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Metabarcoding analysis of regional variation in gut contents of the copepod *Calanus finmarchicus* in the North Atlantic Ocean

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ABSTRACT

The calanoid copepod *Calanus finmarchicus* is one of the most abundant and ecologically important species of the zooplankton assemblage of the North Atlantic Ocean and occupies a pivotal position in the pelagic food web. This study used metabarcoding analysis (high throughput DNA sequencing of target gene regions) to examine the diversity of the copepod gut content, including both eukaryotic and prokaryotic components of the diet and microbiome. Zooplankton samples were collected during the 2013 EuroBASIN cruise of the R/V *G.O. Sars*, which crossed the North Atlantic to survey in the Norwegian, Icelandic, Irminger, and Labrador Seas. Zooplankton samples were examined microscopically for *C. finmarchicus*; species identification was confirmed by genetic markers based on insertion-deletion sequence variation. DNA was extracted from the dissected gut contents of adult female copepods and sequenced for eukaryotic 18S V4 and prokaryotic 16S V3–V4 rRNA hypervariable regions. Prokaryotes identified in the gut contents of all copepods analyzed included Cyanobacteria, Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, and Acidobacteria. The eukaryotic gut content assemblage was diverse, dominated by Ocrophyta (diatoms), Dinophyta (dinoflagellates), Ciliophora (ciliates), as well as Cnidaria and Ctenophora. The diverse assemblage revealed by metabarcoding analysis of copepod gut contents likely represents prey, microbiome, parasites, symbionts, and pathogens. Significant differences in prokaryotic and eukaryotic diversity of the gut contents of copepods collected from four regional seas of the North Atlantic Ocean reflect and contribute to basin-scale differences in the pelagic food web of these ecosystems. This study provides evidence that diversity and variation of the copepod gut contents may both reflect and impact the functioning of pelagic food webs and regional variation in ocean ecosystems.

1. Introduction

1.1. *Calanus finmarchicus* in the North Atlantic foodweb

The calanoid copepod *Calanus finmarchicus* is abundant throughout the North Atlantic, dominating mesozooplankton communities in multiple regions where it can comprise as much as 90% of its biomass (Melle et al., 2014). The diet of a key species like *C. finmarchicus* may have significant effect on nutrient cycles, as local carbon export (via sinking of fecal pellets) is dramatically increased when this species has a diatom-heavy diet (Urban-Rich, 2001). Given the importance of *C. finmarchicus* to the North Atlantic food web, many attempts have been made to determine the diet of the species via direct observation (e.g., incubation experiments in which prey input and output are monitored; Koski and Wexels Riser, 2006) and indirect observation (e.g., identifying prey items in the gut contents or fecal pellets; Nejstgaard et al., 2003). The composition of copepod gut contents is an important source of information about their feeding behaviors. Collection and immediate preservation of the specimens ensures less degradation than the remains found in feces, although the small size of prey and rapid digestion rates limit the usefulness of morphological examination. Genetic methods provide a way to overcome many of these limitations, since DNA may not degrade as rapidly as morphological features of soft-bodied plankton or protists. Using DNA found in the gut contents of copepods may thus be an effective way to determine the diet of *C. finmarchicus*.

The omnivorous feeding behavior of *C. finmarchicus* affects multiple

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trophic levels; populations of phytoplankton and grazing microzooplankton are subject to grazing/predation pressure by this species, which in turn affects the grazing rates of microzooplankton and the phytoplankton species composition (Nejstgaard et al., 2001). The species exerts top-down pressure through its grazing behavior on marine protists to such an extent that it is thought to regulate phytoplankton blooms (Friedland et al., 2016). *Calanus finmarchicus* also exerts bottom-up influence through the nutritional value that this species offers to higher trophic levels, which is critical to the first-year survival of economically-important fish species such as mackerel (Fortier and Villeneuve, 1996; Jansen, 2016; Paradis et al., 2012). Nutritional quality of *C. finmarchicus* can vary; lipid content, which is significantly correlated with energy content, was observed to vary by as much as 13% between study years in the Bay of Fundy (McKinstry et al., 2013), which can have cascading trophic effects, including impacts on the critically endangered North Atlantic right whale populations (Greene and Pershing, 2004; Meyer-Gutbrod et al., 2015).

The diversity of prokaryotic and eukaryotic organisms found in the guts of copepods can have important impacts on the quality and efficiency of energy transferred throughout the marine food web. The physical environment of the copepod gut as a habitat for microbes can be modified by its diet; for example, a diatom-heavy diet has been shown to acidify the gut of some calanoid copepods more so than a diet of cryptophytes (Tang et al., 2011). This change to the internal environment, as well as any undigested food items, alters the microbial diversity in the copepod gut and the chemical reactions they mediate (Tang et al., 2009) and may impact global biogeochemical cycles, such as iron dissolution (Tang et al., 2011). Copepods have been known to harbor bacteria that serve as a sink of dimethyl sulfoniopropionate (DMSP) in the sulfur cycle (Dong et al., 2013), and has the potential to alter biogeochemical cycles in the pelagic ecosystem.

The copepod gut provides a unique microhabitat for microbial growth in the ocean, as this enclosed environment is acidic and suboxic relative to average ocean conditions (Tang et al., 2011). Bacteria hitchhiking on copepod prey items are likely to survive the digestive process, since the lowest pH measured (5.40) in copepod guts is within the range of survivability for most bacteria (Tang et al., 2011). Although active bacteria are still detectable in the fecal pellets of copepods (Hansen and Bech, 1996), the composition of this community is likely altered during its passage through the copepod gut, resulting in a community that is different from that of the ambient water. The acidic and sometimes anoxic conditions along the copepod gut (Tang et al., 2011) influence the metabolism and reproduction of bacteria contained therein (Shoemaker and Moisander, 2017). Conditions in the copepod gut – shaped by both copepod feeding choices and the resident microbiota – influence the microbial community of the surrounding ocean. Given the abundance of copepods throughout the global ocean, processes occurring in the guts of these tiny creatures could play key roles in the functioning of ocean food webs and ecosystems on a wide range of temporal and spatial scales. However, potential impact on this transient microbial community is limited by rapid copepod gut clearance rates, which can be on the order of minutes to hours (Atkinson et al., 1996; Dam and Peterson, 1988; Tirelli and Mayzaud, 2005).

1.2. Approaches to the analysis of copepod diets

Prior studies of copepod feeding selectivity have largely been limited to incubation studies, with analysis employing non-metabarcoding techniques. Several studies have used polymerase chain reaction (PCR), usually with cloning of the PCR products, of copepod gut contents, including studies of *Calanus* species (Haley et al., 2011; Nejstgaard et al., 2003; Vestheim et al., 2005) and other copepods (Craig et al., 2014; Hu et al., 2014). Quantitative PCR (qPCR) studies of copepod gut contents have been carried out for species of *Calanus* (Nejstgaard et al., 2008; Ray et al., 2016; Troedsson et al., 2009) and other copepods (Durbin et al., 2008, 2012). Additional studies have focused on laboratory-incubation experiments using field-collected specimens of species of *Acartia* (Durbin et al., 2012; Hu et al., 2014) and *Calanus* (Nejstgaard et al., 2008, 2003; Ray et al., 2016; Troedsson et al., 2009). PCR analysis of gut contents of field-caught copepods has been completed for species of *Pseudocalanus* from the Eastern Bering Sea (Cleary et al., 2015), *Centropages typicus* from Narragansett Bay (Durbin et al., 2008), and *Calanus helgolandicus* from the mid-Atlantic Bight (Vestheim et al., 2005). The advances in our understanding of copepod diet brought about by genetic methods can be further expanded through the use of metabarcoding techniques.

Metabarcoding approaches (high throughput DNA sequencing of target gene regions) have the advantage of comprehensive analysis based on short DNA sequences (Amaral-Zettler et al., 2009). The diversity of pelagic communities has be assessed through metabarcoding (Bucklin et al., 2016; de Vargas et al., 2015), with homogenization of complex environmental samples for analysis (Durbin et al., 2012). With an output of sequences that can number in the hundreds of thousands to millions, metabarcoding dramatically increases the likelihood that rare sequences will be detected that would otherwise have been missed using standard PCR, cloning and sequencing techniques. The use of highly conserved primers, which can detect most members of large groups such as eukaryotes and prokaryotes, has allowed researchers to determine gut contents semi-quantitatively (Amaral-Zettler et al., 2009; Elbrecht and Leese, 2015). These attributes render metabarcoding a more powerful genetic approach for the characterization of gut contents in copepod diet studies. High throughput metabarcoding methods are increasingly employed to study the composition of copepod diets, including *Pseudocalanus* spp. in the Eastern Bering Strait (Cleary et al., 2015), and *Calanus* spp. in Japan (Hirai et al., 2017) and Norway (Ray et al., 2016). One study, Ray et al. (2016), used both qPCR and metabarcoding to examine the gut contents of field-caught copepod samples from the Norwegian Sea.; Conducting metabarcoding studies for this species across more locations and timepoints has the potential to provide a detailed understanding of *C. finmarchicus* feeding behavior.

1.3. Basin-scale variation of the North Atlantic Ocean

The North Atlantic Ocean comprises several distinct heterogeneous, dynamic, and productive ecosystems that are distinguished by both bathymetry and circulation, and include the Labrador, Irminger, Icelandic, and Norwegian Seas. These regional seas or basins are characterized by diverse environmental conditions and plankton dynamics. The Labrador and Icelandic Seas are generally cooler, due to the stronger influence of Arctic water inputs from the East Greenland current, which brings cold and relatively fresh water into the region. The Irminger and Norwegian Seas are warmer, due to the stronger influence of tropical inputs from the North Atlantic current, which brings water that is warm and relatively salty into the region. Basin-scale differences in sea surface temperature, which may reach ~10° C, have been correlated with significant differences in organismal size and life-history between populations of *C. finmarchicus* in the Labrador and Norwegian basins (Head et al., 2013).

The Euro-BASIN Program, led by the Norwegian Institute of Marine Research, was designed to advance understanding on the variability, potential impacts, and feedbacks of global change and anthropogenic forcing on the structure, function and dynamics of the North Atlantic and associated shelf sea ecosystems. Field collecting and sample analysis have focused on key groups of the pelagic food web, including diatoms and other phytoplankton; species of the copepod genus *Calanus*; and various pelagic fish, including herring (*Clupea harengus*), mackerel (*Scomber scombrus*), and blue whiting (*Micromesistius poutassou*) which represent some of the largest fish stocks on the planet.

1.4. Goals and objectives of this study

This study seeks to examine the diet of *C. finmarchicus,* and to

characterize differences in the trophic relationships of the copepod among basins of the North Atlantic Ocean, based upon molecular (metabarcoding) analysis of prokaryotic and eukaryotic diversity of the gut contents of the copepods. Metabarcoding methods were designed to allow characterization of the full breadth of taxonomic diversity detectable within the copepod gut. including prey, pathogens, parasites, and symbionts.

2. Methods

2.1. Sample collection

Zooplankton samples and environmental data used for this study were collected during a cruise of the R/V *G.O. Sars* from May 1 – June 14, 2013 (Fig. 1) as part of the EuroBASIN Program led by the Institute of Marine Research, Bergen and Tromsoë, Norway. Hydrographic data were collected during down- and up-casts of a SeaBird CTD (SBE 911plus) with sensors for temperature, conductivity, oxygen (SBE43), fluorescence (Aqua-III), and irradiance/PAR (Biospherical/Licor).

Sea surface temperature (SST) was measured during this time period by the CoastWatch Program, NOAA National Environmental Satellite Data and Information Service (NESDIS). SST data analysis and figure preparation were done using the M_map tool box in MatLab (Ver. 15B, MathWorks, Inc., Natick, MA). The sampling grid selected for analysis spanned 55–70 °N latitude and 60–7 °E longitude, where data were collected in one-month intervals. Data from the SST, NOAA POES AVHRR, GAC, 0.1°, Global, Day and Night dataset were utilized.

Zooplankton samples analyzed for this study were collected at 6 stations along the cruise track (Fig. 1) from 0 to 200 m depth using one of three types of ring nets: WP-2 with 180-μm mesh, 1.5m ring net with 180-μm mesh, or T80 with 375-μm mesh (Table 1). Samples were preserved immediately after collection in 95% ethanol, which was changed 24 h after initial preservation. Samples were maintained at 4 °C for long-term storage. Each sample was examined under a dissecting microscope to identify and select adult female *C. finmarchicus* with full guts.

2.2. Species identification confirmation

To confirm species identification of *C. finmarchicus* and discriminate the co-occurring congeneric species *C. glacialis*, genetic markers based on Insertion-Deletion (InDel) variation were used (Smolina et al., 2014). One antenna was excised from each copepod and individual DNA extractions were performed using Qiagen DNeasy DNA extraction kit (Hilden, Germany) according to the manufacturer's instructions. PCR reactions were done using reagents from the GoTaq Flexi Reaction Buffer kit (Promega Life Science, Madison, WI). Purified DNA (15 ng) was added to each reaction with the following reagents: 5.0 μl 5X Green GoTaq Flexi Buffer, 2.5 μl $MgCl_2$, 0.7 μl dNTPs (10 mM), 0.25 μl GoTaq Flexi DNA Polymerase, and 1 μl of each primer (10 μM). The G_150 forward and reverse primer set (Smolina et al., 2014) was used in the following PCR reaction: 1 cycle of denaturation at 95 °C for 10 min; followed by 40 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 25 s; a final extension cycle at 72 °C for 20 min; and an indefinite hold at 4 °C. The PCR product was run in 2.8% MetaPhor high-resolution agarose gel (Lonza Group Ltd., Basel, Switzerland) against a 50 base pairs (bp) Gel Pilot molecular weight marker (Qiagen, Hilden, Germany). The species were distinguished on amplicon size: *C. finmarchicus* (131 bp) versus *C. glacialis* (161 bp; Smolina et al., 2014).

2.3. DNA extraction

After confirmation of species identification of *C. finmarchicus*, 10 specimens were selected for analysis from each sample and triple-washed in 95% ethanol prior to gut dissection. The gut of each copepod was dissected using sterile dissection needles on an autoclaved microscope slide under a dissecting microscope. Samples used for metabarcoding of eukaryotic diversity V4 18S rRNA sequencing included only the foregut, to limit the signal of more degraded DNA from digested material in the hindgut; samples used for analysis of prokaryotic diversity included the whole digestive tract. Dissected guts of copepods from each sample were pooled, with 5 guts in each pool, to create 2 technical replicates for each sample. Pools of copepod foreguts were incubated for 24 h at 56 °C in SDS lysis buffer (10 mM Tris pH 8.0; 100 mM EDTA pH 8.0; 0.5% SDS; 200 μg/ml proteinase K was added immediately before use). Then, 82.5 μl of CTAB (Cetrimonium bromide, 10% w/v) and a full volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the incubated solution. The supernatant of this centrifuged solution was then treated following the kit instructions of the E.Z.N.A. Mollusc DNA (Omega Bio-Tek Inc., Norcross, GA) kit to produce purified DNA for subsequent analyses.

2.4. PCR and sequencing

Purified DNA (12.5 ng for analysis of eukaryotes, 20 ng for analysis of prokaryotes) was added to a mixture with the following PCR reagents (Promega GoTaq® PCR Core Systems, Madison, WI): 5.0 μl 5X colorless

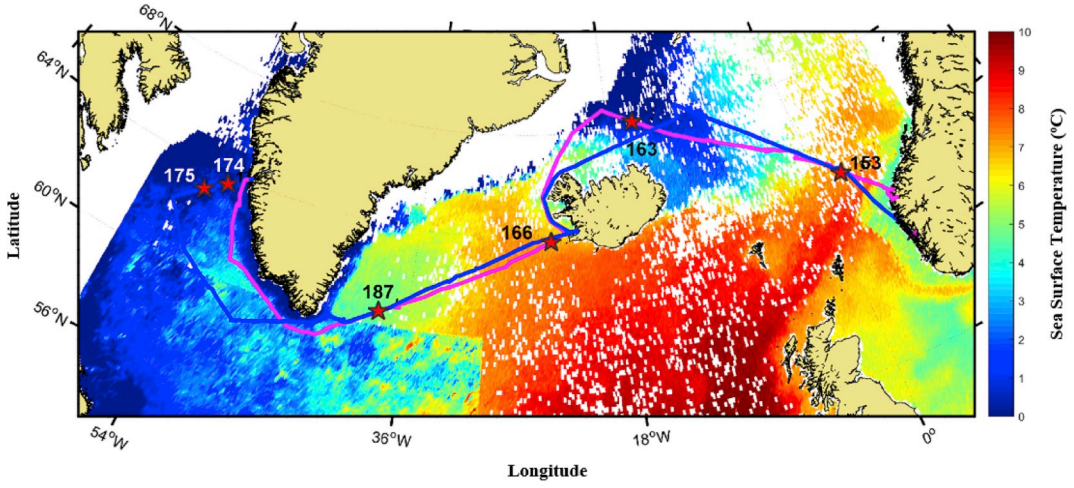

Fig. 1. Regional Temperature Variation. Sea surface temperature across the sampled regions of the North Atlantic Ocean, including the Norwegian, Icelandic, Irminger, and Labrador Seas. Lines show the cruise tracks of the EuroBASIN 2013 R/V G.O. Sars (westbound in mauve, eastbound in blue), with stars indicating stations at which samples were collected for analysis for this study. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Environmental Data. Environmental data at stations where zooplankton samples were collected for examination of *C. finmarchicus* gut contents.

Date	Station	Region/ Sea	Latitude (N)	Longitude	GMT	Net System	Depth (m)	Temp (°C)	Salinity (PSU)	Fluorescence (ug/ L)	Oxygen (mL/ L)
04-May-13	153	Norwegian	63.76	2.28 E	124.46	T80	0–200	7.34	35.21	0.19	6.30
11-May-13	163	Icelandic	68.17	15.33 W	131.44	WP2	0–200	1.88	34.90	0.13	7.28
14-May-13	166	Irminger	63.83	24.29 W	134.97	WP2	0–200	7.37	35.15	0.99	6.84
25-May-13	174	Labrador	63.69	53.4265 W	145.25	1.5m Ring Net	0–200	0.92	33.41	0.28	8.32
26-May-13	175	Labrador	63.08	55.1507 W	146.01	1.5m Ring Net	0–200	2.30	34.19	2.54	8.23
02-Jun-13	187	Irminger	60.61	38.3308 W	152.94	WP2	0–200	5.85	34.89	0.51	6.89

buffer, 2.5 μl $MgCl_2$, 0.7 μl dNTPs (10 mM), 0.25 μl Taq Polymerase, and 1 μl of each primer (10 μM). The metabarcoding of eukaryotic diversity used the 18S rRNA V4 hypervariable region, which was amplified using the Reuk454FWD1 and ReukREV3 primers (Table 2; Stoeck et al., 2010) using the following PCR protocol: 1 cycle of denaturation at 95 °C for 10 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; final extension cycle at 72 °C for 5 min; and an indefinite hold at 4 °C. The metabarcoding analysis of prokaryotic diversity used the 16S rRNA V3–V4 hypervariable region, which was amplified using the Bact 341F/Bact 785R primers (Table 2) and the protocol from Moisander et al. (2015), with the same ratio of PCR reagents as the 18S rRNA reactions. Primers were designed with adapter sequence overhangs at the 5′ end (Table 2). A second PCR was performed to attach a matching overhang plus Illumina p5/p7 and dual indexes (Lange et al., 2014), for which the following PCR protocol was used: 1 cycle of denaturation at 95 °C for 3.5 min; 8 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 90 s; final extension cycle at 72 °C for 10 min. PCR products were pooled for quantification and visualization using the QIAxcel DNA Fast Analysis (Qiagen, Hilden, Germany). PCR products were normalized based on the concentration of DNA from 360 to 440 bp then pooled using the QIAgility liquid handling robot. The pooled PCR products were cleaned using the Gene Read Size Selection kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The cleaned pool was sequenced on the MiSeq using Ver. 2 2 × 250 base pair kit (Illumina, Inc., San Diego, CA).

2.5. Bioinformatics and statistics

Custom scripts were executed using the computational resources of the Xanadu computing cluster of the University of Connecticut Health Center (UCHC). A reference database for the specific hypervariable region used in this study was prepared for taxonomic identifications from the Protist Ribosomal Reference (PR2) database (Guillou et al., 2013) for eukaryotes and the SILVA reference database (Ver. 132; Quast et al., 2013) for prokaryotes using the 'pcr.seqs' command (oligo option) of Mothur (Ver. 1.40.1; Schloss et al., 2009). Following database trimming, the database sequences were aligned in MAFFT (Ver. 7.305, Berkeley Software Distribution, Berkeley, CA), using the 'retree 1' option.

The custom databases for the specific hypervariable regions were used for taxonomic assignments of the MiSeq-generated sequence reads in Mothur. Contiguous sequences were created from bi-directional read data ('make.contigs' command). Low quality sequences were removed that contained any ambiguities or homopolymer runs of a length 8 bp or greater and sequences less than 270 bp for 18S V4 rRNA and 406 bp for 16S V3–V4 rRNA were removed using the 'screen.seqs' command. Chimeras were identified using UCHIME (Edgar et al., 2011) and removed from analysis. Pairwise distances were calculated ('dist.seqs') and OTUs clustered ('cluster') according to the current common practices for the respective gene regions: distances of 0.006 (2 bp difference in 18S V4 rRNA, ~99% similarity) for eukaryotes and 0.03 (~12 bp difference in 16S V3–V4 rRNA, 97% similarity) for prokaryotes. Taxonomic classification was performed using the 'classify.seqs' command to compare the quality-controlled sequences to the custom database for the specific hypervariable region.

Following quality control and taxonomic classification steps, stacked bar graphs were produced to highlight the contributions of selected taxonomic groups to the overall number of sequence reads. Samples were subsampled and merged by region to identify OTUs shared among all groups. A Venn diagram was created to visualize the prokaryotic OTUs shared between each combination of the regions.

Relationships among the diversity of gut content samples from different North Atlantic regions were evaluated in R (Ver. 3.4.1, The R Foundation for Statistical Computing) using the VEGAN package for community ecology statistics (Oksanen et al., 2017) and Phyloseq (McMurdie and Holmes, 2013). Similarities of the copepod gut content among regions was visualized in a Principal Coordinates Analysis (PCoA) plot, a two-dimensional representation of dissimilarity values between samples. The significance of the most parsimonious arrangement of samples along unconstrained axes was evaluated using "Adonis" (PERMANOVA) and "betadisper" (Permutation test for homogeneity of multivariate dispersions) tests. The percentage of the variability in the unconstrained ordination explained by the relationship between region and gut content diversity was assessed using constrained ordination.

Table 2
Oligonucleotide List. Oligonucleotide primer sequences used to amplify insertion-selection markers from copepods, as well as barcode regions of eukaryotic and prokaryotic genes from copepod gut contents.

Marker	Primer name	Sequence (5′—3′)
Calanus InDel	G_150 Forward	GACGCCATTGACCATCCAGT
	G_150 Reverse	GCTCCAGCGGTTAGGTTTCT
Eukaryotic V4 18S rRNA	Reuk454FWD1	CCAGCASCYGCGGTAATTCC
	ReukREV3	ACTTTCGTTCTTGATYRA
Prokaryotic V3–V416S	Bact 341F	CCTACGGGNGGCWGCAG
	Bact 785R	GACTACHVGGGTATCTAATCC
Adapter overhang	Forward	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	Reverse	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

3. Results

3.1. Environmental data

Sea surface temperature varied markedly across the sampled domain (Fig. 1). Temperatures at the collection sites in the Norwegian (7.3 °C) and Irminger (7.4 and 5.9 °C) Seas were notably warm, with cooler temperatures observed in the Labrador (0.9 and 2.3 °C) and Icelandic (1.9 °C) Seas (Table 1).

3.2. Prokaryotic diversity of gut contents

Prokaryotic diversity of the copepod gut contents was dominated by Proteobacteria (Gamma-proteobacteria and Alpha-proteobacteria), Bacteroidetes, Planctomycetes, Cyanobacteria, Acidobacteria, and Actinobacteria (Fig. 2). Cyanobacteria, specifically *Synechococcus*, dominated the V3–V4 16S rRNA reads in the gut from all Irminger Sea stations and were present in all samples (Fig. 2). Proteobacteria (Gamma-, Alpha-, and Delta-proteobacteria) dominated the remainder of the sequences (Fig. 2). Gammaproteobacteria were most abundant within this group, representing 75% of the sequences, and dominated the proteobacteria in all but one sample: 163B from the Icelandic Sea, in which Alpha- nearly matched Gamma-Proteobacteria abundance. The dominant Gammaproteobacteria classes in order from most abundant to least were: Betaproteobacteriales, Cellvibrionales, Nitrosococcales, Alteromonadales, Oceanospirillales, Pseudomonadales.

Deltaproteobacteria were extremely rare in this dataset but were found at most stations. Bacteroidetes was the second most abundant phylum across the dataset, with a particularly strong presence in the westernmost Labrador Sea samples, where the phylum was ~40% of identified sequence reads for each sample. Flavobacteriaceae, sublevels Aurantivirga and Pseudofulvibacter, accounted for most of these Bacteroidetes sequences.

Acidobacteria played a minor role in each sample, except the Icelandic Sea, where the phylum comprised an average of ~10% of reads. Similarly, Actinobacteria, Firmicutes, Planctomycetes, and other phyla (including those with <0.5% of sequence reads) were relatively rare and were only slightly more abundant in the eastern regions (Fig. 2).

Principle Component Analysis (PCoA) explained 25.3% and 21.6% of the variation in the data along Axis 1 and 2, respectively (Fig. 3). The PERMANOVA indicated that the groupings by North Atlantic region had significantly different centroids ($Pr > F = 0.001$); these did not result from differences in group dispersions, as indicated by a non-significant $Pr > F$ result (0.114). A total of 36 OTUs were found in samples from all regions, based on clustering at 97% similarity threshold and normalized with subsampling to the smallest library size (Fig. 4).

3.3. Eukaryotic diversity of gut contents

Phytoplankton (diatoms, dinoflagellates, green algae) were the dominant groups of Eukaryotes found in the gut contents of copepods collected at most stations (Fig. 5). Among diatom sequences identified to the genus level, *Chaetoceros* and *Thalassiosira* were the most abundant, especially in the Icelandic and Labrador Seas. Dinoflagellate sequences identified to genus level were predominantly of the order Syndiniales, specifically *Hematodinium* from a single sample in the Labrador Sea (174B). Other, less abundant, dinoflagellate genera included *Suessiales, Ceratium, Gymnodinium,* and *Prorocentrum.* Ciliate sequences were prominent in a Labrador Sea sample (175A), with minor appearances in all regions except the Irminger Sea, where they were notably absent. Apostome ciliate (Subclass Apostomatia) sequences were found in one Labrador Sea sample (175A). The ciliates found in the Icelandic Sea, which were only a small portion of these samples (~1%), were spirotrichs.

Several groups of organisms that were not expected to be found in the diet of copepods were detected in the gut contents. Cnidarians (mostly hydrozoans) were detected in samples from the Labrador Sea, with smaller signals detected in most other samples. Ctenophores (phylum Ctenophora) were only found in the Labrador Sea. Fungi represented a large portion of sequences in several samples, most notably the Icelandic Sea. Nematodes (phylum Nematoda) were found in the Icelandic Sea samples (163A and 163B) and a Norwegian Sea sample (153A). Apicomplexa, a clade of parasitic Alveolata, dominated the sequence reads from the gut of copepods in one Norwegian Sea sample (153B) and a trace amount in the Icelandic Sea (163B).

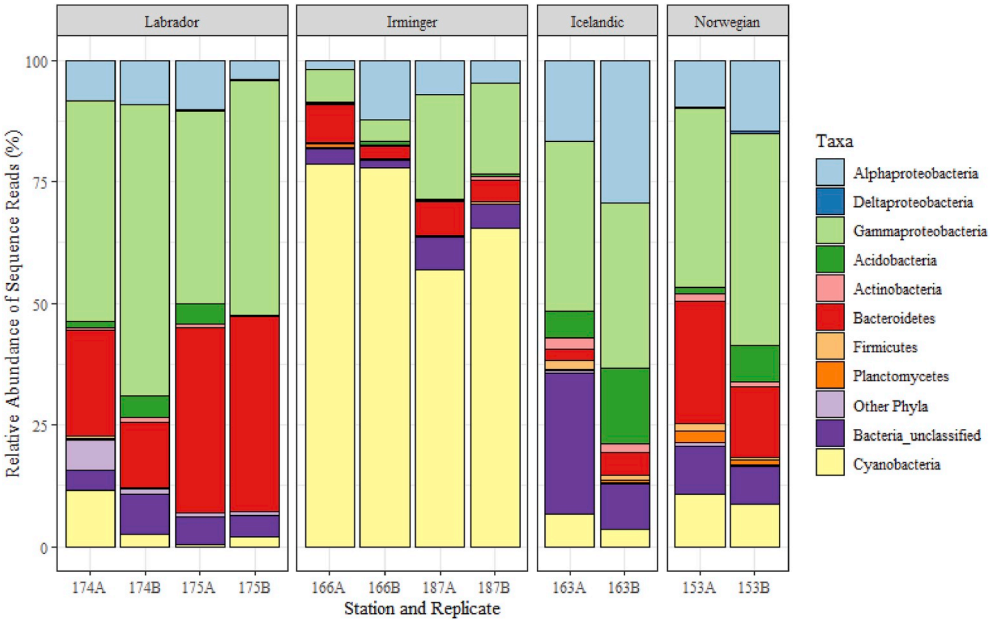

Fig. 2. Prokaryotic Fraction of *C. finmarchicus* Gut Contents. Relative abundances of V3–V4 16S rRNA sequence reads belonging to each bacterial phylum (or class in the case of Protobacteria, which includes Gamma-, Delta-, and Alpha-proteobacteria). From left to right, samples are arranged by station from west to east. "Other" comprises additional phyla that did not exceed 0.5% of the sequence reads for the whole dataset.

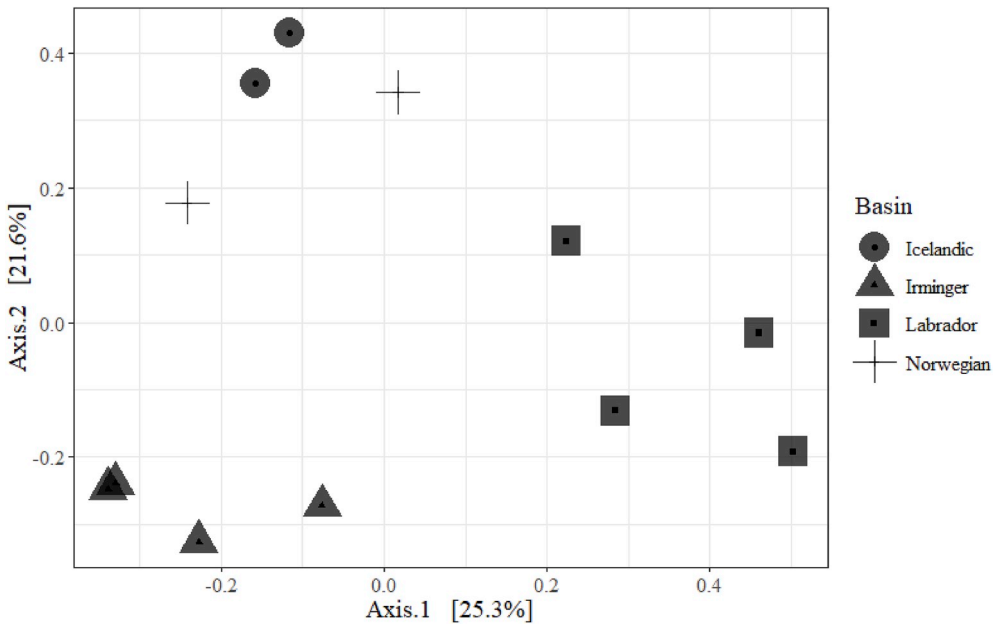

Fig. 3. Variation in Prokaryotic Diversity of *C. finmarchicus* Gut Contents. PCoA unconstrained ordination of *C. finmarchicus* prokaryotic diversity of gut contents by North Atlantic region along an optimal gradient.

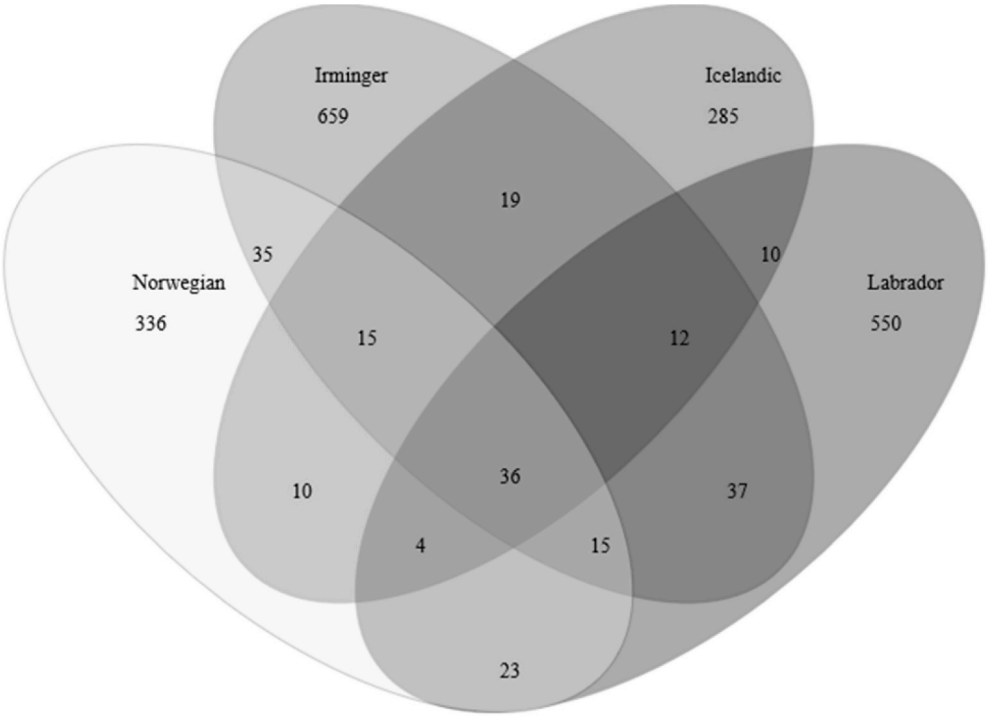

Fig. 4. Venn Diagram Prokaryotic Diversity of *C. finmarchicus* Gut Contents. Venn diagram of OTUs shared among gut contents of *C. finmarchicus* collected from N. Atlantic regions shown. Clustering of sequences used a threshold distance of 0.03.

4. Discussion

4.1. Molecular analysis of C. finmarchicus gut content biodiversity

DNA barcoding (Roslin et al., 2016) and metabarcoding (Bucklin et al., 2016; Pompanon et al., 2012) are valuable tools to clarify trophic interactions and food web dynamics in diverse ecosystems. These molecular approaches allow the detection of DNA from soft-bodied organisms, which may otherwise be unidentifiable. For example, spider DNA was still detectable in the feces of lizards, despite the expectation that these soft-bodied organisms would be degraded too quickly and thoroughly for detection (Kartzinel and Pringle, 2015). Metabarcoding was also successfully employed in the Gulf of Maine to identify phytoplankton consumed by herring that were then consumed by puffins in the Gulf of Maine; the phytoplankton DNA sequences were still detectable in the feces of puffins (Bowser et al., 2013). That study demonstrated that metabarcoding results can be confounded by secondary predation when studying food web interactions at higher trophic levels,

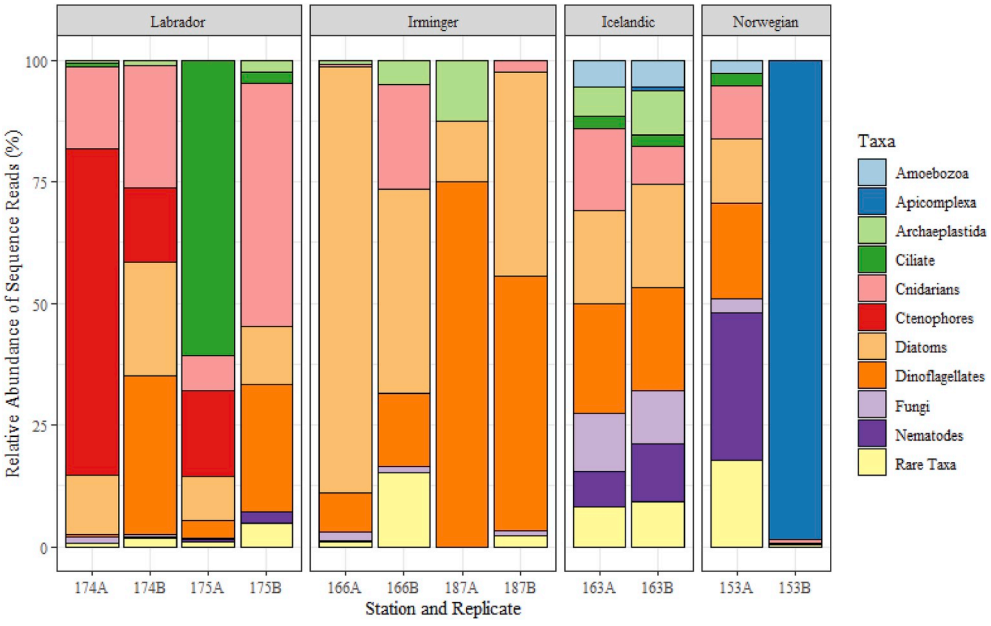

Fig. 5. Eukaryotic Diversity of *C. finmarchicus* Gut Contents. Relative abundances of V4 18S rRNA sequence reads identified as major taxonomic groups found in the gut contents of *C. finmarchicus*. Rare taxa, which comprise additional groups that did not represent more than 1% of total sequences in the dataset, include Hacrobia, Craniata, Rhizaria, Chaetognatha, and Mollusca. From left to right, stations sampled are arranged from west to east. Sequences that could not be classified to phylum level are excluded.

when comparison of predator and prey diets is required (Bowser et al., 2013; Shehzad et al., 2012). Another advantage of metabarcoding gut contents is the detection of unexpected dietary components. The majority of studies on copepod feeding behaviors have been conducted as laboratory incubation experiments, which may be biased by the selection of phytoplankton cultures provided to the copepods. Consequently, unexpected food preferences—such as an affinity for microzooplankton among some copepods—were only recently discovered (Campbell et al., 2016). Metabarcoding offers several unique advantages for studies of diet, potentially achieving high taxonomic resolution despite the short and degraded state of prey DNA sequences, and is useful for comprehensive studies of *C. finmarchicus* diet in the wild.

4.2. Eukaryotic diversity of *C. finmarchicus* gut contents

Calanus finmarchicus has been shown to be predominantly omnivorous, acquiring nutrition through both phytoplankton grazing and predation, including the consumption of potential competitors (Ohman and Runge, 1994). This species is known to consume and exhibit a selective preference for microzooplankton (Campbell et al., 2016), such as ciliates (Leiknes et al., 2014; Nejstgaard et al., 1997), diatoms (Koski and Wexels Riser, 2006; Meyer-Harms et al., 1999), and dinoflagellates (Nejstgaard et al., 1997). The copepod is also known to consume cryptophytes, haptophytes (Meyer-Harms et al., 1999), and rotifers (Nejstgaard et al., 1997). The diet of *C. finmarchicus* is diverse, and possibly impacted by the ever-changing ocean environment in which they reside. The *C. finmarchicus* diet is at least partially dependent upon the physical environment, which plays a role in shaping the planktonic community and will thus impact the assemblage upon which copepods graze (Melle et al., 2014).

Targeted analysis of eukaryotic biota using V4 18S rRNA for metabarcoding analysis allowed detection of items from a wide array of taxonomic groups. Although most items identified in the foregut are likely to be prey items of the copepod, there are clearly some exceptions. Previous studies (Guo et al., 2012; Ray et al., 2016; Shields, 1994) have documented a variety of parasites and symbionts of *C. finmarchicus* and other copepods, which were found in most of the samples examined in this study. Identified sequences revealed the presence of phyla such as Cnidaria and Ctenophora that are not expected as prey of *C. finmarchicus*. In the Pacific Ocean, *Calanus sinicus* has been found to graze on the organic particles/detritus of metazoans such as hydrozoans and ctenophores over continental shelf waters off China, Japan and Korea, when phytoplankton levels are low (Yi et al., 2017). In these situations, it may be assumed that the prey items in question are either the eggs or larvae of the much larger prey, or represent organic detritus collected by the copepod through grazing on marine snow. Regardless of the actual form in which cnidarian and ctenophore prey was ingested by the copepod, this study provides evidence that these taxa include prey items for *C. finmarchicus* in the North Atlantic.

Dinoflagellates are a well-documented component of the *C. finmarchicus* diet (Koski, 2007; Koski and Wexels Riser, 2006; Meyer-Harms et al., 1999; Mullin, 1963), but certain genera can also parasitize copepods. Syndiniales sequences (specifically of the genus *Hematodinium*) were found in the Labrador Sea sample, indicating that at least one copepod sampled was infected by these dinoflagellates. *Hematodinium* is a documented parasitic-castrator of amphipods and copepods, impacting their ability to reproduce (Shields, 1994), and infected zooplankton are believed to be the reservoir and means of transmission of the parasite to economically-important crustaceans (Small and Pagenkopp, 2011). Impacts of parasitism include impaired functioning of muscles, hemolymph, and hepatopancreas, as well as alteration of chitin deposition (Stentiford and Shields, 2005). Although parasitism by the related dinoflagellate *Blastodinium* has been documented in *C. finmarchicus* (Shields, 1994), infection in *C. finmarchicus* by *Hematodinium* has not yet been reported.

Evidence of nematodes was found in multiple samples. Cyclopoid copepods have been found to become infected by feeding on nematode larvae, which then served as a means of parasite transmission to fish feeding on the infected copepods (Hubbard et al., 2016; Moravec, 2009), but nematode infection in *Calanus* has not yet been documented.

Parasitic Apicomplexa have been found to infect amphipods (Prokopowicz et al., 2010), other calanoid copepods, and one species of apicomplexan, *Ganymedes apsteini,* has been found to infect *C. finmarchicus* (Sano et al., 2016). If they are indeed parasites and not a dietary item, this finding leads to new questions about the impact of such parasites on copepods. Gregarine apicomplexan infections in Antarctic krill have been known to negatively impact the microvilli of the gut lumen (Takahashi et al., 2009).

Ciliates have been well-documented as part of the *C. finmarchicus* diet (Koski, 2007; Koski and Wexels Riser, 2006; Nejstgaard et al., 1997), but it is likely that sequences derived from the subclass Apostomatia were not components of the diet. Apostome ciliates are known to

be copepod symbionts and invasive parasites (Guo et al., 2012), so the presence of these sequences in a Labrador Sea sample most likely represents parasitism.

The parasitic taxonomic groups found in the gut contents of *C. finmarchicus* may also be secondary parasites that had infested the prey items of the copepods. It seems most likely that these sequences were not derived from external contamination, since the copepod foregut was dissected, and no external parts of the copepod were included in the DNA extraction. This analysis suggests the occurrence of previously unknown parasitic and symbiotic relationships with *C. finmarchicus*. This finding is significant both for the potential to affect the reproductive rates and nutritional value of the species, as well as for possible transmission of parasites to upper trophic levels.

4.3. Prokaryotic diversity of C. finmarchicus gut contents

The prokaryotic diversity of copepod gut contents is determined by both feeding habits, including prey availability and selectivity, as well as resident species comprising the microbiome (Tang et al., 2009). Parasites are an additional source of prokaryotic diversity of copepod gut contents, as reported by Jepps (1937). Since it may take minutes to hours for food to pass through the copepod gut (Atkinson et al., 1996; Dam and Peterson, 1988; Tirelli and Mayzaud, 2005), the potential for the gut environment to influence the transient microbiota may be limited The prokaryotic diversity found in copepod guts has previously been reported to be dominated by gram-negative Proteobacteria, and this study provides further evidence of this (Møller et al., 2007; Shoemaker and Moisander, 2015; Skovgaard et al., 2015). However, there are conflicting reports about which class within the phylum is dominant, and the differences are thought to be method-dependent (Gerdts et al., 2013; Shoemaker and Moisander, 2017). Clone libraries of the whole body copepod microbiome favored Gammaproteobacteria, whereas PCR-DGGE showed a dominance of Alphaproteobacteria for the same samples (Gerdts et al., 2013). There is also a difference when sampling the excised copepod gut, rather than the whole organism: two studies conducted by the same researchers compared gut to whole-organism samples, and found that Alphaproteobacteria dominated when considering just the gut, while Gammaproteobacteria dominated in analysis of the whole body (Dorosz et al., 2016; Shoemaker and Moisander, 2017, 2015).

The composition and abundance of the microbial community of the copepod gut has been thought to be relatively stable, despite significant variation in diet and trophic ecology over various temporal (e.g. seasonal) and spatial (e.g. regional) scales (Brandt et al., 2010). Comparative studies have shown distinct differences between the microbial diversity of the copepod gut and the surrounding seawater, lower diversity in the gut than in the assemblage of microbes in the surrounding waters (Corte et al., 2014; Gerdts et al., 2013; Møller et al., 2007; Shoemaker and Moisander, 2015, 2017), and a number of studies have supported the concept of a core microbial community of the copepod gut (Shoemaker and Moisander, 2017).

This study found a predominance of Gammaproteobacteria, which could potentially indicate contamination from the exoskeleton microbiome during gut dissection. Alternatively, this could also be an environmentally-driven difference between the *C. finmarchicus* populations of two different regions: the North Atlantic population from this study versus the population from the subtropical North Atlantic Ocean (Shoemaker and Moisander, 2017). Determining which taxonomic groups dominate is important for establishing which metabolisms are favored in the environment of the copepod gut. However, these differences may have limited impact, since both tend to be Gram-negative, aerobic, or facultatively anaerobic and rod-shaped.

Another abundant group was the Alphaproteobacteria, including families that tend to be attached to surfaces, such as Rhodobacterales and Caulobacterales, the latter of which is known to form biofilms (Abraham et al., 2014). Rhizobiales was also found in abundance with sub-taxa that are known to fix nitrogen or are methanotrophs. One of the less abundant taxa was Rickettsiales, a known intracellular pathogen of crustaceans that causes host death following discoloration and weight loss; however, it has been argued that this genus belongs in the Gammaproteobacteria (Cordaux et al., 2007). Classes that dominated among the Gammaproteobacteria include a marine bacteria class with proteorhodopsins: Cellvibrionales, which is known to prefer high nutrient concentrations (Stingl et al., 2007), as can be found in the copepod gut. Additionally, Class Oceanospirillales was abundant, which is a gall-forming Gammaproteobacteria that infects corals, but also lives in copepods, bivalves and snails (Shelyakin et al., 2018).

Bacteroidetes is thought to be derived from diet, as gut populations were correlated with the spring bloom when copepod diets are phytoplankton-heavy (Shoemaker and Moisander, 2017). Although members of the Family Flavobacteriales were numerically dominant, accounting for >91% of Bacteroidetes sequence reads, taxa from the family Chitinophagaceae were the only Bacteroidetes found consistently in the gut of *C. finmarchicus* from all basins. This chitin-degrading group may maintain a constant presence in the chitin-rich environment of the copepod gut, despite the taxon's minor presence in this study. *Pyrinomonas*, a genus of Acidobacteria subdivision-group 4, dominated sequences of this phylum and was also found in samples from every basin. It is heterotrophic and able to use chitin as a carbon source (Foesel et al., 2013; Huber et al., 2014), which provides a likely explanation for a single genus accounting for 5% of all sequence reads observed.

Sequences of the phylum Planctomycetes, which were less than 1% of all sequence reads, were dominated by the family Pirellulaceae: an ammonia-oxidizer, which is thought to be a core bacterial group in the nitrogen cycle, performing nitrification (Kellogg et al., 2016). Despite the low abundance of sequences from this phylum, it was found in gut content samples from all North Atlantic regions, suggesting that copepods carry out key components of the nitrogen cycle in their guts. Phylum Actinobacteria was also rare, with just over 1% of all sequence reads, yet two representatives of several classes were identified in all samples.

Cyanobacteria were extremely abundant in the guts of copepods from the Irminger Sea, with over 50% of 16S rRNA sequence reads in every sample belonging to this group. Cyanobacteria have been reported to be consumed by *C. finmarchicus,* but appear to be avoided when other sources of food are available (Meyer-Harms et al., 1999). Whether Cyanobacteria are a part of the copepod diet or its resident microflora cannot be ascertained without controlled feeding experiments. Regardless, the dominance of this group among the sequence reads in this dataset suggests that Cyanobacteria are an important component of the prokaryotic assemblage found in the gut of *C. finmarchicus* in the North Atlantic.

The prokaryotic diversity of *C. finmarchicus* gut contents varied among the four North Atlantic regions sampled. In addition to the phyla that were consistently found in the copepod gut in the Subtropical North Atlantic (Bacteroidetes, Actinobacteria, and Proteobacteria) (Shoemaker and Moisander, 2017), Acidobacteria and Planctomycetes were found in every sample from the North Atlantic. Additionally, Firmicutes, a group occurring in anaerobic conditions, was described from the guts of *C. finmarchicus* in the Subtropical North Atlantic but represented less than 1% of all sequence reads in this study. The phylum did occur in small numbers in samples from the Icelandic and Norwegian seas. This suggests that copepod gut conditions are mostly aerobic, perhaps with slightly more hypoxic hindguts in the eastern regions.

5. Conclusion

Analysis of eukaryotic and prokaryotic diversity in the gut contents of the copepod *C. finmarchicus* were analyzed by metabarcoding, using hypervariable regions of prokaryotic and eukaryotic rRNA to detect and classify a broad range of taxonomic groups. DNA sequences obtained were analyzed to infer the composition of the copepod diet, microbiome,

parasites, and pathogens. New evidence was found for parasites and pathogens that are known to occur in copepods and other crustaceans, but have not been reported from *C. finmarchicus*. Analysis of copepods collected from the Norwegian, Icelandic, Irminger, and Labrador Seas revealed significant differences in gut content diversity among the North Atlantic regions. Additional research is needed to examine the complex determinants of the temporal and spatial variability of key elements of the diet of *C. finmarchicus*, and to understand impacts on the pelagic ecosystems of the North Atlantic Ocean.

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Declaration of competing interest

None.

CRediT authorship contribution statement

Heidi D. Yeh: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Jennifer M. Questel:** Methodology, Software, Formal analysis. **Kendra R. Maas:** Resources, Methodology, Software. **Ann Bucklin:** Conceptualization, Writing - review & editing.

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References

Abraham, W.-R., Rohde, M., Bennasar, A., 2014. The family *Caulobacteraceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 179–205. https://doi.org/10.1007/978-3-642-30197-1_259.

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W., Huse, S.M., 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PloS One 4, e6372. https://doi.org/10.1371/journal.pone.0006372.

Atkinson, A., Ward, P., Murphy, E.J., 1996. Diel periodicity of Subantarctic copepods: relationships between vertical migration, gut fullness and gut evacuation rate. J. Plankton Res. 18, 1387–1405. https://doi.org/10.1093/plankt/18.8.1387.

Bowser, A.K., Diamond, A.W., Addison, J.A., 2013. From puffins to plankton: a DNA-based analysis of a seabird food chain in the Northern Gulf of Maine. PloS One 8. https://doi.org/10.1371/journal.pone.0083152, 1–1.

Brandt, P., Gerdts, G., Boersma, M., Wiltshire, K.H., Wichels, A., 2010. Comparison of different DNA-extraction techniques to investigate the bacterial community of marine copepods. Helgol. Mar. Res. 64, 331–342. https://doi.org/10.1007/s10152-010-0188-1.

Bucklin, A., Lindeque, P.K., Rodriguez-Ezpeleta, N., Albaina, A., Lehtiniemi, M., 2016. Metabarcoding of marine zooplankton: prospects, progress and pitfalls. J. Plankton Res. 38, 393–400. https://doi.org/10.1093/plankt/fbw023.

Campbell, R.G., Ashjian, C.J., Sherr, E.B., Sherr, B.F., Lomas, M.W., Ross, C., Alatalo, P., Gelfman, C., Keuren, D.V., 2016. Mesozooplankton grazing during spring sea-ice conditions in the eastern Bering Sea. Deep Sea Res. Part II Top. Stud. Oceanogr., Understanding Ecosystem Processes in the Eastern Bering Sea IV 134, 157–172. https://doi.org/10.1016/j.dsr2.2015.11.003.

Cleary, A.C., Durbin, E.G., Rynearson, T.A., Bailey, J., 2015. Feeding by *Pseudocalanus* copepods in the Bering Sea: trophic linkages and a potential mechanism of niche partitioning. Deep Sea Res. Part II Top. Stud. Oceanogr. https://doi.org/10.1016/j.dsr2.2015.04.001.

Cordaux, R., Paces-Fessy, M., Raimond, M., Michel-Salzat, A., Zimmer, M., Bouchon, D., 2007. Molecular characterization and Evolution of arthropod-pathogenic *Rickettsiella* bacteria. Appl. Environ. Microbiol. 73, 5045–5047. https://doi.org/10.1128/AEM.00378-07.

Craig, C., Kimmerer, W.J., Cohen, C.S., 2014. A DNA-based method for investigating feeding by copepod nauplii. J. Plankton Res. 36, 271–275. https://doi.org/10.1093/plankt/fbt104.

Dam, H.G., Peterson, W.T., 1988. The effect of temperature on the gut clearance rate constant of planktonic copepods. J. Exp. Mar. Biol. Ecol. 123, 1–14. https://doi.org/10.1016/0022-0981(88)90105-0.

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horak, A., Jaillon, O., Lima-Mendez, G., Luke, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Tara Oceans Coordinators, Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., Boss, E., Follows, M., Karp-Boss, L., Krzic, U., Reynaud, E. G., Sardet, C., Sullivan, M.B., Velayoudon, D., 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348. https://doi.org/10.1126/science.1261605, 1261605–1261605.

Dong, Y., Yang, G.-P., Tang, K.W., 2013. Dietary effects on abundance and carbon utilization ability of DMSP-consuming bacteria associated with the copepod *Acartia tonsa* Dana. Mar. Biol. Res. 9, 809–814. https://doi.org/10.1080/17451000.2013.765587.

Dorosz, J.N.A., Castro-Mejia, J.L., Hansen, L.H., Nielsen, D.S., Skovgaard, A., 2016. Different microbiomes associated with the copepods *Acartia tonsa* and *Temora longicornis* from the same marine environment. Aquat. Microb. Ecol. 78, 1–9. https://doi.org/10.3354/ame01799.

Durbin, E.G., Casas, M.C., Rynearson, T.A., 2012. Copepod feeding and digestion rates using prey DNA and qPCR. J. Plankton Res. 34, 72–82. https://doi.org/10.1093/plankt/fbr082.

Durbin, E.G., Casas, M.C., Rynearson, T.A., Smith, D.C., 2008. Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. Mar. Biol. 153, 699–707. https://doi.org/10.1007/s00227-007-0843-5.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Elbrecht, V., Leese, F., 2015. Can DNA-based ecosystem Assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PloS One 10, 1–16. https://doi.org/10.1371/journal.pone.0130324.

Foesel, B.U., Rohde, M., Overmann, J., 2013. *Blastocatella fastidiosa* gen. nov., sp. nov., isolated from semiarid savanna soil – the first described species of Acidobacteria subdivision 4. Syst. Appl. Microbiol. 36, 82–89. https://doi.org/10.1016/j.syapm.2012.11.002.

Fortier, L., Villeneuve, A., 1996. Cannibalism and predation of fish larvae by larvae of Atlantic mackerel, *Scomber scombrus*: trophodynamics and potential impact on recruitment. Oceanogr. Lit. Rev. 11, 1155–1156.

Friedland, K.D., Record, N.R., Asch, R.G., Kristiansen, T., Saba, V.S., Drinkwater, K.F., Henson, S., Leaf, R.T., Morse, R.E., Johns, D.G., Large, S.I., Hjøllo, S.S., Nye, J.A., Alexander, M.A., Ji, R., 2016. Seasonal phytoplankton blooms in the North Atlantic linked to the overwintering strategies of copepods. Elem Sci Anth 4. https://doi.org/10.12952/journal.elementa.000099.

Gerdts, G., Brandt, P., Kreisel, K., Boersma, M., Schoo, K.L., Wichels, A., 2013. The microbiome of North Sea copepods. Helgol. Mar. Res. 67, 757–773. https://doi.org/10.1007/s10152-013-0361-4.

Greene, C.H., Pershing, A.J., 2004. Climate and the conservation biology of North Atlantic right whales: the right whale at the wrong time? Front. Ecol. Environ. 2, 29–34. https://doi.org/10.1890/1540-9295(2004)002[0029:CATCBO]2.0.CO;2.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604. https://doi.org/10.1093/nar/gks1160.

Guo, Z., Liu, S., Hu, S., Li, T., Huang, Y., Liu, G., Zhang, H., Lin, S., 2012. Prevalent ciliate symbiosis on copepods: high genetic diversity and wide distribution detected using small subunit ribosomal RNA gene. PloS One 7. https://doi.org/10.1371/journal.pone.0044847.

Haley, S.T., Juhl, A.R., Keafer, B.A., Anderson, D.M., Dyhrman, S.T., 2011. Detecting copepod grazing on low-concentration populations of *Alexandrium fundyense* using PCR identification of ingested prey. J. Plankton Res. 33, 927–936. https://doi.org/10.1093/plankt/fbq151.

Hansen, B., Bech, G., 1996. Bacteria associated with a marine planktonic copepod in culture. I. Bacterial genera in seawater, body surface, intestines and fecal pellets and succession during fecal pellet degradation. J. Plankton Res. 18, 257–273. https://doi.org/10.1093/plankt/18.2.257.

Head, E.J.H., Melle, W., Pepin, P., Bagøien, E., Broms, C., 2013. On the ecology of *Calanus finmarchicus* in the subarctic North Atlantic: a comparison of population dynamics and environmental conditions in areas of the Labrador Sea-Labrador/Newfoundland shelf and Norwegian sea Atlantic and coastal waters. Prog. Oceanogr., Norway-Canada Comparison of Marine Ecosystems (NORCAN) 114, 46–63. https://doi.org/10.1016/j.pocean.2013.05.004.

Hirai, J., Hidaka, K., Nagai, S., Ichikawa, T., 2017. Molecular-based diet analysis of the early post-larvae of Japanese sardine *Sardinops melanostictus* and Pacific round herring *Etrumeus teres*. Mar. Ecol. Prog. Ser. 564, 99–113.

Hu, S., Guo, Z., Li, T., Carpenter, E.J., Liu, S., Lin, S., 2014. Detecting in situ copepod diet diversity using molecular technique: development of a copepod/symbiotic ciliate-excluding eukaryote-inclusive PCR protocol. PloS One 9, e103528. https://doi.org/10.1371/journal.pone.0103528.

Hubbard, I.M., Hill-Spanik, K.M., Knott, D., Buron, I. de, 2016. Development of *Anguillicoloides crassus* in a cyclopoid copepod from the *Acanthocyclops robustus–americanus–vernalis* complex in South Carolina. U.S.A. Comp. Parasitol. 83, 192–196. https://doi.org/10.1654/4835sed.1.

Huber, K.J., Wust, P.K., Rohde, M., Overmann, J., Foesel, B.U., 2014. *Aridibacter famidurans* gen. nov., sp. nov. and *Aridibacter kavangonensis* sp. nov., two novel members of subdivision 4 of the *Acidobacteria* isolated from semiarid savannah soil. Int. J. Syst. Evol. Microbiol. 64, 1866–1875. https://doi.org/10.1099/ijs.0.060236-0.

Jansen, T., 2016. First-year survival of North East Atlantic mackerel (*Scomber scombrus*) from 1998 to 2012 appears to be driven by availability of *Calanus*, a preferred copepod prey. Fish. Oceanogr. 25, 457–469. https://doi.org/10.1111/fog.12165.

Jepps, M.W., 1937. Memoirs: on the Protozoan parasites of *Calanus finmarchicus* in the Clyde sea area. J. Cell Sci. s2–79, 589–658.

Kartzinel, T.R., Pringle, R.M., 2015. Molecular detection of invertebrate prey in vertebrate diets: trophic ecology of Caribbean island lizards. Mol. Ecol. Resour. 15, 903–914. https://doi.org/10.1111/1755-0998.12366.

Kellogg, C.A., Ross, S.W., Brooke, S.D., 2016. Bacterial community diversity of the deep-sea octocoral *Paramuricea placomus*. PeerJ 4. https://doi.org/10.7717/peerj.2529.

Koski, M., 2007. High reproduction of *Calanus finmarchicus* during a diatom-dominated spring bloom. Mar. Biol. 151, 1785–1798. https://doi.org/10.1007/s00227-007-0615-2.

Koski, M., Wexels Riser, C., 2006. Post-bloom feeding of *Calanus finmarchicus* copepodites: selection for autotrophic versus heterotrophic prey. Mar. Biol. Res. 2, 109–119. https://doi.org/10.1080/17451000600684367.

Lange, V., Böhme, I., Hofmann, J., Lang, K., Sauter, J., Schöne, B., Paul, P., Albrecht, V., Andreas, J.M., Baier, D.M., Nething, J., Ehninger, U., Schwarzelt, C., Pingel, J., Ehninger, G., Schmidt, A.H., 2014. Cost-efficient high-throughput HLA typing by MiSeq amplicon sequencing. BMC Genom. 15, 63. https://doi.org/10.1186/1471-2164-15-63.

Leiknes, Ø., Striberny, A., Tokle, N.E., Olsen, Y., Vadstein, O., Sommer, U., 2014. Feeding selectivity of *Calanus finmarchicus* in the trondheimsfjord. J. Sea Res. 85, 292–299. https://doi.org/10.1016/j.seares.2013.05.012.

McKinstry, C.A.E., Westgate, A.J., Koopman, H.N., 2013. Annual variation in the nutritional value of Stage V *Calanus finmarchicus*: implications for right whales and other copepod predators. Endanger. Species Res. 20, 195–204. https://doi.org/10.3354/esr00497.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.

Melle, W., Runge, J., Head, E., Plourde, S., Castellani, C., Licandro, P., Pierson, J., Jonasdottir, S., Johnson, C., Broms, C., Debes, H., Falkenhaug, T., Gaard, E., Gislason, A., Heath, M., Niehoff, B., Nielsen, T.G., Pepin, P., Stenevik, E.K., Chust, G., 2014. The North Atlantic Ocean as habitat for *Calanus finmarchicus*: environmental factors and life history traits. Prog. Oceanogr., North Atlantic Ecosystems, the role of climate and anthropogenic forcing on their structure and function 129, 244–284. https://doi.org/10.1016/j.pocean.2014.04.026.

Meyer-Gutbrod, E.L., Greene, C.H., Sullivan, P.J., Pershing, A.J., 2015. Climate-associated changes in prey availability drive reproductive dynamics of the North Atlantic right whale population. Mar. Ecol. Prog. Ser. 535, 243–258. https://doi.org/10.3354/meps11372.

Meyer-Harms, B., Irigoien, X., Head, R., Harris, R., 1999. Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. Limnol. Oceanogr. 44, 154–165. https://doi.org/10.4319/lo.1999.44.1.0154.

Moisander, P.H., Sexton, A.D., Daley, M.C., 2015. Stable Associations masked by temporal variability in the marine copepod microbiome. PloS One 10, e0138967. https://doi.org/10.1371/journal.pone.0138967.

Møller, E.F., Riemann, L., Søndergaard, M., 2007. Bacteria associated with copepods: abundance, activity and community composition. Aquat. Microb. Ecol. 47, 99–106. https://doi.org/10.3354/ame047099.

Moravec, F., 2009. Experimental studies on the development of *Contracaecum rudolphii* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. Folia Parasitol. Praha 56, 185–193.

Mullin, M.M., 1963. Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol. Oceanogr. 8, 239–250. https://doi.org/10.4319/lo.1963.8.2.0239.

Nejstgaard, J., Gismervik, I., Solberg, P.T., 1997. Feeding and reproduction by *Calanus finmarchicus*, and microzooplankton grazing during mesocosm blooms of diatoms and the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Prog. Ser. 147, 197–217. https://doi.org/10.3354/meps147197.

Nejstgaard, J.C., Frischer, M.E., Raule, C.L., Gruebel, R., Kohlberg, K.E., Verity, P.G., 2003. Molecular detection of algal prey in copepod guts and fecal pellets. Limnol Oceanogr. Methods 1, 29–38. https://doi.org/10.4319/lom.2003.1.29.

Nejstgaard, J.C., Frischer, M.E., Simonelli, P., Troedsson, C., Brakel, M., Adiyaman, F., Sazhin, A.F., Artigas, L.F., 2008. Quantitative PCR to estimate copepod feeding. Mar. Biol. 153, 565–577. https://doi.org/10.1007/s00227-007-0830-x.

Nejstgaard, J.C., Naustvoll, L.-J., Sazhin, A., 2001. Correcting for Underestimation of Microzooplankton Grazing in Bottle Incubation Experiments with Mesozooplankton, pp. 59–75. https://doi.org/10.3354/meps221059.

Ohman, M.D., Runge, J.A., 1994. Sustained fecundity when phytoplankton resources are in short supply: omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnol. Oceanogr. 39, 21–36. https://doi.org/10.4319/lo.1994.39.1.0021.

Oksanen, J., Blanchet, G.F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. Vegan: community ecology package.

Paradis, V., Sirois, P., Castonguay, M., Plourde, S., 2012. Spatial variability in zooplankton and feeding of larval Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. J. Plankton Res. 34, 1064–1077. https://doi.org/10.1093/plankt/fbs063.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21, 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x.

Prokopowicz, A.J., Rueckert, S., Leander, B.S., Michaud, J., Fortier, L., 2010. Parasitic infection of the hyperiid amphipod *Themisto libellula* in the Canadian beaufort sea (Arctic ocean), with a description of *Ganymedes themistos* sp. n. (Apicomplexa, Eugregarinorida). Polar Biol. 33, 1339–1350. https://doi.org/10.1007/s00300-010-0821-0.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Ray, J.L., Althammer, J., Skaar, K.S., Simonelli, P., Larsen, A., Stoecker, D., Sazhin, A., Ijaz, U.Z., Quince, C., Nejstgaard, J.C., Frischer, M., Pohnert, G., Troedsson, C., 2016. Metabarcoding and metabolome analyses of copepod grazing reveal feeding preference and linkage to metabolite classes in dynamic microbial plankton communities. Mol. Ecol. 25, 5585–5602. https://doi.org/10.1111/mec.13844.

Roslin, T., Majaneva, S., Clare, E., 2016. The use of DNA barcodes in food web construction-terrestrial and aquatic ecologists unite!1. Genome 59, 603–628. https://doi.org/10.1139/gen-2015-0229.

Sano, M., Miyamoto, H., Nishida, S., 2016. *Thiriotia euchirellae* n. sp., a new gregarine species (Apicomplexa: Eugregarinorida) from the mesopelagic copepod *Euchirella rostrata* in Sagami Bay, Japan. Mar. Biodivers. 46, 753–760. https://doi.org/10.1007/s12526-015-0423-8.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah, S.A., Pompanon, F., Coissac, E., Taberlet, P., 2012. Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. Mol. Ecol. 21, 1951–1965. https://doi.org/10.1111/j.1365-294X.2011.05424.x.

Shelyakin, P.V., Garushyants, S.K., Nikitin, M.A., Mudrova, S.V., Berumen, M., Speksnijder, A.G.C.L., Hoeksema, B.W., Fontaneto, D., Gelfand, M.S., Ivanenko, V.N., 2018. Microbiomes of gall-inducing copepod crustaceans from the corals *Stylophora pistillata* (Scleractinia) and *Gorgonia ventalina* (Alcyonacea). Sci. Rep. 8 https://doi.org/10.1038/s41598-018-29953-y, 11563.

Shields, J.D., 1994. The parasitic dinoflagellates of marine crustaceans. Annu. Rev. Fish Dis. 4, 241–271. https://doi.org/10.1016/0959-8030(94)90031-0.

Shoemaker, K.M., Moisander, P.H., 2017. Seasonal variation in the copepod gut microbiome in the subtropical North Atlantic Ocean. Environ. Microbiol. 19, 3087–3097. https://doi.org/10.1111/1462-2920.13780.

Shoemaker, K.M., Moisander, P.H., 2015. Microbial diversity associated with copepods in the North Atlantic subtropical gyre. FEMS Microbiol. Ecol. 91 https://doi.org/10.1093/femsec/fiv064.

Skovgaard, A., Castro-Mejia, J.L., Hansen, L.H., Nielsen, D.S., 2015. Host-specific and pH-dependent microbiomes of copepods in an extensive rearing system. PloS One 10, e0132516.

Small, H.J., Pagenkopp, K.M., 2011. Reservoirs and alternate hosts for pathogens of commercially important crustaceans: a review - ScienceDirect. J. Invertebr. Pathol. 153–164.

Smolina, I., Kollias, S., Poortvliet, M., Nielsen, T.G., Lindeque, P., Castellani, C., Møller, E.F., Blanco - Bercial, L., Hoarau, G.G., 2014. Genome- and Transcriptome-Assisted Development of Nuclear Insertion/deletion Markers for *Calanus* Species (Copepoda: Calanoida) Identification. https://doi.org/10.1111/1755-0998.12241.

Stentiford, G.D., Shields, J.D., 2005. A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. Dis. Aquat. Org. 66.

Stingl, U., Desiderio, R.A., Cho, J.-C., Vergin, K.L., Giovannoni, S.J., 2007. The SAR92 clade: an abundant coastal clade of culturable marine bacteria possessing proteorhodopsin. Appl. Environ. Microbiol. 73, 2290–2296. https://doi.org/10.1128/AEM.02559-06.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.-W., Richards, T. A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x.

Takahashi, K.T., Kawaguchi, S., Toda, T., 2009. Observation by electron microscopy of a gregarine parasite of Antarctic krill: its histological aspects and ecological explanations. Polar Biol. 32, 637–644. https://doi.org/10.1007/s00300-008-0563-4.

Tang, K., Dziallas, C., Hutalle-Schmelzer, K., Grossart, H.-P., 2009. Effects of food on bacterial community composition associated with the copepod *Acartia tonsa* Dana. Biol. Lett. 5, 549–553. https://doi.org/10.1098/rsbl.2009.0076.

Tang, K.W., Glud, R.N., Glud, A., Rysgaard, S., Nielsen, T.G., 2011. Copepod guts as biogeochemical hotspots in the sea: evidence from microelectrode profiling of

Calanus spp. Limnol. Oceanogr. 56, 666–672. https://doi.org/10.4319/lo.2011.56.2.0666.

Tirelli, V., Mayzaud, P., 2005. Relationship between functional response and gut transit time in the calanoid copepod *Acartia clausi*: role of food quantity and quality. J. Plankton Res. 27, 557–568. https://doi.org/10.1093/plankt/fbi031.

Troedsson, C., Simonelli, P., Nägele, V., Nejstgaard, J., Frischer, M., 2009. Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). Mar. Biol. 156, 253–259. https://doi.org/10.1007/s00227-008-1079-8.

Urban-Rich, J., 2001. Seston effects on faecal pellet carbon concentrations from a mixed community of copepods in Balsfjord, Norway, and the Antarctic Polar. Front. ICES J. Mar. Sci. 58, 700–710. https://doi.org/10.1006/jmsc.2000.1055.

Vestheim, H., Edvardsen, B., Kaartvedt, S., 2005. Assessing feeding of a carnivorous copepod using species-specific PCR. Mar. Biol. 147, 381–385. https://doi.org/10.1007/s00227-005-1590-0.

Yi, X., Huang, Y., Zhuang, Y., Chen, H., Yang, F., Wang, W., Xu, D., Liu, G., Zhang, H., 2017. In situ diet of the copepod *Calanus sinicus* in coastal waters of the South Yellow Sea and the Bohai Sea. Acta Oceanol. Sin. 36, 68–79. https://doi.org/10.1007/s13131-017-0974-6.