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Metabarcoding successfully tracks temporal changes in eukaryotic communities in coastal sediments

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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.

49

50 **Introduction**

51

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

114 sediments were sampled for organic matter content and bottom water samples were collected from 5 cm
115 above the sediment surface for measurements of dissolved oxygen, ammonium and nitrate. Sedimentary
116 organic matter content was measured as loss on ignition (LOI) and diffusive oxygen utilization (DOU) in
117 bottom waters was inferred from triplicate oxygen micro sensor profiles (Jäntti et al. 2011, Vetterli et
118 al.2015). For additional details on the sampling protocols and site descriptions, see Jäntti et al. 2011 and
119 Vetterli et al. 2015.

120 *DNA extraction and amplification*

121 DNA was extracted from 0.25 g of sediment with the MoBio Powerkit for soil (MoBio, Carlsbad, Germany)
122 according to the manufacturer's instructions. Three replicate DNA extractions were done for each sample
123 moment, all of which were taken from different replicate cores, with the exception of summer 2008 sample
124 from Storfjärden in both datasets and winter 2009 sample from Storfjärden in the V4 dataset, which had only
125 2 replicates available.

126 Two sets of primers were used for DNA amplification, targeting either the V4 (Comeau et al. 2011 and Hugerth
127 et al. 2014) or the V9 (Amaral-Zettler et al.2009) region of the 18S rRNA gene (Supplementary information
128 Table S1). Both forward and reverse primers were modified at the 5' end to include overhang sequences for
129 the downstream sequencing. DNA was amplified with Polymerase Chain Reaction (PCR) using Phusion
130 Mastermix (ThermoFisher) according to manufacturer's instructions. Duplicate PCR products in equal
131 volumes were pooled after amplification and quality-checked with agarose gel electrophoresis.

132 Negative controls were made for the extraction kit used (to test for contamination in the kit reagents) and for
133 all the PCR reactions (to test the contamination in the PCR master mix). All negative controls except one in
134 the V9 dataset did not show a product on agarose gel electrophoresis. The negative control that was visible in
135 gel electrophoresis was subsequently sent for sequencing and analyzed. It contained 110 reads assembled in
136 33 OTUs (< 0.1 % of reads in an average sample in the V9 dataset). The OTUs of the negative control were
137 removed from the final V9 dataset. Furthermore, negative controls were made during the PCR purifications
138 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.

141 *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

217 dataset (Fig. 2c) and 66.4 % in V9 dataset (Fig. 2d), average of both sites). At order-level, V9 region was
218 able to identify three dominant orders in the class Maxillopoda, namely Harpacticoida (25.5% average in all
219 samples, Fig. 2d), Calanoida (5.7%) and Cyclopoida (0.5%), whereas the V4 region could not resolve the
220 lower taxonomic levels of Maxillopoda (mentioned as "unclassified Maxillopoda", Fig. 2c). In total 4.9% of
221 all reads were classified as "unclassified eukaryotes" when using V4 region. Using V9 region none of the
222 reads were classified as "unclassified".

223 Overall the Shannon diversity index (H') and Species richness estimate (S') were higher in average of all
224 samples in the V9 dataset than in the V4 (Fig. 3). The general tendency in both of these diversity indices
225 suggests that the average diversity was overall higher in summer (median H': 1.3 V4, 2.2 V9; S': 200 V4,
226 315 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,
227 1.6 V9; S': 199 V4, 271 V9). In V4 dataset exceptions to the medians were seen in 2008, where in
228 Storfjärden both S' and H' indices were higher in spring and winter in Storfjärden, and in Muncken S' index
229 was highest in winter. In the V9 dataset the S' index of the year 2008 was also higher in winter and spring
230 than in summer at site Storfjörden (Fig. 3). Pielou's evenness values in the V9 dataset were also greater in
231 summer compared with other seasons (median 0.4) with highest values recorded in Storfjärden in summer
232 2009 (0.50), but in the V4 dataset the median for all season was the same (0.2) (Fig. 3).

233 *Community response to temporal environmental changes*

234 Principal coordinate analysis (PCoA) based on Unifrac metric revealed that most of the observed community
235 variance, in both the V4 and V9 datasets, can be explained by the year (Fig. 4). Differences between the two
236 years are mainly highlighted by the separation of 2008 and 2009 samples on the first axis, which explained
237 69.3% of the variance in the V4 dataset and 70.8% of the variance and in the V9 dataset (Fig. 4). The second
238 axis of the PCoA plot appears to depict seasonal changes with summer samples clustering separately from
239 winter samples, explaining 9% of the variance in the V4 dataset (Fig. 4a) and 10.7% of the variance in the
240 V9 dataset (Fig. 4b).

241 The influence of temporal variation on eukaryotic sediment community was confirmed with canonical
242 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

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

303 *Metabarcoding approach for environmental biomonitoring: advantages and recommendations*

304 Our results support recent efforts in research (e.g. Chariton et al. 2015, Lejzerowicz et al. 2015, Aylagas et
305 al. 2018), stating that metabarcoding has various assets compared to traditional morphology-based
306 biomonitoring. Firstly, our datasets were dominated by meio- and microfauna, and inclusion of smaller size
307 fraction of eukaryotes typically neglected in biomonitoring surveys increases the potential of detecting the
308 temporal and environmental variations since higher diversity is captured. Compared to macrofaunal species
309 richness, which is typically very low in the Gulf of Finland (e.g. 22 sub-littoral soft-sediment species,
310 Bonsdorff 2006), the species richness estimate based on our metabarcoding approach is approximately 10-
311 fold higher. Metabarcoding of surface sediment samples also enables simultaneous observations of both
312 benthic and pelagic taxa, which allows linking benthic community observations to events occurring in the
313 water column, such as the phytoplankton bloom magnitude and community composition. Therefore, we
314 support the inclusion of non-metazoans in these types of studies as they provide useful information on
315 temporal environmental variation (Lanzén et al. 2016). Additionally, metabarcoding may ensure taxonomic
316 identification. For example, *Biecheleria baltica* co-exists in the Baltic Sea with *Scrippsiella hangoei* and
317 they can only be identified from each other with molecular methods (Kremp et al. 2005). In our data, the
318 majority of the reads in the class Dinophyceae fall into one OTU, similar to *Biecheleria* (Supplementary
319 information Fig. S2), supporting the idea that *B. baltica* plays the major role in the *Scrippsiella*/*Biecheleria*
320 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.

369

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References

Amaral-Zettler, L., McCliment, E., Ducklow, H. and Huse, S. (2009). A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. *PLoS ONE*, 4(7), p.e6372.

Aylagas, E., Borja, Á., Irigoien, X. and Rodríguez-Ezpeleta, N. (2016). Benchmarking DNA Metabarcoding for Biodiversity-Based Monitoring and Assessment. *Frontiers in Marine Science*, 3.

Aylagas, E., Borja, Á., Muxika, I. and Rodríguez-Ezpeleta, N. (2018). Adapting metabarcoding-based benthic biomonitoring into routine marine ecological status assessment networks. *Ecological Indicators*, 95, pp.194-202.

Bik, H., Sung, W., De Ley, P., Baldwin, J., Sharma, J., Rocha-Olivares, A. and Thomas W. (2011). Metagenetic community analysis of microbial eukaryotes illuminates biogeographic patterns in deep-sea and shallow water sediments. *Molecular Ecology*, 21(5), pp.1048-1059.

Bonsdorff, E. (2006). Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. *Journal of Experimental Marine Biology and Ecology*, 330(1), pp.383-391.

Brannock, P., Learman, D., Mahon, A., Santos, S. and Halanych, K. (2018). Meiobenthic community composition and biodiversity along a 5500 km transect of Western Antarctica: a metabarcoding analysis. *Marine Ecology Progress Series*, 603, pp.47-60.

Caron, D., Countway, P., Savai, P., Gast, R., Schnetzer, A., Moorthi, S., Dennett, M., Moran, D. and Jones, A. (2009). Defining DNA-Based Operational Taxonomic Units for Microbial-Eukaryote Ecology. *Applied and Environmental Microbiology*, 75(18), pp.5797-5808.

Chariton, A., Stephenson, S., Morgan, M., Steven, A., Colloff, M., Court, L. and Hardy, C. (2015). Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution*, 203, pp.165-174.

Comeau, A., Li, W., Tremblay, J., Carmack, E. and Lovejoy, C. (2011). Arctic Ocean Microbial Community Structure before and after the 2007 Record Sea Ice Minimum. *PLoS ONE*, 6(11), p.e27492.

Diaz, R., Solan, M. and Valente, R. (2004). A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management*, 73(3), pp.165-181.

410 Dunthorn, M., Klier, J., Bunge, J. and Stoeck, T. (2012). Comparing the Hyper-Variable V4 and V9 Regions
411 of the Small Subunit rDNA for Assessment of Ciliate Environmental Diversity. *Journal of Eukaryotic*
412 *Microbiology*, 59(2), pp.185-187.

413 Edgar, R., Haas, B., Clemente, J., Quince, C. and Knight, R. (2011). UCHIME improves sensitivity and
414 speed of chimera detection. *Bioinformatics*, 27(16), pp.2194-2200.

415 Forster, D., Dunthorn, M., Mahé, F., Dolan, J., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R.,
416 Claverie, J., Decelle, J., Edvardsen, B., Egge, E., Eikrem, W., Gobet, A., Kooistra, W., Logares, R.,
417 Massana, R., Montresor, M., Not, F., Ogata, H., Pawlowski, J., Pernice, M., Romac, S., Shalchian-Tabrizi,
418 K., Simon, N., Richards, T., Santini, S., Sarno, D., Siano, R., Vaulot, D., Wincker, P., Zingone, A., de
419 Vargas, C. and Stoeck, T. (2016). Benthic protists: the under-charted majority. *FEMS Microbiology Ecology*,
420 92(8), p.fiw120.

421 Giner, C., Forn, I., Romac, S., Logares, R., de Vargas, C. and Massana, R. (2016). Environmental
422 Sequencing Provides Reasonable Estimates of the Relative Abundance of Specific Picoeukaryotes. *Applied*
423 *and Environmental Microbiology,* 82(15), pp.4757-4766.

424 Hu, Y., Karlson, B., Charvet, S. and Andersson, A. (2016). Diversity of Pico- to Mesoplankton along the
425 2000 km Salinity Gradient of the Baltic Sea. *Frontiers in Microbiology*, 7.

426 Hugerth, L., Muller, E., Hu, Y., Lebrun, L., Roume, H., Lundin, D., Wilmes, P. and Andersson, A. (2014).
427 Systematic Design of 18S rRNA Gene Primers for Determining Eukaryotic Diversity in Microbial Consortia.
428 *PLoS ONE*, 9(4), p.e95567.

429 Ininbergs, K., Bergman, B., Larsson, J. and Ekman, M. (2015). Microbial metagenomics in the Baltic Sea:
430 Recent advancements and prospects for environmental monitoring. *AMBIO*, 44(S3), pp. 439-450.

431 Jäntti, H., Stange, F., Leskinen, E. and Hietanen, S. (2011). Seasonal variation in nitrification and nitrate-
432 reduction pathways in coastal sediments in the Gulf of Finland, Baltic Sea. *Aquatic Microbial Ecology*,
433 63(2), pp.171-181.

434 Kaitala, S., Hällfors, S. (2008). Phytoplankton biomass and species succession in the Gulf of Finland,
435 Northern Baltic Proper and Southern Baltic Sea in 2008. HELCOM Baltic Sea Environment Fact Sheets.
436 Online. Viewed online 29.04.2018. http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/.
437

438 Kaitala, S., Hällfors, S. (2009). Phytoplankton biomass and species succession in the Gulf of Finland,
439 Northern Baltic Proper and Southern Baltic Sea in 2009. HELCOM Baltic Sea Environment Fact Sheets.
440 Online. Viewed online 29.04.2018. http://www.helcom.fi/baltic-sea-trends/environment-fact-sheets/.
441 Kennedy, A. and Jacoby, C. (1999). Biological Indicators of Marine Environmental Health: Meiofauna – A
442 Neglected Benthic Component? *Environmental Monitoring and Assessment*, 54(1), pp.47-68.
443

444 Kim, E., Sprung, B., Duhamel, S., Filardi, C. and Kyoon Shin, M. (2016). Oligotrophic lagoons of the South
445 Pacific Ocean are home to a surprising number of novel eukaryotic microorganisms. *Environmental*
446 *Microbiology*, 18(12), pp.4549-4563.
447

448 Kremp, A., Elbrächter, M., Schweikert, M., Wolny, J. and Gottschling, M. (2005). *Woloszynskia halophila*
449 (Biecheler) comb. nov.: A bloom-forming cold-water dinoflagellate co-occurring with *Scrippsiella hangoei*
450 (Dinophyceae) in the Baltic Sea. *Journal of Phycology*, 41(3), pp.629-642.
451

452 Lanzén, A., Lekang, K., Jonassen, I., Thompson, E. and Troedsson, C. (2016). High-throughput
453 metabarcoding of eukaryotic diversity for environmental monitoring of offshore oil-drilling activities.
454 *Molecular Ecology*, 25(17), pp.4392-4406.

455 Lejzerowicz, F., Esling, P., Pillet, L., Wilding, T., Black, K. and Pawlowski, J. (2015). High-throughput
456 sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. *Scientific*
457 *Reports*, 5(1).

458 Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., Chambouvet, A., Christen, R., Claverie,
459 J., Decelle, J., Dolan, J., Dunthorn, M., Edvardsen, B., Forn, I., Forster, D., Guillou, L., Jaillon, O., Kooistra,
460 W., Logares, R., Mahé, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M., Probert, I., Romac, S., Richards,
461 T., Santini, S., Shalchian-Tabrizi, K., Siano, R., Simon, N., Stoeck, T., Vaulot, D., Zingone, A. and de
462 Vargas, C. (2015). Marine protist diversity in European coastal waters and sediments as revealed by high-
463 throughput sequencing. *Environmental Microbiology*, 17(10), pp.4035-4049.

464 McMurdie, P. and Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and
465 Graphics of Microbiome Census Data. *PLoS ONE*, 8(4), p.e61217.

466 Nascimento, F., Lallias, D., Bik, H. and Creer, S. (2018). Sample size effects on the assessment of eukaryotic
467 diversity and community structure in aquatic sediments using high-throughput sequencing. *Scientific*
468 *Reports,* 8(1).

469 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R.
470 B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H. (2017). Vegan: Community
471 Ecology Package. R. package version 2.4-5. https://CRAN.R-project.org/package=vegan

472 Park, S., Park, B., Pham, V., Yoon, D., Kim, S. and Rhee, S. (2008). Microeukaryotic diversity in marine
473 environments, an analysis of surface layer sediments from the East Sea. *The Journal of Microbiology*, 46(3),
474 pp.244-249.

475 Piredda, R., Tomasino, M., D'Erchia, A., Manzari, C., Pesole, G., Montresor, M., Kooistra, W., Sarno, D.
476 and Zingone, A. (2016). Diversity and temporal patterns of planktonic protist assemblages at a
477 Mediterranean Long Term Ecological Research site. *FEMS Microbiology Ecology*, 93(1), p.fiw200.

478 Schloss, P., Westcott, S., Ryabin, T., Hall, J., Hartmann, M., Hollister, E., Lesniewski, R., Oakley, B., Parks,
479 D., Robinson, C., Sahl, J., Stres, B., Thallinger, G., Van Horn, D. and Weber, C. (2009). Introducing mothur:
480 Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing
481 Microbial Communities. *Applied and Environmental Microbiology*, 75(23), pp.7537-7541.

482 Sundström, A., Kremp, A., Tammilehto, A., Tuimala, J. and Larsson, U. (2010). Detection of the bloom-
483 forming cold-water dinoflagellate Biecheleria baltica in the Baltic Sea using LSU rRNA probes. *Aquatic*
484 *Microbial Ecology*, 61(2), pp.129-140.

485 Tragin, M., Zingone, A. and Vaulot, D. (2018). Comparison of coastal phytoplankton composition estimated
486 from the V4 and V9 regions of the 18S rRNA gene with a focus on photosynthetic groups and especially
487 Chlorophyta. *Environmental Microbiology*, 20(2), pp.506-520.

488 Vainio, J. 2008. The ice season 2007-2008. HELCOM Baltic Sea Environment Fact Sheets. Online. Date
489 Viewed 29.03.2018, http://www.helcom.fi/baltic-seatrends/environment-fact-sheets/.

490 Finnish Meteorological Institute. Vainio, J., Eriksson, P. http://en.ilmatieteenlaitos.fi/ice-winter-2008-2009.
491 Page viewed 11.04.2018.

492 van Dijk, E., Auger, H., Jaszczyszyn, Y. and Thermes, C. (2014). Ten years of next-generation sequencing
493 technology. *Trends in Genetics*, 30(9), pp.418-426.

494 Vetterli, A., Hyytiäinen, K., Ahjos, M., Auvinen, P., Paulin, L., Hietanen, S. and Leskinen, E. (2015).
495 Seasonal patterns of bacterial communities in the coastal brackish sediments of the Gulf of Finland, Baltic
496 Sea. *Estuarine, Coastal and Shelf Science*, 165, pp.86-96.

497 Wu, S., Xiong, J. and Yu, Y. (2015). Taxonomic Resolutions Based on 18S rRNA Genes: A Case Study of
498 Subclass Copepoda. *PLOS ONE*, 10(6), p.e0131498.

499 Zhang, W., Pan, Y., Yang, J., Chen, H., Holohan, B., Vaudrey, J., Lin, S. and McManus, G. (2018). The
500 diversity and biogeography of abundant and rare intertidal marine microeukaryotes explained by
501 environment and dispersal limitation. *Environmental Microbiology*, 20(2), pp.462-476.

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