. Latitude given is based on the collection location for each sample. Correlation coefficient ® and significance level (p) are shown for each graph.

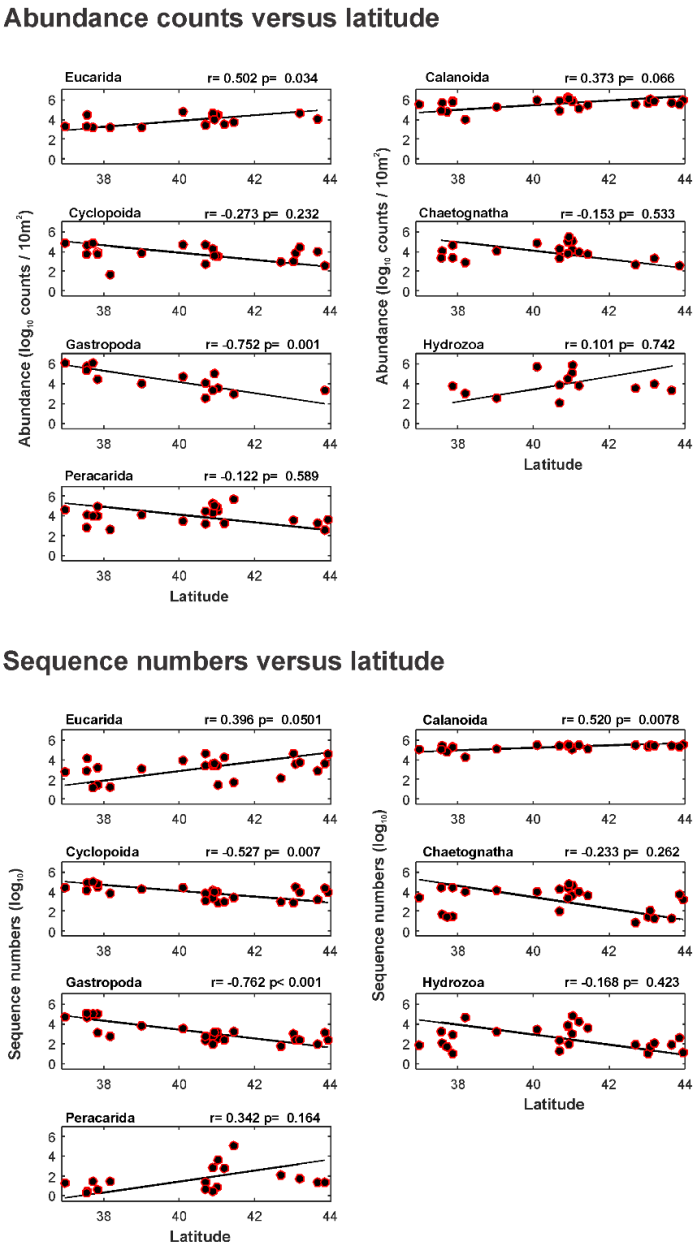
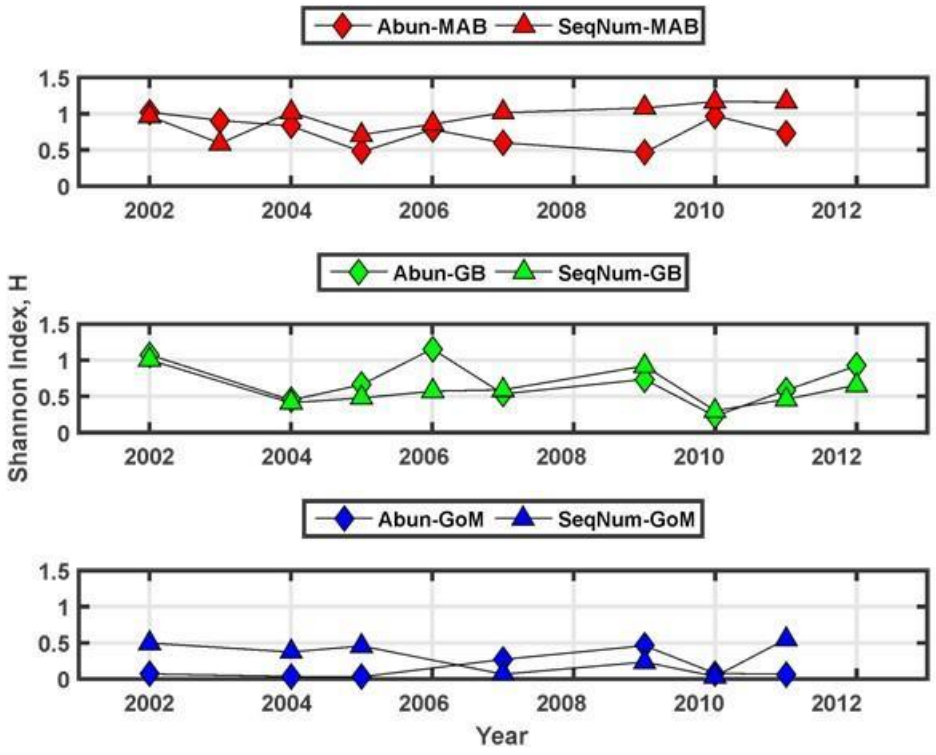


**Figure 7.** Comparison of the Shannon Diversity Index (H) in Mid-Atlantic Bight (MAB), Georges Bank (GB), and Gulf of Maine (GoM), based on 7 taxonomic groups for which both morphological abundance counts (Abun) and numbers of sequences (SeqNum) were available. Data were $\log_{10}$ transformed before analysis.



**Supplementary Table 1.** Numbers of sequences for samples from each EcoMon station for the 28 taxonomic groups resolved by V9 metabarcodes based on analysis and alignment of sequence reads to a customized reference file based on the SILVA database (Release 132).

**Suppl. Table 1. Sequence numbers by station for 28 taxonomic groups.**

| | Taxonomic Group | Total Unique | AL020611 | AL020675 | AL0206108 | DE030515 | DE030538 | AL040514 | AL040553 | AL0405117 | AL050524 | AL050570 | AL0505111 | AL060514 | AL060553 | DE070615 | DE070676 | DE0706112 | DE090535 | DE090573 | DE0905112 | DE100403 | DE100473 | DE1004129 | DE110525 | DEL110583 | DEL1105127 | HB120271 | Taxonomic Group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Eucarida | 197687 | 13100 | 25 | 39777 | 2819 | 1234 | 638 | 2591 | 3426 | 1633 | 2476 | 37391 | 1117 | 7747 | 25 | 19111 | 738 | 14 | 56 | 5113 | 15 | 3472 | 142 | 742 | 45684 | 4303 | 4279 | Eucarida |
| 2 | Calanoida | 5598963 | 255179 | 168516 | 202502 | 121138 | 118373 | 113326 | 257418 | 317257 | 235102 | 382846 | 339132 | 130625 | 318103 | 172663 | 286320 | 267125 | 62580 | 134684 | 273678 | 18695 | 259823 | 300513 | 111166 | 263103 | 203478 | 284822 | Calanoida |
| 3 | Cyclopoida | 497555 | 76517 | 701 | 758 | 10513 | 3904 | 25578 | 6936 | 32937 | 33065 | 13638 | 8982 | 18887 | 26674 | 62711 | 946 | 1760 | 103789 | 2769 | 8385 | 7642 | 2278 | 1055 | 14237 | 1190 | 21540 | 10145 | Cyclopoida |
| 4 | Chaetognatha | 260657 | 52 | 39496 | 26 | 4080 | 3789 | 2820 | 17977 | 120 | 26203 | 25694 | 1518 | 13576 | 9472 | 33 | 10210 | 19 | 26 | 4081 | 18 | 10136 | 2134 | 7 | 25708 | 94 | 5483 | 57872 | Chaetognatha |
| 5 | Salpida | 76326 | 1 | 0 | 0 | 2 | 0 | 75559 | 2 | 24 | 1 | 30 | 161 | 2 | 0 | 0 | 0 | 6 | 0 | 17 | 10 | 5 | 7 | 7 | 486 | 3 | 3 | 0 | Salpida |
| 6 | Teleostei | 4783 | 18 | 65 | 0 | 6 | 663 | 150 | 118 | 77 | 1 | 1251 | 2 | 0 | 25 | 2 | 384 | 1 | 0 | 0 | 0 | 55 | 1 | 3 | 1956 | 0 | 3 | 2 | Teleostei |
| 7 | Echinoidea | 9382 | 62 | 1281 | 0 | 47 | 606 | 2 | 312 | 19 | 178 | 925 | 41 | 4 | 116 | 3 | 3 | 4 | 7 | 3 | 20 | 5433 | 228 | 1 | 20 | 0 | 66 | 1 | Echinoidea |
| 8 | Bivalvia | 22826 | 32 | 13946 | 0 | 783 | 642 | 31 | 92 | 701 | 198 | 1528 | 35 | 28 | 1705 | 158 | 289 | 32 | 7 | 305 | 20 | 933 | 444 | 2 | 273 | 16 | 151 | 475 | Bivalvia |
| 9 | Gastropoda | 427439 | 45616 | 343 | 1177 | 1397 | 313 | 54395 | 222 | 241 | 1195 | 685 | 243 | 6651 | 3358 | 95325 | 250 | 100 | 96763 | 1832 | 254 | 609 | 98 | 61 | 112922 | 551 | 1340 | 1472 | Gastropoda |
| 10 | Anthozoa | 23923 | 2 | 6978 | 1 | 227 | 241 | 9 | 1838 | 3 | 9775 | 337 | 52 | 0 | 547 | 0 | 3 | 0 | 2 | 2 | 23 | 3599 | 4 | 1 | 275 | 2 | 0 | 1 | Anthozoa |
| 11 | Hydrozoa | 109460 | 105 | 22310 | 6 | 1105 | 3343 | 66 | 166 | 52 | 737 | 5489 | 12 | 1654 | 2663 | 9 | 17300 | 84 | 46 | 3940 | 117 | 47659 | 300 | 80 | 1664 | 19 | 437 | 95 | Hydrozoa |
| 12 | Scyphozoa | 44590 | 8 | 37107 | 3 | 14 | 15 | 1 | 39 | 0 | 4 | 2 | 1 | 0 | 21 | 0 | 20 | 4 | 8 | 215 | 3 | 5 | 6957 | 1 | 152 | 1 | 2 | 7 | Scyphozoa |
| 13 | Ciliophora | 1935 | 39 | 601 | 57 | 12 | 29 | 3 | 6 | 6 | 91 | 62 | 414 | 37 | 42 | 0 | 33 | 21 | 3 | 5 | 20 | 235 | 11 | 26 | 90 | 33 | 51 | 8 | Ciliophora |
| 14 | Dinoflagellata | 133404 | 115 | 5932 | 30 | 708 | 1349 | 145 | 1702 | 52 | 5891 | 2645 | 111 | 108 | 1508 | 241 | 253 | 73 | 470 | 82 | 495 | 85263 | 607 | 65 | 11663 | 150 | 13174 | 570 | Dinoflagellata |
| 15 | Syndiniales | 3412 | 814 | 31 | 11 | 143 | 25 | 78 | 101 | 1 | 246 | 65 | 11 | 28 | 263 | 173 | 6 | 67 | 76 | 15 | 20 | 730 | 6 | 9 | 215 | 4 | 243 | 31 | Syndiniales |
| 16 | Cercozoa | 753 | 2 | 231 | 6 | 1 | 6 | 10 | 2 | 24 | 30 | 37 | 3 | 0 | 10 | 0 | 3 | 1 | 2 | 87 | 28 | 98 | 57 | 3 | 58 | 2 | 45 | 7 | Cercozoa |
| 17 | Diatomea | 37072 | 18 | 6863 | 1 | 37 | 42 | 32 | 167 | 39 | 158 | 372 | 89 | 11 | 70 | 5 | 23 | 186 | 21 | 39 | 8086 | 16944 | 2217 | 10 | 65 | 6 | 732 | 839 | Diatomea |
| 18 | Annelida | 25629 | 110 | 8170 | 505 | 276 | 124 | 10 | 410 | 288 | 28 | 968 | 135 | 151 | 591 | 1 | 258 | 535 | 9 | 684 | 527 | 5563 | 1022 | 597 | 3882 | 24 | 471 | 290 | Annelida |
| 19 | Nemertea | 894 | 8 | 187 | 1 | 9 | 141 | 0 | 138 | 5 | 0 | 209 | 0 | 0 | 11 | 0 | 3 | 0 | 10 | 0 | 1 | 85 | 37 | 0 | 44 | 0 | 5 | 0 | Nemertea |
| 20 | Platyhelminthes | 27270 | 319 | 112 | 1190 | 8 | 6 | 1052 | 0 | 6 | 15 | 11 | 17 | 144 | 35 | 21348 | 1198 | 185 | 257 | 4 | 18 | 10 | 3 | 0 | 1316 | 5 | 1 | 8 | Platyhelminthes |
| 21 | Peracarida | 115462 | 2 | 4589 | 0 | 7 | 4 | 18 | 23 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 644 | 21 | 26 | 109206 | 54 | 29 | 688 | 120 | 1 | 4 | 21 | 0 | Peracarida |
| 22 | Harpacticoida | 127 | 0 | 23 | 9 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 83 | 3 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | Harpacticoida |
| 23 | Siphonostomatoida | 1264 | 0 | 979 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 215 | 0 | 4 | 30 | 0 | 27 | 0 | 0 | 7 | Siphonostomatoida |
| 24 | Thecostraca | 1694 | 0 | 286 | 0 | 1 | 0 | 2 | 91 | 72 | 2 | 4 | 4 | 31 | 5 | 0 | 5 | 5 | 1 | 20 | 76 | 27 | 68 | 2 | 14 | 0 | 924 | 54 | Thecostraca |
| 25 | Ostracoda | 355 | 0 | 9 | 0 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 221 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | Ostracoda |
| 26 | Insecta | 141 | 0 | 14 | 0 | 0 | 0 | 107 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 19 | 0 | 0 | 0 | Insecta |
| 27 | Bryozoa | 341 | 0 | 97 | 0 | 0 | 57 | 0 | 8 | 3 | 12 | 92 | 0 | 1 | 5 | 3 | 7 | 0 | 0 | 31 | 0 | 0 | 4 | 0 | 11 | 0 | 3 | 7 | Bryozoa |
| 28 | Ctenophora | 24689 | 5 | 0 | 0 | 59 | 331 | 15 | 32 | 6 | 1749 | 3355 | 291 | 54 | 116 | 4 | 685 | 249 | 10 | 15 | 1978 | 14243 | 8 | 30 | 1249 | 8 | 175 | 22 | Ctenophora |
| | Totals | 7648033 | 392124 | 318892 | 246060 | 143482 | 135239 | 274047 | 290391 | 355360 | 316314 | 442723 | 388645 | 173109 | 373308 | 352707 | 337958 | 271216 | 264127 | 258390 | 298947 | 218018 | 280543 | 302735 | 288255 | 310899 | 252651 | 361014 | Totals |

**Supplementary Table 2.** Numbers of operational taxonomic units (OTUs) for samples from each EcoMon station for 28 taxonomic groups resolved by V9 metabarcodes based on analysis and alignment to reference and taxonomy files based on the SILVA database (Release 132).

**Suppl. Table 2. OTU numbers by station for 28 taxonomic groups.**

| | Taxonomic Group | Total | AL020611 | AL020675 | AL0206108 | DE030515 | DE030538 | AL040514 | AL040553 | AL0405117 | AL050524 | AL050570 | AL0505111 | AL060514 | AL060553 | DE070615 | DE070676 | DE0706112 | DE090535 | DE090573 | DE0905112 | DE100403 | DE100473 | DE1004129 | DE110525 | DEL110583 | DEL1105127 | HB120271 | Taxonomic Group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Calanoida | 6812 | 934 | 490 | 553 | 692 | 643 | 650 | 1099 | 706 | 737 | 1301 | 775 | 556 | 1212 | 846 | 610 | 731 | 471 | 437 | 707 | 169 | 589 | 589 | 850 | 673 | 905 | 890 | Calanoida |
| 2 | Eucarida | 2696 | 594 | 6 | 976 | 233 | 181 | 80 | 291 | 302 | 156 | 330 | 551 | 98 | 431 | 4 | 727 | 60 | 2 | 11 | 356 | 8 | 290 | 19 | 111 | 787 | 374 | 286 | Eucarida |
| 3 | Cyclopoida | 634 | 212 | 12 | 13 | 67 | 61 | 108 | 61 | 107 | 133 | 125 | 39 | 87 | 138 | 189 | 16 | 16 | 232 | 34 | 48 | 52 | 23 | 9 | 139 | 13 | 158 | 73 | Cyclopoida |
| 4 | Chaetognatha | 2059 | 13 | 645 | 8 | 258 | 294 | 157 | 632 | 22 | 617 | 844 | 136 | 439 | 411 | 7 | 488 | 5 | 7 | 255 | 4 | 351 | 109 | 3 | 752 | 17 | 375 | 950 | Chaetognatha |
| 5 | Salpida | 497 | 1 | 0 | 0 | 2 | 0 | 492 | 1 | 5 | 1 | 5 | 17 | 1 | 0 | 0 | 0 | 1 | 0 | 4 | 4 | 1 | 2 | 3 | 49 | 3 | 2 | 0 | Salpida |
| 6 | Teleostei | 273 | 8 | 8 | 0 | 3 | 49 | 16 | 14 | 8 | 1 | 82 | 2 | 0 | 4 | 2 | 32 | 1 | 0 | 0 | 0 | 7 | 1 | 1 | 156 | 0 | 1 | 2 | Teleostei |
| 7 | Echinoidea | 173 | 6 | 58 | 0 | 6 | 74 | 1 | 30 | 1 | 16 | 82 | 7 | 2 | 13 | 2 | 1 | 1 | 2 | 1 | 3 | 116 | 13 | 1 | 6 | 0 | 5 | 1 | Echinoidea |
| 8 | Bivalvia | 470 | 6 | 206 | 0 | 60 | 52 | 5 | 14 | 38 | 17 | 87 | 10 | 7 | 108 | 20 | 32 | 9 | 5 | 43 | 6 | 91 | 27 | 2 | 34 | 6 | 18 | 47 | Bivalvia |
| 9 | Gastropoda | 2197 | 681 | 46 | 104 | 136 | 47 | 589 | 29 | 31 | 82 | 87 | 33 | 264 | 200 | 682 | 26 | 12 | 530 | 101 | 27 | 59 | 18 | 12 | 1281 | 61 | 134 | 108 | Gastropoda |
| 10 | Anthozoa | 201 | 2 | 139 | 1 | 22 | 31 | 3 | 99 | 1 | 134 | 38 | 5 | 0 | 41 | 0 | 1 | 0 | 1 | 1 | 4 | 112 | 2 | 1 | 29 | 1 | 0 | 1 | Anthozoa |
| 11 | Hydrozoa | 919 | 8 | 253 | 4 | 63 | 175 | 12 | 17 | 5 | 18 | 232 | 4 | 14 | 108 | 2 | 192 | 4 | 10 | 146 | 9 | 259 | 21 | 7 | 115 | 2 | 52 | 12 | Hydrozoa |
| 12 | Scyphozoa | 245 | 1 | 227 | 1 | 2 | 5 | 1 | 5 | 0 | 2 | 1 | 1 | 0 | 4 | 0 | 1 | 1 | 1 | 16 | 1 | 2 | 128 | 1 | 15 | 1 | 1 | 2 | Scyphozoa |
| 13 | Ciliophora | 162 | 13 | 38 | 15 | 5 | 13 | 2 | 5 | 4 | 18 | 17 | 34 | 13 | 11 | 0 | 15 | 6 | 3 | 5 | 10 | 33 | 3 | 8 | 14 | 15 | 15 | 5 | Ciliophora |
| 14 | Dinoflagellata | 1437 | 20 | 211 | 7 | 42 | 97 | 25 | 41 | 9 | 199 | 136 | 16 | 19 | 86 | 21 | 26 | 11 | 30 | 22 | 45 | 776 | 41 | 15 | 213 | 11 | 373 | 30 | Dinoflagellata |
| 15 | Syndiniales | 127 | 24 | 10 | 4 | 17 | 9 | 10 | 10 | 1 | 23 | 25 | 7 | 6 | 21 | 9 | 5 | 2 | 10 | 7 | 9 | 30 | 5 | 6 | 26 | 2 | 39 | 8 | Syndiniales |
| 16 | Cercozoa | 142 | 2 | 67 | 4 | 1 | 5 | 4 | 2 | 6 | 9 | 15 | 2 | 0 | 5 | 0 | 3 | 1 | 1 | 6 | 7 | 16 | 7 | 3 | 13 | 2 | 18 | 3 | Cercozoa |
| 17 | Diatomea | 613 | 10 | 179 | 1 | 12 | 21 | 12 | 19 | 8 | 18 | 45 | 20 | 7 | 17 | 4 | 10 | 21 | 12 | 14 | 137 | 278 | 65 | 5 | 16 | 4 | 67 | 43 | Diatomea |
| 18 | Annelida | 1046 | 8 | 362 | 51 | 46 | 31 | 4 | 68 | 31 | 7 | 136 | 21 | 15 | 80 | 1 | 40 | 48 | 5 | 80 | 62 | 202 | 74 | 58 | 203 | 4 | 67 | 37 | Annelida |
| 19 | Nemertea | 61 | 2 | 14 | 1 | 2 | 16 | 0 | 14 | 1 | 0 | 25 | 0 | 0 | 2 | 0 | 1 | 0 | 3 | 0 | 1 | 11 | 3 | 0 | 6 | 0 | 1 | 0 | Nemertea |
| 20 | Platyhelminthes | 254 | 26 | 7 | 67 | 3 | 3 | 22 | 0 | 2 | 2 | 2 | 4 | 9 | 5 | 91 | 66 | 13 | 15 | 2 | 3 | 2 | 3 | 0 | 38 | 4 | 1 | 2 | Platyhelminthes |
| 21 | Peracarida | 771 | 1 | 195 | 0 | 3 | 2 | 5 | 5 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 34 | 1 | 3 | 642 | 7 | 5 | 17 | 8 | 1 | 1 | 4 | 0 | Peracarida |
| 22 | Harpacticoida | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | Harpacticoida |
| 23 | Siphonostomatoida | 13 | 0 | 8 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 1 | Siphonostomatoida |
| 24 | Thecostraca | 33 | 0 | 7 | 0 | 1 | 0 | 1 | 4 | 5 | 1 | 1 | 1 | 3 | 2 | 0 | 2 | 2 | 1 | 1 | 6 | 1 | 3 | 1 | 1 | 0 | 18 | 3 | Thecostraca |
| 25 | Ostracoda | 73 | 0 | 3 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | Ostracoda |
| 26 | Insecta | 12 | 0 | 2 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | Insecta |
| 27 | Bryozoa | 35 | 0 | 12 | 0 | 0 | 8 | 0 | 1 | 2 | 3 | 10 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 2 | 0 | 2 | 4 | Bryozoa |
| 28 | Ctenophora | 115 | 2 | 0 | 0 | 4 | 7 | 4 | 3 | 3 | 21 | 27 | 6 | 2 | 6 | 1 | 8 | 9 | 3 | 4 | 22 | 55 | 3 | 5 | 25 | 2 | 6 | 3 | Ctenophora |
| | **Totals** | **22072** | **2574** | **3206** | **1811** | **1705** | **1825** | **2210** | **2464** | **1299** | **2215** | **3655** | **1691** | **1543** | **2963** | **1883** | **2338** | **955** | **1347** | **1844** | **1479** | **2639** | **1451** | **757** | **4098** | **1609** | **2636** | **2511** | **Totals** |

**Supplementary Table 3.** Abundance counts for 18 taxonomic groups for which data are available from both morphological taxonomic and metabarcoding analysis. Abundance counts by group are from the EcoMon Plankton Database (ftp://ftp.nefsc.noaa.gov/pub/hydro/zooplankton_data/). Counts are given as numbers per $10m^2$.

**Suppl. Table 3. Morphological counts for 18 taxonomic groups.**

| | Taxonomic Group | Totals | AL0206-11 | AL0206-75 | AL0206-108 | DE0305-15 | DE0305-38 | AL0405-14 | AL0405-53 | AL0405-117 | AL0505-24 | AL0505-70 | AL0505-111 | AL0605-14 | AL0605-53 | DE0706-15 | DE0706-76 | DE0706-112 | DE0905-35 | DE0905-73 | DE0905-112 | DE1004-25 | DE1004-73 | DE1004-129 | DE1105-25 | DEL1105-83 | DEL1105-127 | HB1202-71 | Taxonomic Group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Eucarida | 301561 | 30682 | 32430 | 0 | 27682 | ND | 1915 | 3321 | 0 | 0 | 18070 | 0 | 1647 | 55962 | 0 | 3400 | 12336 | 1680 | 5015 | 45470 | 1773 | 45288 | 0 | 1890 | 2536 | 0 | 10464 | Eucarida |
| 2 | Calanoida | 15205135 | 515014 | 830212 | 396549 | 650514 | ND | 346694 | 76362 | 1174349 | 811619 | 1722710 | 897022 | 185774 | 935823 | 610300 | 144484 | 468753 | 58785 | 307945 | 663447 | 10257 | 1549476 | 365248 | 81632 | 776272 | 332009 | 1293885 | Calanoida |
| 3 | Cyclopoida | 377116 | 41639 | 3243 | 936 | 3460 | ND | 72787 | 492 | 6672 | 8935 | 18070 | 0 | 7576 | 46636 | 5252 | 0 | 10280 | 67183 | 0 | 26869 | 48 | 0 | 922 | 5291 | 46932 | 405 | 3488 | Cyclopoida |
| 4 | Chaetognatha | 726037 | 10958 | 110263 | 0 | 15571 | ND | 0 | 2090 | 0 | 41698 | 102399 | 0 | 11528 | 68399 | 2101 | 8499 | 0 | 0 | 5015 | 2067 | 719 | 6470 | 461 | 2268 | 17758 | 405 | 317368 | Chaetognatha |
| 5 | Salpida | 249,404 | 0 | 0 | 0 | 0 | ND | 241345 | 0 | 0 | 0 | 0 | 0 | 329 | 6218 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 1512 | 0 | 0 | 0 | Salpida |
| 7 | Echinoidea | 44773 | 0 | 0 | 0 | 15571 | ND | 0 | 0 | 0 | 1489 | 2008 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6200 | 96 | 19409 | 0 | 0 | 0 | 0 | 0 | Echinoidea |
| 8 | Bivalvia | 72,610 | 0 | 0 | | 0 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40,512 | 0 | 0 | 0 | 0 | 2,984 | 29,113 | 0 | 0 | 0 | 0 | 0 | Bivalvia |
| 9 | Gastropoda | 3199398 | 563228 | 3243 | 0 | 0 | ND | 1086055 | 369 | 0 | 26806 | 2008 | 0 | 9882 | 55963 | 27311 | 0 | 0 | 1078280 | 1003 | 0 | 0 | 0 | 0 | 229400 | 12684 | 2027 | 101139 | Gastropoda |
| 10 | Anthozoa | 0 | 0 | 0 | 0 | 0 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Anthozoa |
| 11 | Hydrozoa | 1342908 | 0 | 687520 | 0 | 108996 | ND | 0 | 123 | 0 | 5957 | 32125 | 0 | 329 | 478794 | 0 | 6799 | 2056 | 0 | 0 | 8267 | 1103 | 0 | 3228 | 0 | 7611 | 0 | 0 | Hydrozoa |
| 12 | Scyphozoa | 0 | 0 | 0 | 0 | 0 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Scyphozoa |
| 18 | Annelida | 23,089 | 0 | 0 | | 1,730 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3,109 | 0 | 5,949 | 5,140 | 0 | 1,003 | 4,134 | 0 | 0 | 0 | 756 | 1,268 | 0 | 0 | Annelida |
| 21 | Peracarida | 1169638 | 13149 | 35673 | 3745 | 83044 | ND | 40224 | 1599 | 0 | 10424 | 170665 | 4153 | 13505 | 3109 | 96640 | 1983 | 2056 | 10077 | 514580 | 0 | 431 | 22644 | 0 | 756 | 29174 | 405 | 111602 | Peracarida |
| 24 | Thecostraca | 38,630 | 0 | 9,729 | | 0 | ND | 0 | 0 | 0 | 0 | 497 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,067 | 14,922 | 0 | 0 | 0 | 11,416 | 0 | 0 | Thecostraca |
| 25 | Ostracoda | 827 | 0 | 0 | | 0 | ND | 0 | 0 | 0 | 0 | 497 | 0 | 329 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Ostracoda |
| 27 | Bryozoa | 0 | 0 | 0 | | 0 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Bryozoa |
| 28 | Ctenophores | 378 | 0 | 0 | 0 | 0 | ND | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 378 | 0 | 0 | 0 | Ctenophores |