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2 Metabarcoding successfully tracks temporal changes in eukaryotic communities in coastal
3 sediments

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34 **Abstract**

35 Metabarcoding is a method that combines high-throughput DNA sequencing and DNA based identification.

36 Previously, this method has been successfully used to target spatial variation of eukaryote communities in

37 marine sediments, however, the temporal changes in these communities remain understudied. Here, we

38 follow the temporal changes of the eukaryote communities in Baltic Sea surface sediments collected from

39 two coastal localities during three seasons of two consecutive years. Our study reveals that the structure of

40 the sediment eukaryotic ecosystem was primarily driven by annual and seasonal changes in prevailing

41 environmental conditions, whereas spatial variation was a less significant factor in explaining the variance in

42 eukaryotic communities over time. Therefore, our data suggests that shifts in regional climate regime or

43 large-scale changes in the environment are the overdriving factors in shaping the coastal eukaryotic sediment

44 ecosystems rather than small-scale changes in local environmental conditions or heterogeneity in ecosystem

45 structure. More studies targeting temporal changes are needed to further understand the long-term trends in

46 ecosystem stability and response to climate change. Furthermore, this work contributes to the recent efforts

47 in developing metabarcoding applications for environmental biomonitoring, proving a comprehensive option

48 for traditional monitoring approaches.

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50 **Introduction**

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52 Metabarcoding has expanded our knowledge of the eukaryote community composition and diversity across

53 marine habitats (e.g. Park et al. 2008, Massana et al. 2015, Forster et al. 2016). However, in general the

54 benthic realm has received much less attention than the marine pelagic environments, even though the

55 eukaryotes in sediments form complex and diverse assemblages (Bik et al. 2011, Forster et al. 2016, Kim et

56 al. 2016) and respond to environmental change (e.g. Chariton et al. 2015, Zhang et al. 2018). In addition, the

57 existing metabarcoding studies have focused more on the spatial diversity of benthic eukaryotes (e.g. Bik et

58 al. 2011, Aylagas et al. 2016, Brannock et al. 2018) and so far metabarcoding application to track temporal

59 changes in sediment eukaryote communities remains understudied, yet it could provide useful data for

60 biomonitoring and environmental assessment applications.

61 Traditionally benthic biomonitoring has been based on morphological assessment of macrofauna (≥ 0.5 mm

62 fraction), and many of the existing biodiversity indices used in biomonitoring are based on macrobenthos

63 (e.g. Diaz et al. 2004). Including meiofauna (e.g. small metazoans) would increase the accuracy of

64 monitoring, due to its high diversity and the fast response to anthropogenic impacts (Kennedy and Jacoby,

65 1999). However, such an approach is often neglected due to a number of practical reasons. For example,

66 morphological environmental biomonitoring is already time-consuming, expensive and requires skilled

67 taxonomic expertise. Inclusion of traditional meiobenthos approach to monitoring practices would make the

68 work even more laborious and costly. Metabarcoding, however, has the advantage of being able to target

69 macrofauna along with smaller eukaryotes (< 0.5 mm), as well as being cost-efficient, time-saving and

70 readily applicable (Aylagas et al. 2018). Furthermore, recent studies suggest that metabarcoding can perform

71 well as an environmental assessment tool (Lejzerowicz et al. 2015, Piredda et al. 2016, Aylagas et al. 2016,

72 Lanzén et al. 2016, Aylagas et al. 2018) and it has been successfully applied to identify sediment eukaryote

73 composition in a wide range of marine environments, such as the Norwegian continental shelf (Lanzén et

74 al.2016), sandy beaches at the coast of China and USA (Zhang et al.2018) and marine sediments from

75 shallow to deep waters in the Atlantic and the Pacific (Bik et al. 2011).

76 The use of metabarcoding in biomonitoring may be especially useful in environments, such as the Western

77 Gulf of Finland, Baltic Sea, which are characterized by natural low biodiversity due to brackish waters (e.g.

78 Bonsdorff, 2006, Ininbergs et al. 2015). The macrofaunal assemblages of the Baltic Sea have been

79 intensively studied morphologically, whereas the general eukaryote diversity and community composition in

80 sediments via metabarcoding has to our knowledge received no attention to date. In the Baltic Sea water

81 column, however, Hu et al. (2016) showed that metabarcoding can be used to track spatial changes in

82 eukaryote communities across salinity gradients, and even detect taxonomic groups previously un-observed

83 in the Baltic Sea. In addition, sediment bacterial communities in the Baltic Sea have been shown to vary

84 seasonally and annually (Vetterli et al. 2015). Yet, when it comes to eukaryote communities in sediments,

85 the potential of metabarcoding remains until now unexplored.

86 One of the crucial steps in metabarcoding studies is the choice of the targeted region, since this may affect

87 the community composition obtained (Dunthorn et al. 2012, Aylagas et al.2016, Giner et al.2016, Piredda et

88   al. 2016, Tragin et al. 2018). In the 18S rRNA gene, the short (around 150 bp) hypervariable region V9 is

89   one of the most commonly targeted and thus well-represented in reference databases (Amaral-Zetterel et al.

90   2009). Improvements in high-throughput sequencing technologies allow for bigger amplicon sizes, thus the

91   use of the longer V4 region of 18 S rRNA gene is continuously increasing (van Dijk et al. 2014).

92   Metabarcoding studies comparing both of these regions have been conducted (e.g. Dunthorn et al. 2012,

93   Piredda et al. 2016, Giner et al. 2016, Tragin et al. 2018) but, so far, a consensus of the most suitable 18S

94   hypervariable region remains a matter of debate.

95   In this study, a metabarcoding approach, targeting both the V4 and V9 hypervariable regions of the 18S

96   rRNA gene, is used to investigate the eukaryote communities in two localities from the Western Gulf of

97   Finland, Baltic Sea, coastal sediments over a period of two years. The aim of the study is to identify temporal

98   changes at these two localities and evaluate the overdriving factors in shaping the sediment eukaryotic

99   communities through time. In addition, we demonstrate the potential use of the metabarcoding approach for

100   environmental assessment in a coastal settings.

101

102   **Materials and methods**

103   *Study site and sampling*

104   The samples were collected from two sites, located less than one kilometer apart from each other near

105   Tvärminne Zoological Station, on the Finnish coast of the Gulf of Finland, the Baltic Sea (Vetterli et al.

106   2015). Storfjärden sampling site is a muddy accumulation basin (59˚51.310' N, 23˚18.810' E) with a depth

107   of 33m. In contrast, Muncken is a sandier, shallow (11 m) transportation channel (59˚51.140' N, 23˚14.700'

108   E). The samples were collected three times a year during two consecutive years, in 2008 (April, August and

109   November) and 2009 (April, August and December). From here on, we refer to the sampling seasons as

110   spring, summer and winter. Intact, undisturbed sediment cores were retrieved with a Gemax twin corer.

111   Surface sediment (0.0-0.5 cm) was collected for molecular analysis and immediately after sampling frozen at

112   -70˚C. Several replicate cores were taken at each sampling moment. Salinity and temperature were measured

113   using a conductivity, temperature and depth device (CTD). In addition to molecular samples, surface

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sediments were sampled for organic matter content and bottom water samples were collected from 5 cm

above the sediment surface for measurements of dissolved oxygen, ammonium and nitrate. Sedimentary

organic matter content was measured as loss on ignition (LOI) and diffusive oxygen utilization (DOU) in

bottom waters was inferred from triplicate oxygen micro sensor profiles (Jäntti et al. 2011, Vetterli et

al.2015). For additional details on the sampling protocols and site descriptions, see Jäntti et al. 2011 and

Vetterli et al. 2015.

DNA extraction and amplification

DNA was extracted from 0.25 g of sediment with the MoBio Powerkit for soil (MoBio, Carlsbad, Germany)

according to the manufacturer's instructions. Three replicate DNA extractions were done for each sample

moment, all of which were taken from different replicate cores, with the exception of summer 2008 sample

from Storfjärden in both datasets and winter 2009 sample from Storfjärden in the V4 dataset, which had only

2 replicates available.

Two sets of primers were used for DNA amplification, targeting either the V4 (Comeau et al. 2011 and Hugerth

et al. 2014) or the V9 (Amaral-Zettler et al.2009) region of the 18S rRNA gene (Supplementary information

Table S1). Both forward and reverse primers were modified at the 5' end to include overhang sequences for

the downstream sequencing. DNA was amplified with Polymerase Chain Reaction (PCR) using Phusion

Mastermix (ThermoFisher) according to manufacturer's instructions. Duplicate PCR products in equal

volumes were pooled after amplification and quality-checked with agarose gel electrophoresis.

Negative controls were made for the extraction kit used (to test for contamination in the kit reagents) and for

all the PCR reactions (to test the contamination in the PCR master mix). All negative controls except one in

the V9 dataset did not show a product on agarose gel electrophoresis. The negative control that was visible in

gel electrophoresis was subsequently sent for sequencing and analyzed. It contained 110 reads assembled in

33 OTUs (< 0.1 % of reads in an average sample in the V9 dataset). The OTUs of the negative control were

removed from the final V9 dataset. Furthermore, negative controls were made during the PCR purifications

and attachment of barcodes during the MiSeq library preparations. The V9 sequencing control consisted of 3

139 OTUs with > 100 reads which were present in low numbers in the actual samples, and they were removed from

140 the dataset.

Sequencing and sequence analysis

142 Samples were sequenced in the Laboratory of DNA sequencing and Genomics in the Institute of

143 Biotechnology at the University of Helsinki (http://www.biocenter.helsinki.fi/bi/dnagen/index.htm). PCR

144 products were purified prior to sequencing and custom barcodes for later sample de-multiplexing were

145 attached in a second PCR reaction. Samples were sequenced on the Illumina MiSeq platform. Raw reads

146 were grouped into samples and primers, MiSeq overhangs and barcode sequences removed. Sequences were

147 assembled to paired-end reads and quality-filtered in Mothur version 1.36.1 (Schloss et al. 2009). Maximum

148 length was set to 349 and 150 base pairs (bp) in the V4 and V9 datasets, respectively. No ambiguous

149 sequences were allowed and maximum number of homopolymers was set to 8. Quality filtered reads were

150 aligned against the SILVA database (release 132) and chimeric sequences were removed in Mothur with the

151 UCHIME tool (version 4.2.40, Edgar et al. 2011). Operational taxonomic units (OTUs) were created using

152 95% similarity as threshold (as suggested by Caron et al. 2009). Taxonomic classification of OTUs was

153 performed in Mothur against the SILVA database. The distance matrix created at the OTU generation stage

154 was used to define representative sequences for each OTU, by selecting the reads with the smallest

155 maximum distance to other sequences. In case of a tie, the read with the smallest average distance was

156 selected. Sequence data is available in the NCBI Sequence Read Archive

157 (https://www.ncbi.nlm.nih.gov/sra/docs/) under BioProject accession number PRJNA459491.

158 Before further analysis, we removed taxa (OTUs) likely to create noise to our dataset, e.g. OTUs that are

159 unlikely to provide any useful information because they are very rare in our samples or they contain only a

160 small amount of reads (singletons, doubletons etc.). Additionally, we reasoned that the presence of OTUs

161 with low amounts of reads may be the result of the clustering process rather than the presence of real

162 unique/rare taxa, thus leading to overestimates of the community diversity. In order to determine a filtering

163 threshold, the total counts (total number of observations of an OTU across all samples) in both datasets were

164 calculated. This indicated that many of the OTUs obtained contain only a small amount of the total reads,

165 whereas the majority of reads are spread across a few OTUs. Subsequently, the cumulative sum of OTUs that

166 would be filtered was plotted against the total counts (Supplementary information Fig. S1), which plateaus at

167 about total counts 50 in both datasets. In order to be as inclusive as possible, a lower filtering threshold of

168 >24 reads per OTUs was applied for both datasets. This means that by excluding OTUs observed less than 25

169 times across our samples, we excluded 2 872 OTUs in the V4 dataset and 5 148 in the V9, consisting only of

170 < 1% of the total reads.

171 *Statistical analysis*

172 Statistical analysis was conducted in R (version 3.4.2, 2017-09-28). Alpha diversity and rarefaction analysis

173 were performed using the package Vegan (version 2.4-5, Oksanen et al. 2017). Principal coordinate analysis

174 (PCoA) and non-metric multidimensional scaling analysis (NMDS) were computed with the package

175 Phyloseq (version 1.22.3, McMurdie and Holmes, 2013). The analysis was based on the weighted Unifrac

176 metric with Bray-Curtis distance, including a phylogenetic tree built in Mothur. Canonical correspondence

177 analysis (CCA) was conducted using the Phyloseq package and including the variables of year, season and

178 site and the environmental parameters, including bottom water NH_4^+, salinity and temperature, diffusive

179 oxygen utilization (DOU) and sediment organic matter content based on loss on ignition (LOI). Significance

180 of these variables was determined using the ANOVA function in Vegan (Oksanen et al. 2017).

181

182 **Results**

183 The number of total sequence reads before / after quality filtering was 11 237 993 / 7 708 041 in the V4

184 dataset, and 6 409 150 / 4 169 688 in the V9 dataset. UCHIME (Edgar et al. 2011) removed 3.4 % of the V4

185 and 0.03% of the V9 sequences. Clustering at 95% similarity produced 3717 operational taxonomic units

186 (OTUs) using the V4 region and 5 194 OTUs using the V9 region. After OTUs with <25 reads were removed

187 (Supplementary information Fig. S1), the V4 dataset contained 885 OTUs (retaining 99.7% of total reads in

188 the dataset) and the V9 dataset contained 613 OTUs (retaining 99.7% of total reads in the dataset).

189 *Community structure and diversity*

190   The majority of samples the in the V9 dataset reached a satisfactory sequencing depth, as indicated by the

191   leveling rarefaction curves (Fig. 1). In the V4 dataset, 7 replicates (all from year 2009) were relatively low in

192   the number of reads (Fig. 1). Some variation was also seen in the distribution of these replicates in non-

193   metric multidimensional scaling (NMDS) analysis of the V4 dataset, which shows that the replicates from

194   2008 generally plot closer to one another than the replicates from 2009 (Supplementary information, Fig.

195   S3a). However, replicate was found to be significant parameter for all samples (PERMANOVA, p=0.001),

196   even when V4 samples from the year 2009 were analyzed separately (PERMANOVA, p=0.049). Therefore,

197   all replicates were included in the subsequent analysis.

198   The V4 region targeted 60 eukaryote classes, and the V9 region 68 (Supplementary information Table S3).

199   The most dominant class in all samples was on average Dinophyceae (70% V4, 42.6% V9), of which the

200   genus *Biecheleria* accounted for 87.5% in the V4 dataset and 74.4% in the V9 dataset (Supplementary

201   information Fig. S2). Other relatively abundant classes were Maxillopoda (average 12.9% V4, 31.6% V9),

202   and Diatomea (average 6.4% V4, 17.9% V9) (Fig. 2). In the year 2008, Dinophyceae had 89.5% relative

203   abundance on average across all seasons and both sites in the V4 dataset (Fig. 2a), and 67.9% in the V9

204   dataset (Fig. 2b). Diatomea was the second most abundant class (average 3% in V4 dataset (Fig. 2a), and

205   16.7% in V9 dataset (Fig. 2b). In the year 2009, based on V4 region, the class Dinophyceae was still the

206   relatively most abundant class in Muncken and Storfjärden (48.1% average of all samples, Fig. 2a).

207   However, based on the V9 region (Fig. 2b), the most relatively abundant class was Maxillopoda, with the

208   average of 57.7% in all 2009 samples. Other differences between the primers observed at the class level were

209   noticed in classes, such as Ostracoda, Perkinsidae and Ulvophyceae (Fig. 2a), which were more clearly

210   targeted by V4 region and only observed in low (<1%) relative abundance in the V9 dataset. In V9 dataset,

211   fungal class Agaricomycetes and metazoan class Chromadorea were more abundant than in the V4 dataset

212   (Fig. 2b).

213   Altogether 101 eukaryote orders were found using the V4 region, and 112 using the V9 region

214   (Supplementary information Table S3). The most relatively abundant order was Gymnodiniphycidae, which

215   accounted for 61.8% of the V4 and 32.6% of the V9 dataset. Gymodiniphycidae was particularly common in

216   the year 2008 (81.4% V4, 53.1% V9, Fig. 2c-d), with the greatest relative abundance in winter (84.6% in V4

dataset (Fig. 2c) and 66.4 % in V9 dataset (Fig. 2d), average of both sites). At order-level, V9 region was

able to identify three dominant orders in the class Maxillopoda, namely Harpacticoida (25.5% average in all

samples, Fig. 2d), Calanoida (5.7%) and Cyclopoida (0.5%), whereas the V4 region could not resolve the

lower taxonomic levels of Maxillopoda (mentioned as "unclassified Maxillopoda", Fig. 2c). In total 4.9% of

all reads were classified as "unclassified eukaryotes" when using V4 region. Using V9 region none of the

reads were classified as "unclassified".

Overall the Shannon diversity index (H') and Species richness estimate (S') were higher in average of all

samples in the V9 dataset than in the V4 (Fig. 3). The general tendency in both of these diversity indices

suggests that the average diversity was overall higher in summer (median H': 1.3 V4, 2.2 V9; S': 200 V4,

315 V9) than in winter (median H': 1.1 V4, 1.4 V9; S': 194 V4, 280 V9) or in spring (median H': 1.2 V4,

1.6 V9; S': 199 V4, 271 V9). In V4 dataset exceptions to the medians were seen in 2008, where in

Storfjärden both S' and H' indices were higher in spring and winter in Storfjärden, and in Muncken S' index

was highest in winter. In the V9 dataset the S' index of the year 2008 was also higher in winter and spring

than in summer at site Storfjörden (Fig. 3). Pielou's evenness values in the V9 dataset were also greater in

summer compared with other seasons (median 0.4) with highest values recorded in Storfjärden in summer

2009 (0.50), but in the V4 dataset the median for all season was the same (0.2) (Fig. 3).

Community response to temporal environmental changes

Principal coordinate analysis (PCoA) based on Unifrac metric revealed that most of the observed community

variance, in both the V4 and V9 datasets, can be explained by the year (Fig. 4). Differences between the two

years are mainly highlighted by the separation of 2008 and 2009 samples on the first axis, which explained

69.3% of the variance in the V4 dataset and 70.8% of the variance and in the V9 dataset (Fig. 4). The second

axis of the PCoA plot appears to depict seasonal changes with summer samples clustering separately from

winter samples, explaining 9% of the variance in the V4 dataset (Fig. 4a) and 10.7% of the variance in the

V9 dataset (Fig. 4b).

The influence of temporal variation on eukaryotic sediment community was confirmed with canonical

correspondence analysis (CCA), where 51.6% of the total observed community variance was explained by

243   constrained variables in the V4 dataset and 72.3% of the variance in the V9 dataset, respectively (Fig. 5).

244   The most significant factor for the V4 dataset was year ($p < 0.001$) followed by season ($p < 0.004$). For the

245   V9 dataset, the most significant factors were year, season, diffusive oxygen utilization (DOU) and loss on

246   ignition (LOI, reflecting sediment organic matter content) ($p < 0.001$ for all), followed by site ($p < 0.002$).

247

248   **Discussion**

249   *Temporal and environmental impact on eukaryote communities in sediments*

250   Our data shows that in this study the driving factor for the observed eukaryote community variance was time,

251   firstly the sampling year, followed by the season (Figs. 3, 4). The difference between the sampling years was

252   observed as a change from a strongly phytoplankton (mainly dinoflagellate, class Dinophyceae) dominated

253   community in 2008 to a more metazoan (class Maxillopoda) dominated community in 2009 (Fig. 2).

254   However, this trend appears to be more evident in the V9 dataset then in the V4 dataset, which may be

255   related to differences in the ability of the two regions to target various eukaryote groups (e.g. Giner et al.

256   2016, Piredda et al. 2016).

257   The temporal changes in the sediment eukaryote communities may be related to changes in prevailing

258   weather/climatic conditions in the study region. Based on available Baltic Marine Environment Protection

259   Commission – Helsinki Commission (HELCOM) monitoring data, the winter of 2007-2008 was warm and

260   the ice cover season at the Baltic Sea started late and was exceptionally short (Vainio, 2008). By contrast, the

261   ice season of 2008-2009 was colder (Finnish Meteorological Institute, http://en.ilmatieteenlaitos.fi/ice-

262   winter-2008-2009). The ice-free conditions at the Gulf of Finland affected the community composition and

263   the phytoplankton bloom biomass, which was higher-than-average especially in July in year 2008, whereas

264   in 2009 the phytoplankton bloom was close to long-term average (Kaitala and Hällfors, 2008, 2009). The

265   increase in phytoplankton blooms and subsequently the dominance of Dinophyceae in our 2008 dataset is

266   possibly related to the warmer than average winter of 2007-2008, and the greater relative abundance of other

267   classes, such as Maxillopoda, due to the smaller phytoplankton bloom in 2009. Because the observed

268   temporal changes are linked to larger-scale phenomena, the impact is seen equally prominent at both study

269   sites.

270   In addition to annual changes, season was also a significant factor in explaining community variance in our

271   dataset (Figs 3, 4). As seasonal changes equally affect both sampling sites, this further supports the idea that

272   the large-scale temporal changes, rather spatial heterogeneity are the overdriving factors in shaping the

273   sediment ecosystem structure. The seasonal changes were clearly captured in the relative abundance of

274   different phytoplankton classes. The peak abundance of class Diatomea was typically observed in the spring

275   in both years (Fig. 2), while Dinophyceae was found throughout the year 2008 and mainly during the

276   summer in 2009. This is in accordance with the HELCOM monitoring data, which shows that the spring

277   bloom succession is first dominated by diatoms followed by dinoflagellates (Kaitala and Hällfors 2008,

278   2009). Other seasonal differences were seen in the species diversity and richness, which were generally

279   higher in summer than in spring and winter (Fig. 3). This is likely to be related to optimum environmental

280   conditions, including high temperature and nutrient and organic matter availability (measured as LOI), yet

281   with sufficient oxygen availability in bottom waters to sustain diverse eukaryotic communities

282   (Supplementary information Table S2). Diversity was exceptionally high in the summer of 2009 at the site

283   Storfjärden, which is also seen in our CCA analyses (Fig. 5) as these samples are clearly separated from the

284   others. The diversity here may have been additionally influenced by an upwelling event, which occurred just

285   before the summer sampling of 2009, and was recorded in the CTD data showing more than 10 degrees

286   lower temperatures and increased salinity compared to sampling in 2008 (Vetterli et al.2015)

287   (Supplementary information Table S2). The community structure in V9 dataset was also significantly

288   influenced by the environmental parameters, DOU and LOI (Fig. 5). Similarly, Vetterli et al. (2015) showed

289   that the bacterial communities, which were sampled simultaneously at the same sites, showed a comparable

290   response to these same parameters.

291   The effect of seasonal and annual variations on sediment eukaryotic communities is generally understudied

292   in comparison to spatial distribution and heterogeneity. However, as our findings demonstrate, the sediment

293   eukaryote community structure is heavily dependent on sampling time and relatively large changes in the

294   community structure may take place in response to changes in prevailing climatic conditions, i.e. temperature

and ice volume, even within a period of few years. In contrast, the spatial heterogeneity and variation in the community composition was less evident in our dataset and only visible in the V9 dataset (Fig. 5). Therefore, our results support the importance of temporal surveys, contributing to our understanding of prominent environmental changes in any given environment and allowing us to untangle a potential anthropogenic signal from more naturally occurring events. In addition, climatic and anthropogenic factors are known to cause environmental stress that has been documented to manifest as regime shifts in marine environments across the globe (DeYoung et al. 2008). To distinguish these shifts and evaluate their persistence in an environment, long-term temporal surveys are crucially needed.

Metabarcoding approach for environmental biomonitoring: advantages and recommendations

Our results support recent efforts in research (e.g. Chariton et al. 2015, Lejzerowicz et al. 2015, Aylagas et al. 2018), stating that metabarcoding has various assets compared to traditional morphology-based biomonitoring. Firstly, our datasets were dominated by meio- and microfauna, and inclusion of smaller size fraction of eukaryotes typically neglected in biomonitoring surveys increases the potential of detecting the temporal and environmental variations since higher diversity is captured. Compared to macrofaunal species richness, which is typically very low in the Gulf of Finland (e.g. 22 sub-littoral soft-sediment species, Bonsdorff 2006), the species richness estimate based on our metabarcoding approach is approximately 10-fold higher. Metabarcoding of surface sediment samples also enables simultaneous observations of both benthic and pelagic taxa, which allows linking benthic community observations to events occurring in the water column, such as the phytoplankton bloom magnitude and community composition. Therefore, we support the inclusion of non-metazoans in these types of studies as they provide useful information on temporal environmental variation (Lanzén et al. 2016). Additionally, metabarcoding may ensure taxonomic identification. For example, *Biecheleria baltica* co-exists in the Baltic Sea with *Scrippsiella hangoei* and they can only be identified from each other with molecular methods (Kremp et al. 2005). In our data, the majority of the reads in the class Dinophyceae fall into one OTU, similar to *Biecheleria* (Supplementary information Fig. S2), supporting the idea that *B. baltica* plays the major role in the *Scrippsiella*/*Biecheleria* complex in the Gulf of Finland (Sundström et al. 2010).

321   One advantage of metabarcoding, in comparison to traditional monitoring methods, is the small sediment

322   sample that is easy to process and allows for replication. However, recent metabarcoding survey by

323   Nascimento et al. (2018) suggested that the sediment sample size should be approximately 14 g instead of <1

324   g applied by many benthic surveys, including ours, to achieve sufficient beta diversity. Based on our data,

325   even small (< 1 g) sample sizes are able to capture abundant single-celled eukaryotes comprehensively.

326   However, as mentioned in Nascimento et al. (2018), larger metazoans are likely to have a more heterogenous

327   distribution in sediment, and hence cause some variation between the replicates. This was also seen in our

328   datasets where in 2009 the samples, which were more abundant with metazoan classes such as Maxillopoda,

329   also had a bigger heterogeneity between replicates (Supplementary information, Fig. S3). Therefore, when

330   targeting large metazoans or macrofauna, a bigger sample size may be advisable.

331   The choice of the targeted region has a potentially significant influence in metabarcoding surveys. The V4

332   and V9 regions of the 18S gene are two of the most commonly targeted in environmental surveys. V4 has the

333   benefit of being the largest 18S region in eukaryotes with high variability, which makes it well suited to

334   estimate genetic distances (Dunthorn et al. 2012). However, despite being much shorter, V9 has the

335   advantage of capturing virtually all eukaryote phyla (Amaral-Zettler et al. 2009, Pawlowski et al. 2011). This

336   is also seen in our study where estimated species richness was clearly higher in the V9 dataset than in the V4

337   (Fig. 3). Despite this, the two 18S regions targeted in this study were able to provide a relatively similar

338   overview of the community composition through time (Fig. 2). Nevertheless, a considerable difference was

339   observed in the ability of the V4 region in targeting the class Maxillopoda. The difference was most striking

340   in 2009 summer samples, when the V9 samples indicated that the assemblage was dominated by

341   Maxillopoda and the V4 by Dinophyceae. Furthermore, V9 was also able to identify different Maxillopoda

342   orders (Fig. 2). This is consistent with previous studies, which showed that V9 region is able to better target

343   and resolve the taxonomy of the class Maxillopoda than the V4 region (Wu et al. 2015, Tragin et al. 2018).

344   In addition, the reference database used has potentially a big impact on the obtained eukaryote community.

345   For example, benthic protist diversity is still largely undescribed, and thus it may lead to underestimation of

346   such taxa (Forster et al. 2016). Compared to the V9 dataset, the V4 region gave a higher number of taxa

347   identified as "unclassified", which may be an issue related to available references in the database. However,

348 new metabarcoding data is continuously contributing to the existing databases, so the situation is likely to

349 improve in the future. We conclude, that targeting the V4 region instead of V9 may be justifiable due to the

350 larger size and greater variability of this region, which may help to tell closely related taxa apart from one

351 another. However, as our data shows, despite the increasing use of the V4 region, it still fails to identify all

352 eukaryote taxonomic groups. This is especially prominent when targeting the large and abundant class of

353 Maxillopoda. Therefore, in environments where Maxillopoda contributes significantly to the eukaryotic

354 community, the use of V9 target region is advisable.

355

356 **Conclusions**

357 Here, we demonstrate for the first time that 18S metabarcoding approach can be successfully applied to track

358 temporal changes in sediment eukaryote communities resulting from shifts in regional climate regime or

359 large-scale changes in the environment. These results have important implications for future metabarcoding-

360 based monitoring programs. Firstly, based on the high significance of the seasonal and annual changes, long-

361 term surveys are recommended. Recent metabarcoding studies have focused on spatial variations, providing

362 us only with snap-shot views of environmental status of study locations. To gain a comprehensive

363 perspective of the influence of the prevailing conditions on sediment eukaryotic composition, temporal

364 trends must be taken into account. Secondly, monitoring programs should be carefully designed in respect to

365 sample moment as the sediment eukaryotic communities show large seasonal changes in their composition.

366 Therefore, the timing and frequency of the sampling strategy should reflect the monitoring aims. For

367 example, if the focus is related to impact of coastal eutrophication on sediment community, the sampling

368 should be systematically carried out towards the end of the growth season in order to capture the signal.

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