Metabarcoding Targets Functional Group Diversity of Micro- and Mesozooplankton in Pelagic Food Webs

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Abstract

The ability for marine ecosystems to maintain productivity and functionality under long term changes in resource availability relies on the diversity of functional groups. Nevertheless, the complexity of zooplankton interactions is rarely considered in trophic studies because of the lack of detailed information about feeding interactions in nature. In this study, we used DNA metabarcoding to detect trophic interactions of a wide range of micro- and mesozooplankton including ciliates, rotifers, cladocerans, copepods and their prey, by sequencing 16- and 18S rRNA genes. Our study demonstrates that functional group diversity goes beyond both phylogeny and size and reinforces the importance of diversity in resource use for stabilizing food web efficiency by allowing for alternative pathways of energy transfer. We further demonstrate the importance of ciliates and rotifers in recycling organic matter from degraded filamentous cyanobacteria within the pelagic zone, contributing to ecosystem production. The approach used in this study is a suitable entry point to ecosystem-wide food web modeling considering species-specific resource use of key consumers.

Introduction

The ability for ecosystems to maintain productivity and functionality under seasonal and long term changes in resource availability relies on the diversity of functional groups (Cadotte *et al.* 2011). In marine food webs, functionally diverse assemblages of heterotrophic bacteria, heterotrophic protists and zooplankton transfer the organic matter from primary producers to higher trophic levels (Sommer 1989). Zooplankton regulate the flow of energy and matter in the food web through several mechanisms including grazing, respiration, excretion, and as food to support higher trophic levels (Calbet & Landry 2004; Mitra & Davis 2010; Steinberg & Landry 2017). Variation in temporal abundance, feeding traits, size, phenotypic plasticity, growth rate and predation resistance all contribute to the total diversity of zooplankton functional groups in marine food webs (Petchey & Gaston 2006). A high diversity of functional groups contributes to a large variety of resource use that is crucial for the maintenance of ecosystem services under changing conditions (Cadotte *et al.* 2011). To generate accurate predictions of vulnerability and estimate the resilience of marine ecosystems, a mechanistic understanding of resource use by zooplankton is needed (Bindoff *et al.* 2019). However, most trophic studies are based on size or phylogeny, and the complexity of zooplankton interactions is rarely considered in trophic studies because of the lack of detailed information about feeding interactions in nature. Consequently, the functional diversity of the zooplankton community and their ability to exploit similar resources is typically not accurately considered (Mitra *et al.* 2014).

The diversity of zooplankton allows for maintaining the biomass of fish stocks over the seasons by a shift from a phytoplankton to detritus-based food webs at times when the biomass of phytoplankton is low or inedible (D'Alelio *et al.* 2016). While crustacean zooplankton (e.g. copepods and cladocerans) constitute the primary link between phytoplankton and planktivorous fish (Cushing 1990), microzooplankton (i.e. heterotrophic

flagellates, ciliates and rotifers) can at times dominate ocean's carbon respiration in productive coastal ecosystems(Sherr & Sherr 2002; Calbet & Landry 2004). By utilizing matter recycled by heterotrophic bacteria in the microbial loop (Azam*et al.* 1983), the microzooplankton serve as an additional link between primary producers and crustacean zooplankton (Gifford 1991). The possibility to switch between alternative food web states may be particularly critical in coastal ecosystems that experience an increase in filamentous cyanobacteria due to climate warming (Paerl & Huisman 2008; Cloern *et al.* 2016).

While most trophic studies have clustered zooplankton into broad phylogenetic groups (Mitra *et al.* 2014), recent studies suggest that models based on traits, particularly size, reflect the true ecosystem structure more effectively (Sommer & Stibor 2002; Boyce*et al.* 2015). However, none of these approaches consider the entire functional group diversity of zooplankton. As an example, the rotifer phylum contains members of different size classes (belonging to both the micro- and mesozooplankton) (Arndt 1993), as well as organisms with different feeding behaviors such as micro-filtering feeders (Pourriot 1977), selective feeders (Bogdan *et al.* 1980; Bogdan & Gilbert 1982; Gilbert & Jack 1993), and in some cases even carnivores (Gilbert 1980). Similarly, copepods and cladocerans can perform different feeding strategies ranging from feeding-current feeding to passive/active ambush feeding (Kiørboe 2011), utilizing a wide spectrum of resources.

The difficulty in resolving plankton food webs lies within method limitations. Traditional methods to study plankton food webs, such as grazing dilution techniques (Landry & Hassett 1982), biogeochemical tracers or microscopic observations (Post 2002), may not display its full complexity with enough resolution, and has created a biased knowledge towards larger organisms in the food web that are more frequently studied (Gutiérrez-Rodríguez *et al.* 2014). Molecular techniques, including DNA sequencing targeting plankton communities, has highlighted the complexity and diversity of plankton interactions on a global scale (Lima-Mendez *et al.* 2015). Further, DNA metabarcoding of gut content or selected organisms has proven to be a useful tool for resolving trophic interactions (Pompanon *et al.*2012) and for zooplankton, barcoding of whole organisms can resolve both trophic, parasitic and mutualistic interactions among crustacean zooplankton (De Corte *et al.* 2017; Zamora-Terol *et al.*2020). To our knowledge no study has so far aimed to estimate the diversity of functional groups of zooplankton spanning both phylum and size, using targeted DNA metabarcoding.

In this study, we aimed to investigate the functional group diversity of the most abundant zooplankton genera in the Baltic Sea, a temperate coastal sea with strong seasonal variability, and where both micro- and mesozooplankton are at times dominating with well-defined abundance peaks (Fig. 1). We hypothesize that diet composition between zooplankton consumers constitutes a more realistic proxy for functional diversity compared to size and phylogeny. By sequencing *18S rRNA* and*16S rRNA* barcoding genes, we analyzed zooplankton-associated prey of selected individuals of different size classes including a ciliate and rotifers, and compared them with the most abundant crustacean zooplankton (copepods and cladocerans). We demonstrate a larger functional group diversity in resource use within zooplankton in the Baltic Sea than previously acknowledged. The functional group diversity goes beyond both phylogenetic diversity and size and is crucial for the understanding of key ecological processes and maintenance of ecosystem functions.

Methods

Sampling

Zooplankton and water samples were collected at Landsort Deep monitoring station BY31(58'35, N18'14 E) located in the eastern Baltic Sea proper, which is an offshore station at the deepest location of the Baltic Sea with 495 m depth. To capture the seasonality of zooplankton (Fig. 1), samples were collected in June and August 2017, and in March 2018, synchronized with the Swedish national pelagic monitoring program (Naturvårdsverket 2009).

Water samples were collected with 10L Niskin bottles with 5 m depth intervals above the thermocline (0-30

m depth). The depths were mixed, and 1-3L were sequentially filtered onto 25 mm diameter polycarbonate filters with 0.2, 2 and 20 µm pore size. Filters were stored frozen at -80°C until further analysis. The three size fractions were sequenced separately but pooled after sequencing. Zooplankton samples were collected with vertical hauls from 0-30 m using a 90 µm-WP2 plankton net (Hydrobios, Kiel, Germany). Ciliates were sampled with a 55 µm hand-towed plankton net in the upper 10 m layer (Hydrobios, Kiel, Germany). The zooplankton and ciliate samples were immediately preserved in 95% ethanol.

Zooplankton sorting and DNA extraction

The rotifers *Synchaeta baltica, Synchaeta monopus* and*Keratella* spp.*,* cladocerans *Evadne nordmanni* and*Bosmina* spp. and the copepods *Temora longicornis, Acartia*spp.*, Pseudocalanus* spp. and *Centropages hamatus* were identified and sorted from the zooplankton samples under a stereomicroscope (400X magnification). All individual rotifers were rinsed five times in ethanol, crustaceans were rinsed five times in miliQ water, and thereafter soaked for 30 seconds in a 1% bleach solution to remove contamination of external DNA. Only individuals without visible external parasites or symbionts were used. 5-12 individuals from each species were randomly pooled into one sample tube and stored in 180µl ALT lysis buffer (Qiagen). The ciliate*Helicostomella* was transferred from the zooplankton samples onto a PET-membrane coated glass slide (Zeiss) and covered with raisin based liquid cover glass (Zeiss). Single cells of *Helicostomella* were collected using a Laser Capture Microdissection Microscope (Zeiss) and 10-15 individuals per sample pooled into 10µl ALT lysis buffer (Qiagen). All of the sorted zooplankton samples were prepared in at least five replicates, that were treated separately in all downstream analyses.

DNA from zooplankton samples was extracted using QIAamp DNA Micro Kit (Qiagen), including 1µg carrier RNA according to manufacturer's instructions for tissue samples (rotifers, cladocerans and copepods) or the instructions for laser-micro dissected samples (ciliates). Genomic DNA was extracted from the water filters using the DNeasy Plant Mini Kit (Qiagen) with an additional step of bead beating with 1mm glass beads, and an overnight incubation at 56°C with proteinase K (Qiagen).

Illumina library preparation and sequencing

Illumina sequencing library preparation was performed according to best practices described by Hu *et al.* (2016). We amplified a 400 bp long fragment of the V4 region of the *18S rRNA* gene (*18S*) in a polymerase chain reaction (PCR) using universal primers 528F (GCGGTAATTCCAGCTCCAA) and 706R (AATCCRAGAATTTCACCTCT) (Ho *et al.*2017). For prokaryotes and photoautotrophic eukaryotes, a 500 bp long fragment of the V3-V4 region of the *16S rRNA* gene (*16S*) was amplified using universal primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) (Herlemann *et al.* 2011; Hu *et al.*2016).

Each PCR reaction contained 10 µl of HiFi HotStart Ready Mix (Roche, KAPA Biosystems), 1 µl of each primer 10nM (with attached adapter sequence) (Eurofins Genomics) 2 µl of template DNA and 16µl water. Thermal cycling conditions were as follows: 98ºC initial denaturation for 2 min followed by 25 cycles of 98ºC denaturation for 20 s, 63ºC (*16S*) or 54ºC (*18S*) annealing for 20 s, 72ºC elongation for 15 s, and final extension step of 2 min at 72ºC. PCR products were cleaned using Agencourt AMPure XP magnetic beads according to the manufacturer's instructions (Beckman Coulter).

An outer PCR step followed to attach unique index sequences, to facilitate sample pooling. Reactions contained 14 µl of KAPA HiFi HotStart Ready Mix (Roche, KAPA Biosystems), 1 µl Handle1 (index_forward)-Adapter1 (10µM), 1 µl Handle2 (index_reverse)-Adapter2 (10µM) and 12 µl of cleaned PCR product. The thermocycling conditions were: 98ºC for 2 min followed by 10 cycles of 98ºC for 20 s, 62ºC for 30 s, 72ºC for 30 s, and final extension step of 2 min at 72ºC. PCR products were pooled at equimolar amounts and purified using XP magnetic beads (Agencourt AMPure XP, Beckman Coulter). DNA concentration and quality were determined using a Qbit fluorometer (Qbit dsDNA BR Assay, Thermo Fisher) and Bioanalyzer assay

(Agilent). Sequence clustering was done "onboard" and sequenced on MiSeq (MSC 2.5.0.5/RTA 1.18.54) pair-end setup (2x300 bp, version 3, Illumina) with the addition of 10% genomic PhiX.

Bioinformatics

Results were converted from Bcl to FastQ (Sanger/phred33/Illumina quality scale) using "bcl2fastq2" from the Casava software. Primers were truncated in the Cutadapt software (removing sequences without primers) (Martin 2011), and further analysis conducted in R (R Core Team 2018). Quality control and filtering, error rate modeling, sequence dereplication, ribosomal sequence variant (RSV) inference and taxonomic assignment were done using the DADA2 R package (Callahan *et al.*2016) (for detailed settings, see supplementary information). *18S*sequences were assigned to the Protist Ribosomal Reference database (Guillou *et al.* 2013) and the *16S* sequences were assigned to a custom made database combining the SILVA *16S* reference database (Pruesse *et al.* 2007) with the PhytoREF database (Decelle *et al.* 2015), in order to get an adequate taxonomic resolution for both prokaryotes and photoautotrophic eukaryotes.

Data validation

For each step in the library preparation procedure, a negative control was included. The negative controls were analyzed with Qbit and gel electrophoresis after the full library preparation and did not result in observable bands. Water samples from the sequencing analysis were compared with zooplankton and phytoplankton abundance data from the Swedish national pelagic monitoring (Naturvårdsverket 2009), available from Svenskt Havsarkiv (www.sharkdata.se). The *16S* data showed a correlation in relative abundance with phytoplankton counts (See Appendix1, Fig S1). Due to a general overrepresentation of crustacean reads in the *18S* sequences of all samples, all crustacean reads were removed from the dataset.

Data analysis and visualization

Data filtering and statistical analysis were facilitated by the Phyloseq R package (McMurdie & Holmes 2013). All sequences originating from the respective zooplankton consumer species in each sample were removed prior to data visualization. Heterogenic sequencing depth was controlled for using subsampling (rarefaction), and subsequent conversion to relative abundance. We used Schoener's Index (Formula 1) (Schoener 1968) as a measure of the percentage of dietary overlap, α , between two consumer species x and y :

$$\alpha = 1 - 0.5 \sum_{i=1}^{n} |P_{x,i} - P_{y,i}| * 100 \qquad (1)$$

where n is the number of diet groups, and$P_{x,i}$ is the proportion of diet species $i$in consumer species x . Differences in diet overlap and differences in the proportion of specific diet of consumers were modeled with one-way ANOVA on ranks, using the "kruscal.test" function from the MASS R-package. Multiple testing in pairwise analyses was controlled with Benjamin-Hochberg P-value correction in the "pairwise.wilcox.test" function. Nonmetric multidimensional scaling plots were based on Bray-Curtis distances and calculated with the "metaMDS" function in the Vegan R package (Oksanen *et al.*2007). Figures were made in the ggplot2 R package (Wickham 2016). The most important prevalent taxa (determined as taxa occupying at least 0.1 percent of the sequences in at least 70 percent of the samples in each sample group) were visualized in bipartite networks made in the Circlize R package (Gu *et al.* 2014).

Results

Diversity of biotic associations

The Illumina sequencing effort produced over 37 million sequence reads that passed quality control. The *16S rRNA* gene (*16S*) that targets bacteria and photoautotrophic eukaryotes (plastids), generated 1483 unique ribosomal sequence variants (RSVs) of which 2771 were found in the bulk water samples and 1799 found in the selective zooplankton samples. The *18S rRNA* gene (*18S*) that targets all eukaryotes, generated 1170 RSVs, of which 1078 were in the bulk water samples and 201 found in the zooplankton samples. Associated with the zooplankton organisms we found a broad range of organisms including heterotrophic and autotrophic bacteria, phytoplankton, protozoans and metazoans.

We found that on average, 90% of the *16S* sequence reads associated with the zooplankton samples were heterotrophic bacteria, which varied between zooplankton species and season (See Appendix1, Fig. S2A). Among photoautotrophic taxa (cyanobacteria and plastic-containing eukaryotes) associations of zooplankton consumer samples were dominated by cyanobacteria, green algae (Chlorophyta), diatoms (Bacillariophyta) and dinoflagellates (Dinophyceae) (Fig. 2A).

The zooplankton samples were associated with a diversity of eukaryotic organisms, based on the *18S* reads, including both photoautotrophic and heterotrophic plankton, as well as a diversity of potential symbiotic or parasitic organisms including oomycetes and dinoflagellates.

Functional diversity of zooplankton in spring

In spring, the rotifer *Synchaeta baltica* was the dominating zooplankton species in the Baltic Sea proper, accompanied by less abundant copepod species (Fig 1A). The main primary producers in March were bloom-forming dinoflagellates and diatoms, but also the photoautotrophic ciliate *Myrionecta* (Fig 1B). Diet overlap between the zooplankton species was relatively low according to the*16S* reads. The rotifer *S. baltica* had between 13-25% diet overlap with the copepod groups, while the highest diet overlap was found between the copepods *Temora* and *Pseudocalanus* (67%) (Fig. 3A). The rotifer *S. baltica* was mainly associated with the bloom-forming dinoflagellate *Peridiniella* (occupying on average 76% of the *16S* reads) (Fig. 3B). The copepods *Temora* and*Pseudocalanus* were associated with fewer sequences of*Peridiniella* , compared to the rotifer (on average 6% of*16S* reads, W=70, p=0.001), but instead associated with various groups of small phytoplankton and picocyanobacteria. *Acartia* was almost exclusively associated with filamentous cyanobacteria. The*18S* sequences support the association between *S. baltica*and *Peridiniella* but reveal also associations with the ciliate*Myrionecta* . The *18S* sequences further revealed associations between all zooplankton species and diatoms (Fig. S3).

Synchaeta baltica reached its peak abundance in the Baltic Sea towards the end of the spring, coordinated with the decline of*Peridiniella* in June (Fig 1). Diet overlap between zooplankton species became more apparent but did not cluster according to phylogenetic affiliation. In June *S. baltica* had a higher diet overlap with the copepod *Centropages* (61%) and the cladoceran*Evadne* (73%), compared to the sister species *S. monopus*(49%). Similarly, the copepod *Acartia* had a higher diet overlap with *S. monopus* (64%) than with the other copepods (only 10% overlap with *Temora*) (Fig 3A). At the end of spring, cyanobacteria became more apparent in the diet of the rotifers, indicating a transition from a spring to a summer prey community (Fig 3B).

Functional diversity of zooplankton in summer

In summer, the abundance and diversity of crustacean zooplankton increased in the Baltic Sea, and *Keratella* was the most abundant rotifer. The rotifer *Synchaeta baltica* was still present, but with low abundance (Fig. 1A). The primary production was characterized by extensive blooms of filamentous cyanobacteria (Fig. 1B). In summer, the zooplankton groups clustered into four distinct functional niches based on their diet (when a threshold level of 75% similarity was applied to the diet overlap of *16S* reads). The four defined niche clusters did not follow taxonomic affiliation but spanned over both phyla and kingdoms (Fig. 3A). The separation of the four functional groups was also supported by non-metric multidimensional scaling (Fig. S3).

The first cluster, consisting of the heterotrophic ciliate*Helicostomella* and the rotifer *Keratella,* was mostly associated with filamentous cyanobacteria (occupying on average 76% of*16S* reads) (Fig. 3B). In contrast, the second cluster, containing the larger rotifer *Synchaeta baltica* together with the cladoceran*Bosmina* and the copepod *Acartia,* was associated with a lower proportion of filamentous cyanobacteria than the first cluster (on average 39%, W=145, p=0.002), but with a larger proportion of picocyanobacteria (45%, W=3, p<0.001) as well as a diversity of small phytoplankton. Thus, diet overlap between the two rotifer species *Keratella* and *S. baltica* , was lower (48%) than the overlap both between *Keratella* and the heterotrophic ciliate*Helicostomella* (78%, χ^2=11, P=0.001), and between *S. baltica* and the copepod *Acartia* (75%, χ^2=7, P=0.007). The third cluster, consisting of the large copepods *Temora* and *Centropages* , was associated with an even lower proportion of filamentous cyanobacteria than the second cluster (9%, W=, p=) and was almost exclusively associated with a higher relative proportion of picocyanobacteria than the other clusters (75%, W=32, p=0.007). Consequently, the copepod *Acartia* had a higher diet overlap with the cladoceran *Bosmina* sp. (91%) than with the other copepods (e.g. *Temora* , 52%, χ^2=8, P=0.005). Finally, *Pseudocalanus* , clustering alone, was associated with a significant proportion of unclassified organisms (up to 35% of *16S* reads).

The *18S* sequences revealed various groups of heterotrophic flagellates associated with *S. baltica* , *Keratella* , and*Helicostomella* (Fig. 3B). Small phytoplankton (chlorophytes and eustigmatophytes), heterotrophic protozoans of different phyla, as well as metazoans, dominated the *18S* sequences of the cladocerans and copepods in summer (Fig. S3).

Discussion

In order to resolve the functional group diversity of consumers in plankton food webs, we analyzed trophic associations of several micro- and mesozooplankton species using *18S* and *16S rRNA*gene sequencing of selected zooplankton. Our results demonstrate a complexity of species interactions that are dynamic and differ both between consumer species and seasons. The results exemplify that clustering zooplankton by size or phylogeny does not capture the true differences in diet niche and leads to an underestimation of the functional group diversity of consumers in the pelagic food web.

In the Baltic Sea, rotifers have so far generally clustered together as microzooplankton (Motwani & Gorokhova 2013) and referred to as obligate filter feeders (Grinienė *et al.* 2016). Despite this, we show that the two most common rotifer genera have little diet overlap and represent distinct functional niches (Fig. 3). Being the only rotifer present over the whole year (Fig. 1), *Synchaeta baltica* has a functional niche more similar to cladocerans and copepods, than to the other rotifer species *Keratella* and *S. monopus* , a distinction already proposed by Arndt (1993). Bloom-forming phytoplankton (the dinoflagellate *Peridiniella* , with a size range of 20-35 µm, and the photoautotrophic ciliate *Myrionecta,* 45-55 µm) appears to be more abundant in the diet of *S. baltica* (c. 350 µm), compared to the surrounding water in spring (Fig. 2), suggesting a selective rather than passive feeding behavior. These results are in line with previous studies that have observed predation on large phytoplankton and protozoa up to 50 µm by *Synchaeta* (Pourriot 1977; Bogdan *et al.* 1980; Bogdan & Gilbert 1982; Gilbert & Jack 1993). This is further supported by a study from the Mediterranean Sea, where *Synchaeta* was estimated to consume up to 80% of the daily production of a dinoflagellate bloom (Calbet *et al.* 2003).

As copepods and cladocerans are temporarily decoupled from the spring bloom (Fig. 1), the rapid decline of phytoplankton at the end of the spring bloom in the Baltic Sea has been described as a result of nutrient limitation in the upper water column (Tamminen & Andersen 2007). However, as the decline of *Peridiniella* coincides with the peak of *Synchaeta* (Fig. 1), we propose that the spring bloom decline is a result of both nutrient limitation and grazing by the rotifer.

The rotifer *Keratella* peaks in abundance during the summer, when*S. baltica* is low in abundance (Fig 1). *Keratella* was mainly associated with larger filamentous cyanobacteria, thus revealing a higher diet overlap

with the tintinnid ciliate *Helicostomella*than with *S. baltica* (Fig. 3). The size of the *Keratella*(150 µm) and *Helicostomella* (100 µm) compared to cyanobacteria filaments that often exceed 1mm suggests that these consumers do not feed directly on the filamentous cyanobacteria. *Keratella* is a filter feeder (Arndt 1993) that prefers partially degraded food (detritus) over living cells (Starkweather & Bogdan 1980). Filamentous cyanobacteria likely contribute to a pool of particulate organic matter, that is both available and attractive for detritus-eating rotifers and ciliates. The detritivorous feeding niche of *Keratella* and*Helicostomella* suggested here is further supported by a relatively high proportion of associated crustacean DNA (Fig S2), which for similar reasons is unlikely to be preyed upon directly.

Our study confirms that both filamentous cyanobacteria and picocyanobacteria are important food-resources in the summer community of the Baltic Sea, although consumed by different zooplankton groups (Fig 3B). Stable isotope studies have shown that nitrogen fixed by filamentous cyanobacteria is enriched in copepods and enhances productivity in the Baltic Sea food web (Karlson *et al.* 2015), but at the same time, these filamentous cyanobacteria are described as unpalatable and not consumed by copepods (Engstrom 2000). The contradiction has been explained by incorporation of diazotrophic nitrogen by mesozooplankton through an enhanced microbial loop during summer (Uitto *et al.* 1997; Motwani & Gorokhova 2013; Wannicke*et al.* 2013; Eglite *et al.* 2018) as production of heterotrophic bacteria and flagellates increases during summer months (Bunse *et al.* 2018). This explanation fits with the copepods*Temora* and *Centropages* that in this study were almost exclusively associated with picocyanobacteria without the ability to fix nitrogen (Klawonn *et al.* 2016) (Fig 3B). Yet, the mechanism of diazotrophic nitrogen transfer in the food web remains unclear.

Given the high proportion of filamentous cyanobacteria associated with*Keratella* and *Helicostomella,* we propose that these filter-feeding detritivores have an important role in the utilization of diazotrophic nitrogen in the Baltic Sea food web. The ecosystem function of *Keratella* is demonstrated in an experiment by Arndt (1993), showing how the growth of heterotrophic flagellates in a microcosm, together with bacteria and algae, sustained in a nutrient-poor media, is facilitated by the addition of *Keratella.* Arndt proposed that*Keratella* through its feeding enhances leaking of dissolved organic matter (DOM) from the algae, thereby supporting increased biomass of both bacteria and the flagellates. Similarly, we propose that*Keratella* and *Helicostomella* through the degradation of detritus stimulates the production of heterotrophic bacteria and picocyanobacteria in the Baltic Sea. By making nitrogen fixed by filamentous cyanobacteria available as dissolved organic nitrogen, the detritivores likely support the productivity of higher trophic levels during blooms of filamentous cyanobacteria. Although mechanisms of organic matter recycling by zooplankton are highlighted in several studies (D'Alelio *et al.* 2016; Steinberg & Landry 2017), pathways of detrital degradation are seldom taken into account (Moore*et al.* 2004). We suggest that detritivores are likely to be the main link (except passive leaking) between cyanobacterial POM and DOM in the Baltic Sea (Fig 4). The recycling of POM by microzooplankton further has the potential to increase the retention time of sedimenting cyanobacteria, thereby preventing loss of organic material to the sediment and the formation of anoxic bottoms, which are strongly linked with the cyanobacteria blooms in the Baltic Sea.

Synchaeta monopus, Evadne and *Acarita* in June, and*S. baltica* , *Bosmina* and *Pseudocalanus* in August consumed a broad range of primary producers, including filamentous cyanobacteria, picocyanobacteria and other phytoplankton, as well as heterotrophic protists (Fig. 3B). This multitrophic feeding further contribute to the diversity of functional groups that is important for creating stability in the dynamic food web (De Ruiter *et al.*2005). Zooplankton studied here showed the ability to utilize resources both, directly from filamentous cyanobacteria, and from picocyanobacteria via the consumption of heterotrophic protists (Fig. 4). Diverse pathways of resource use by zooplankton balance out ecosystem effects of the increased blooms of filamentous cyanobacteria in the Baltic Sea (Fig. 4), and likely other ecosystems with high dominance of inedible phytoplankton.

By putting weight on the relative comparison between groups of samples rather than the absolute read counts, DNA metabarcoding is a feasible tool for estimating utilization of resources by zooplankton in the pelagic food web. Without the assumption of a relationship between read counts and biomass, metabarcoding allows for highlighting rudimentary differences between functional zooplankton groups. In combination with

count data validation and support from experimental studies, DNA metabarcoding has a strong potential to resolve the ecosystem function of diverse groups of zooplankton.

Our study shows that focusing on functional group diversity rather than phylogenetic diversity or size has implications on our interpretation of food web structure. The results highlight a large variation in resource use between groups of zooplankton, and reinforce the importance of functional group diversity in stabilizing energy transfer in food webs by allowing for alternative trophic pathways, particularly during seasons when primary producers include filamentous cyanobacteria. The presence of multitrophic species with the ability to prey on different components of the food web further contributes to ecosystem resilience, as well as season-dependent phenotypic plasticity that allows zooplankton populations to survive under varying resource availability. Our results emphasize the importance of understanding the diversity of resource use of key zooplankton taxa to generate accurate predictions about ecosystem functioning. Food web models based on size or phylogeny may not capture the important role of individual species and may not be detailed enough to predict energy pathways of plankton food webs, and thus the vulnerability of ecosystems to environmental change. The approach used in this study is therefore a suitable entry point to food web modeling and ecosystem network analysis.

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Competing interests

The authors declare no competing interests.

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Figure legends

Figure 1: (A) Abundance of zooplankton and **(B)**biovolume of phytoplankton at Landsort Deep in the Baltic Sea. Interpolated daily means over the years 2006-2018. The data is retrieved from the Swedish marine monitoring program (Naturvardsverket 2009). Samples are taken weekly to bi-weekly during the spring and summer period and monthly during winter.

Figure 2: Relative abundance of sequence counts per taxa of**(A)** *16S rRNA* gene reads (photoautotrophic organisms only) and **(B)** *18S rRNA* (eukaryotic organisms, excluding crustaceans and Syndiniales), for

different zooplankton consumer species and months in the Baltic Sea. The bars represent unique biological replicates.

Figure 3: (A) Heatmap of Shoener's Diet Overlap Index (%) between zooplankton species and photoautotrophic organisms in March, June and August, based on *16S rRNA* reads. Black squares indicate distinct clustering of zooplankton species in August: 1)*Helicostomella* and *Keratella* ; 2) *Synchaeta baltica* ,*Bosmina* spp., and *Acartia* spp.*; 3) Centropages hamatus* and *Temora longicornis;* and 4)*Pseudocalanus* spp. **(B)** Zooplankton consumer species (upper) with their most prevalent food taxa (lower) based on*16S rRNA* reads. The thickness of the bars is proportional to relative rRNA read abundance. The taxa shown here are present in at least 60% of the samples in at least one sample group. 1) Nostocaceae, 2) Chaetoceraceae, 3) Bacillariophyta fam., 4) Attheyaceae, 5) Peridiniaceae, 6) Monodopsidaceae, 7) Chlamydomonadales fam., 8) Cocomyxaceae ,9) Oocystaceae, 10) Ochrophyta fam., 12) Monodopsidaceae, 13) Chlorellaceae, 14) Chlorellales fam., 15) Trebouxiophyceae fam., 16) Pyramimonadaceae, 17) Dictyochophyceae fam., 18) Chrysochromulinaceae, 19) Cyanobiaceae, 20) Unknown Chlorophyta, 21) Unknown Archaeplastida, 22) Unknown Phytoplankton.

Figure 4: Functional group diversity in the Baltic Sea plankton food web during summer. The thick green line highlights a possible pathway of diazotrophic nitrogen. Filamentous cyanobacteria are degraded to smaller pieces (1) that are consumed by microzooplankton and filter feeders (2). The feeding and excretion contribute to a pool of DOM (3) that stimulates the production of hetero- and autotrophic bacteria (4) that are consumed by heterotrophic protozoa (5) and mesozooplankton (6).

Figure 1

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Figure 2

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Figure 3

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Figure 4