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Mamiellophyceae shift in seasonal predominance in the Baltic Sea

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ABSTRACT: The green algae Mamiellophyceae are a group of marine picoeukaryotes. We studied the succession of Mamiellophyceae in the Baltic Sea water column and ice from autumn to summer, using the hypervariable V4 region of the 18S ribosomal RNA gene. We show that species of Mamiellophyceae shift in seasonal predominance and that different species characterize sea ice, under-ice water and the water column in the Baltic Sea.

KEY WORDS: 18S rRNA gene · Hypervariable V4 region · Green algae · Picoeukaryotes · Succession

1. Introduction

Mamiellophyceae, a class of green algae, includes 3 described orders: Mamiellales, Dolichomastigales and Monomastigales (Marin & Melkonian 2010). Dolichomastigales and Monomastigales host many lineages, but they are not abundant in marine waters (Monier et al. 2016). In contrast, the most common Mamiellophyceae in coastal waters—species from the Mamiellales genera *Ostreococcus*, *Bathycoccus* and *Micromonas*—may contribute significantly to the primary production of picoeukaryotes (Worden et al. 2004, Tragin & Vaulot 2018). This has raised research interest in Mamiellophyceae recently, and with the help of molecular methods, the contributions and seasonal patterns of different lineages have become possible to investigate in more detail (e.g. Foulon et al. 2008, Demir-Hilton et al. 2011).

Mamiellophyceae as a class does not show preferences to environmental conditions globally (Tragin & Vaulot 2018), and Mamiellophyceae biogeography is driven largely by geographical location rather than water depth (Monier et al. 2016). For example, species of *Ostreococcus* are globally distributed but are not always found together with usually co-occurring *Bathycoccus* and *Micromonas* (Demir-Hilton et al. 2011), and *Ostreococcus* species are absent in Arctic waters (Tragin & Vaulot 2019). *Ostreococcus* is, however, present in adjacent seasonally sea ice covered areas, for example the Baltic Sea and the White Sea (Majaneva et al. 2012, Belevich et al. 2018).

At the species and genetic strain level, Mamiellophyceae occupy different ecological niches. For example, within the morphospecies *Micromonas pusilla*, different genetic lineages recently divided into several species (Simon et al. 2017) show shifts in abundance along local and basin-wide environmental gradients (Foulon et al. 2008). Similarly, *Ostreococcus* strains show differences in distribution, and co-occurrence at the same geographical location is rare

(Demir-Hilton et al. 2011). Metagenomes of the genetically less variable *Bathycoccus prasinos* indicate the same (Vannier et al. 2016).

In the Baltic Sea, species of *Monomastix*, *Dolichomastix*, *Mamiella*, *Mantoniella* and *Micromonas* are reported (Hällfors 2004, Majaneva et al. 2012). *Mantoniella* sp. appears characteristic for ice, while *B. prasinos* is characteristic for under-ice water (Majaneva et al. 2017). However, there are no studies on seasonal patterns or environmental preferences of Mamiellophyceae in the Baltic Sea. Here, we take advantage of our bi-weekly sampling of cold water season protists and describe Mamiellophyceae succession based on V4 reads of the 18S ribosomal RNA (18S rRNA) gene in Baltic Sea water and sea ice during the period from autumn to summer.

2. Materials and Methods

Samples were collected from 2 coastal brackish water (salinity 3.5–6) sites in the northwestern Gulf of Finland, Baltic Sea. Sampling was carried out from 8 October 2012 to 8 May 2013, with extra samples on 3 September 2012 and 1 July 2013. The sampling protocol, DNA extraction, PCR setup and sequencing are described in Enberg et al. (2018) and in Supplement 1 at www.int-res.com/articles/suppl/mXXXpXXX_supp/. Enberg et al. (2018) outlined the eukaryotic communities using microscopy and a 310 bp long fragment of the V4 region of the 18S rRNA gene (hereafter short V4). Here, we focus on Mamiellophyceae short V4 reads and a longer fragment of the V4 region that was amplified using 574*F and 1132R (Hugerth et al. 2014) primers (hereafter long V4). This long V4 was approximately 540 bp long. The raw reads are available at the ENA SRA repository with the study names PRJEB21047, PRJEB23628 and PRJEB25089.

The paired-end sequenced short and long V4 reads were merged using the -fastq_mergepairs command in vsearch v.2.6.2 (Rognes et al. 2016). The quality filtering using the -fastq_filter command discarded reads shorter than 320 bases (short V4) and 550 bases (long V4), reads longer than 380 bases (short V4) and 610 bases (long V4), reads with ambiguous bases and reads with over 1 maximum expected error. Primer sequences were removed using the command trim.seqs in mothur v.1.39.5 (Schloss et al. 2009). Exact duplicates were removed using the command -derep_fulllength in vsearch. Chimeric reads were searched using the command -uchime_denovo in vsearch. A read was treated as chimeric and removed if its abundance was 5 times lower than its assumed parental reads. Operational taxonomic units (OTUs) were searched using the option fastidious in swarm v.2.1.6 (Mahé et al. 2015). The abundance of each OTU in each sample was searched using the -usearch_global command (-id 0.0) in vsearch.

The OTUs were assigned taxonomically searching all OTUs first against the NCBI non-redundant nucleotide database (short V4: 9 February 2018, long V4: 10 February 2018) and second against the SILVA_132_SSURef database (Quast et al. 2013), using BLAST v.2.6.0+ (Zhang et al. 2000). All OTUs affiliated with Mamiellophyceae were selected for the subsequent analyses. Read abundance was normalized to the total number of Mamiellophyceae reads in each sample.

To place the OTUs phylogenetically, we took all available sequences of described Mamiellophyceae species together with some relevant uncultured sequences (e.g. Mamiellophyceae DSGM-81) and 6 Pyrenomonadaceae sequences as an outgroup from the SILVA and NCBI Nucleotide databases (23 January 2019). The datasets were combined and aligned with the MAFFT online service (Katoh et al. 2017), and the alignment was cut to the length of the long V4. Bayesian phylogenetic analysis was performed with MrBayes v.3.2.6 (Ronquist et al. 2012). Two independent runs with 4 Markov chains and 1500000 generations were carried out. We did not choose the model prior to the analysis but sampled across the general time-reversible model space with gamma-distributed rate variation across sites and a proportion of invariable sites. The resulting estimates (e.g. tree topology) were posterior probability weighted averages of the models. The scripts for methods are provided in Supplement 2. The reads and read abundance are provided in Table S1 in Supplement 3.

3. Results and Discussion

In the dataset, 59 short and 46 long V4 Mamiellophyceae OTUs were present, and after manually checking the alignment and combining identical short and long V4 OTUs, 59 OTUs were used in the analyses. Mamiellophyceae OTU richness was significantly lower in sea ice than in under-ice water and the water column (Kruskal-Wallis $\chi^2 = 13.7$, $p = 0.003$, followed by pairwise comparisons using the Mann-Whitney *U*-test). Mamiellophyceae contributed 3.3% of the total abundance of taxonomically assigned OTUs, and they were present in all samples. The lowest Mamiellophyceae read abundance was in the Krogarviken April ice sample (<0.1% of total abundance), and the highest contribution was in the Storfjärden March bottom water sample (17% of total abundance). Mamiellophyceae read abundance was significantly lower in sea ice and under-ice water than in the water column (Kruskal-Wallis $\chi^2 = 26.3$, $p < 0.001$, followed by pairwise comparisons using the Mann-Whitney *U*-test).

The Bayesian phylogenetic tree (Fig. 1) resembled the latest phylogenies of Mamiellophyceae (Marin & Melkonian 2010, Monier et al. 2016, Simon et al. 2017, Yau et al. unpubl.; preprint doi:10.1101/506915), and the OTUs represented all 3 described orders with 35 Mamiellales OTUs, 23 Dolichomastigales OTUs and 1 Monomastigales OTU (Fig. 1). OTUs that referred to the genus *Mamiella* were not found. In accordance with an earlier global study (Monier et al. 2016), Dolichomastigales were diverse but rare in the Baltic Sea, and the abundant OTUs (>100 reads in total, 22 OTUs) were mainly from Mamiellales. The abundant OTUs were associated with *Bathycoccus* (86923 reads in total, 3 OTUs), *Ostreococcus* (59313 reads, 3 OTUs), *Micromonas* (36640 reads, 4 OTUs) and *Mantoniella* (25982 reads, 7 OTUs). The rest of the abundant OTUs were affiliated with DSGM-81 Mamiellophyceae (1147 reads, 3 OTUs) and with *Crustomastix* (362 reads, 2 OTUs) from Dolichomastigales.

Different Mamiellophyceae OTUs characterized sea ice, under-ice water and the water column (generalized discriminant analysis of 4 first principal coordinates analysis axes, analysis based on ln[x + 1] transformed Bray-Curtis dissimilarities, squared correlations 0.80 and 0.58, $p < 0.001$, misclassification error 43.9%).

The Mamiellophyceae OTU associated with an uncultured Baltic Sea ice clone FN690723 characterized sea ice (Fig. 2a,d, Otu07). This species is clearly sea ice associated, and it is present in the sea ice of the Gulf of Bothnia and the Gulf of Finland in the Baltic Sea (Majaneva et al. 2012) and in the White Sea (Belevich et al. 2018). Otu07 and the uncultured Baltic Sea ice clones form a clade of their own, basal to the *Mantoniella* and *Micromonas* clade in our Bayesian phylogenetic tree (Fig. 1) but which are classified as *Mantoniella* in Tragin & Vaulot (2019). *Mantoniella squamata* (Otu14) was the most abundant *Mantoniella* species in the water column in September and October, as was *M. beaufortii* (Otu04) in November to May and OTUs close to *M. antarctica* (Otu21, Otu22) in July (Fig. 3c). *M. beaufortii* and several *Crustomastix* OTUs characterized under-ice water. The presence of freshwater *Crustomastix* species in low abundance in under-ice water is an indication of river discharge under ice in the area (Kaartokallio et al. 2007).

The abundant *Bathycoccus*, *Ostreococcus* and *Micromonas* species characterized the water column but shifted in predominance during the sampling season (Fig. 2c,f,g). *Ostreococcus mediterraneus* (Otu02) was the most abundant species in October to December, although *Bathycoccus* and *Micromonas* species had a relatively high abundance as well. Otu01, closely related to *Bathycoccus prasinos*, predominated distinctly in January to April, *Micromonas polaris* (Otu06) in May and *M. commoda* (Otu03) in July (Fig. 2c,f).

The 4 species of *Micromonas* alternated in dominance in the water column: the predominant species was *M. pusilla* (Otu09) in October surface water and *M. bravo* (Otu10) in bottom water, *M. polaris* (Otu06) in January to May and *M. commoda* (Otu03) in summer and early autumn water (Fig. 3a,b). These shifts in predominance are in line with the thermal niches of *Micromonas* species (Demory et al. 2019).

The 2 abundant *Ostreococcus* OTUs—*O. mediterraneus* (Otu02) and the Otu05 basal to the clade of *O. tauri* and *O. lucimarinus*—co-occurred in autumn, and both OTUs were practically absent after March (Fig. 2). The co-occurrence is in accordance with the earlier findings of co-occurring coastal *Ostreococcus* strains (Demir-Hilton et al. 2011) and the absence of *Ostreococcus* in cold polar waters (Tragin & Vaulot 2019). *B. prasinos* had a similar occurrence in the Baltic Sea, being present and abundant only in the autumn (e.g. Fig. 2c). The 2 *Ostreococcus* OTUs and *B. prasinos* appear to be characteristic species of the autumn water community (Enberg et al. 2018).

The most abundant OTU in our dataset (Otu01, 1 base difference to clone FN690721 from Majaneva et al. 2012) was present in all samples. It is closely related to *B. prasinos* (Fig. 1), which is the only described species within the genus currently and whose cultures show identical 18S rRNA gene sequences but differing genomes (Vannier et al. 2016, Tragin & Vaulot 2019). It is beyond this note, but the uncorrected interspecific 18S rRNA sequence divergence within the other Mamiellales genera is 1.1 to 5.6%, and the 1.1% uncorrected interspecific divergence of Otu01 and *B. prasinos* implies that Otu01 could be an uncultured, undescribed species of *Bathycoccus*.

Here, we have shown that species of Mamiellophyceae shift in seasonal predominance in a coastal brackish water locality in the Baltic Sea and that different species characterize sea ice, under-ice water and the water column during the sea ice covered season. Our findings support the earlier research showing geographic niche partition within Mamiellophyceae (Foulon et al. 2008, Demir-Hilton et al. 2011, Monier et al. 2016) and show that the niche partition holds also for populations in the water and sea ice of the Baltic Sea.

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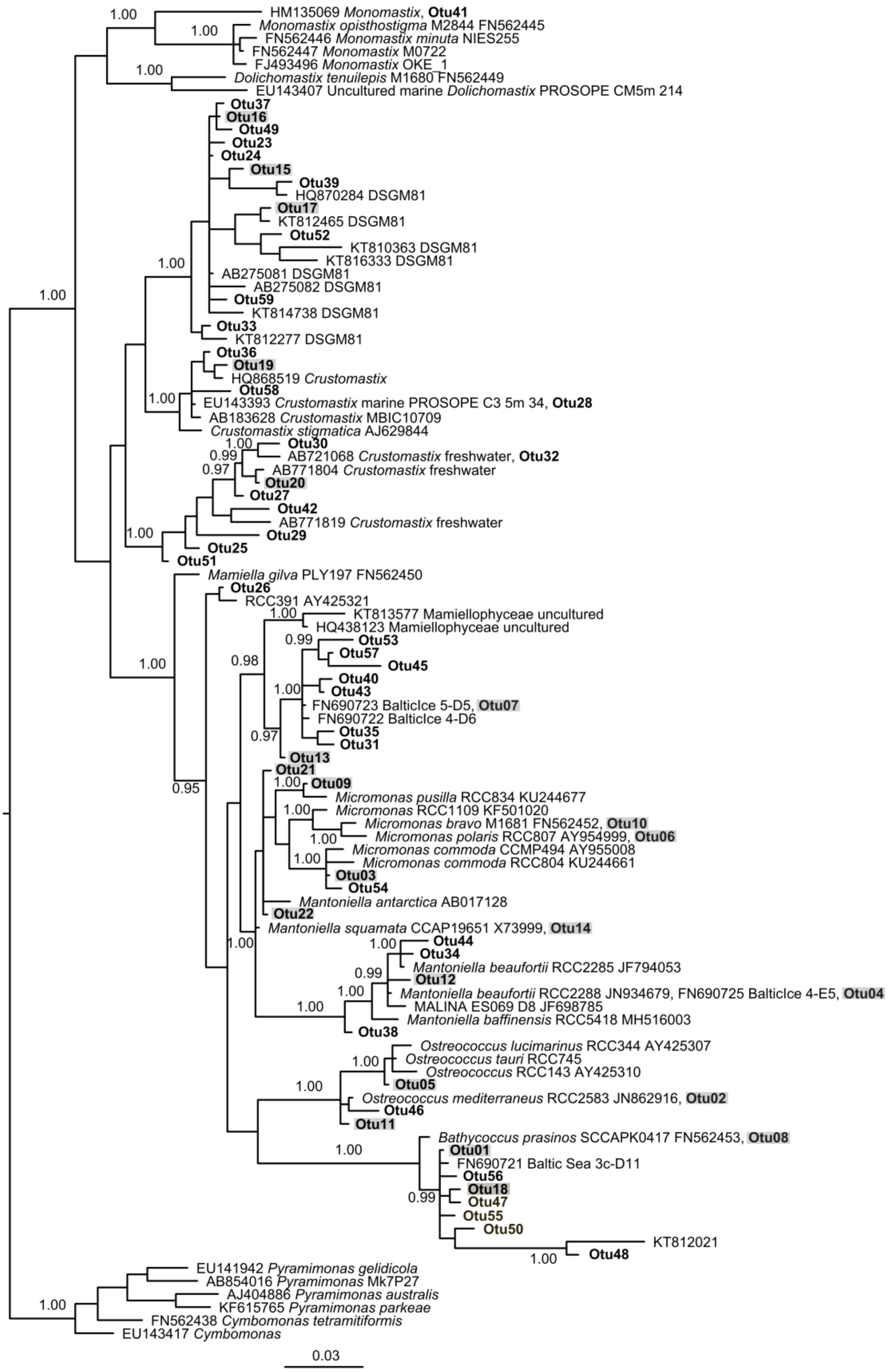

Fig. 1. Bayesian phylogenetic tree of Mamiellophyceae. Posterior probabilities >0.95 are shown on the branches. The operational taxonomic units (OTUs) of the current research are named Otu01, Otu02, etc., in decreasing order by their abundance in the total dataset, and are in **bold**. OTUs with >100 reads in total have a grey background

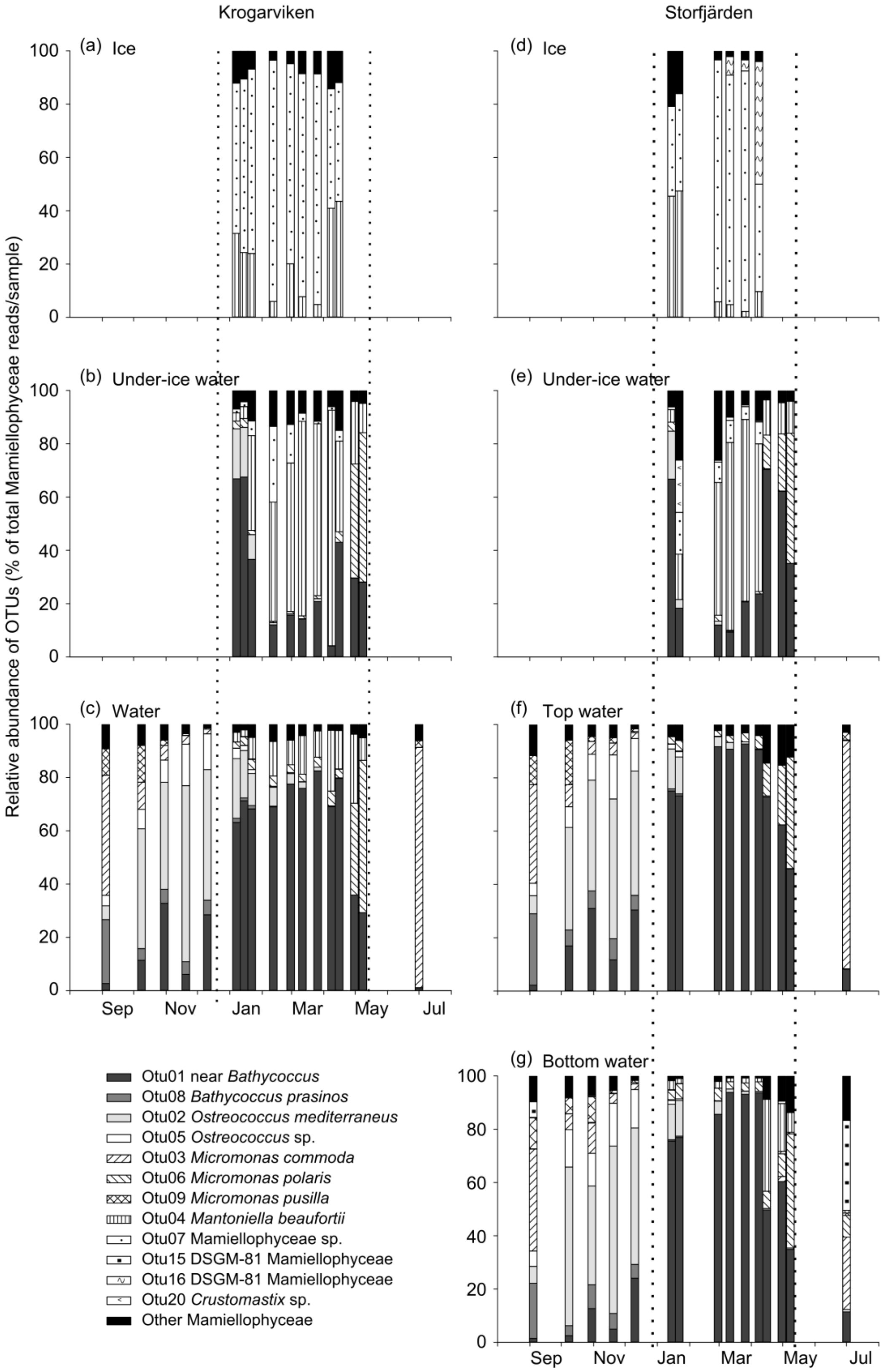

Fig. 2. Relative read abundance of the most abundant Mamiellophyceae operational taxonomic units in sea ice, under-ice water and in the water column at Krogarviken and Storfjärden throughout the sampling season. The water depth was 3 m at Krogarviken and ca. 30 m at Storfjärden. The top water was 0 to 15 m and the bottom water 15 to 30 m at Storfjärden. The time between dotted lines represents the ice-covered season

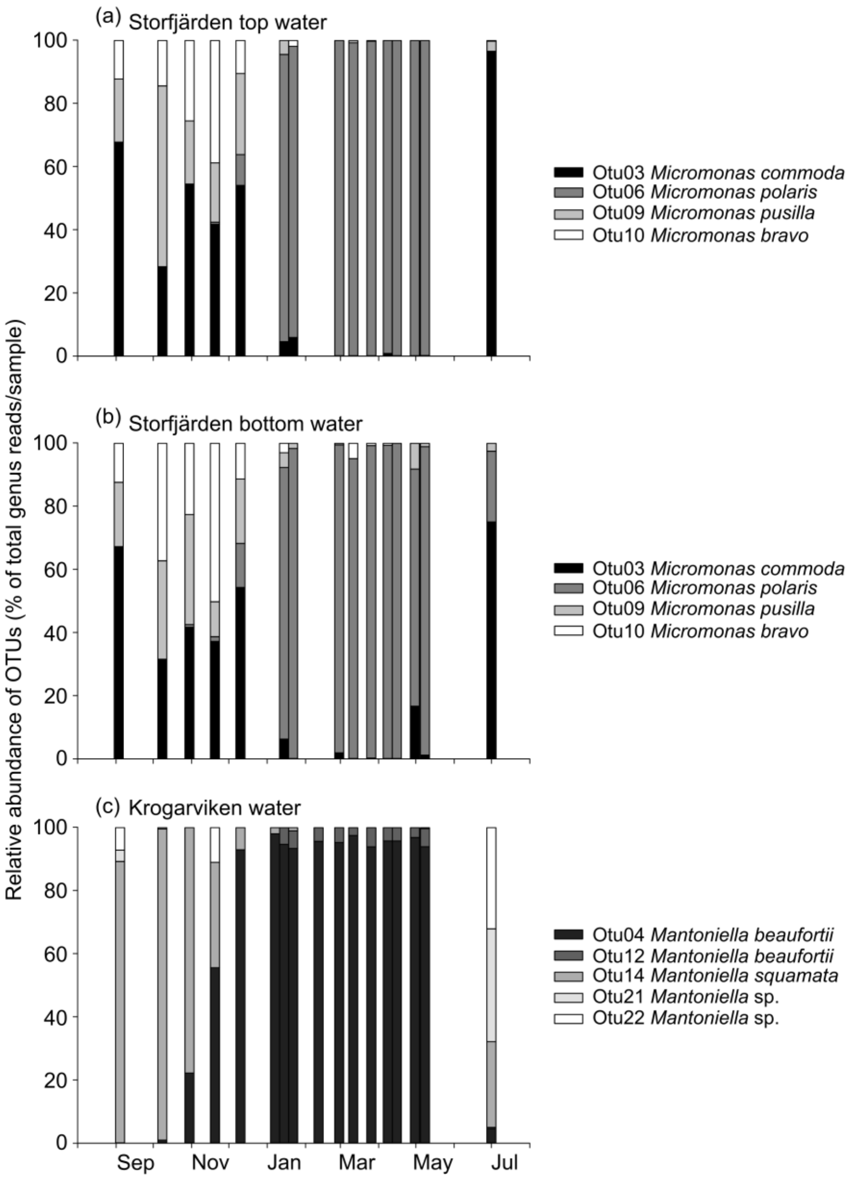

Fig. 3. Relative read abundance of *Micromonas* and *Mantoniella* operational taxonomic units in the water column