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1 # Metabarcoding reveals different zooplankton
2 # communities in northern and southern areas of the
3 # North Sea

4

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16

17 ## Highlights

18 - Zooplankton communities are different in northern and southern areas of the North Sea

19 - Metabarcoding results are consistent with known species distributions and abundance

20 - Metabarcoding allows for fast identification of meroplanktonic species

21

22 ## Abstract

23 Zooplankton are key players in marine ecosystems, linking primary production to higher
24 trophic levels. The high abundance and high taxonomic diversity renders zooplankton ideal
25 for biodiversity monitoring. However, taxonomic identification of the zooplankton assemblage

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26 is challenging due to its high diversity, subtle morphological differences and the presence of
27 many meroplanktonic species, especially in coastal seas. Molecular techniques such as
28 metabarcoding can help with rapid processing and identification of taxa in complex samples,
29 and are therefore promising tools for identifying zooplankton communities. In this study, we
30 applied metabarcoding of the mitochondrial cytochrome c oxidase I gene to zooplankton
31 samples collected along a latitudinal transect in the North Sea, a shelf sea of the Atlantic
32 Ocean. Northern regions of the North Sea are influenced by inflow of oceanic Atlantic
33 waters, whereas the southern parts are characterised by more coastal waters. Our
34 metabarcoding results indicated strong differences in zooplankton community composition
35 between northern and southern areas of the North Sea, particularly in the classes
36 Copepoda, Actinopterygii (ray-finned fishes) and Polychaeta. We compared these results to
37 the known distributions of species reported in previous studies, and by comparing the
38 abundance of copepods to data obtained from the Continuous Plankton Recorder (CPR).
39 We found that our metabarcoding results are mostly congruent with the reported distribution
40 and abundance patterns of zooplankton species in the North Sea. Our results highlight the
41 power of metabarcoding to rapidly assess complex zooplankton samples, and we suggest
42 that the technique could be used in future monitoring campaigns and biodiversity
43 assessments.

44

45 ## Introduction

46

47 Zooplankton are key players in marine ecosystems, linking primary production to higher
48 trophic levels (Suthers & Rissik, 2009; Beaugrand, Edwards & Legendre, 2010; Turner,
49 2015; Steinberg & Landry, 2017). Due to their abundance and high taxonomic diversity,
50 zooplankton can be used for ecosystem assessments and biomonitoring (Bucklin et al.,
51 2016; Chain et al., 2016; Chiba et al., 2018). However, studying zooplankton is a challenging
52 task, as obtaining samples and taxonomic identification can be difficult (Schminke, 2007;

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53 McManus & Katz, 2009; Cornils & Held, 2014). Especially meroplanktonic species, i.e. taxa
54 which are part of the plankton only during their larval stages, can be difficult to identify
55 (Mathivat-Lallier & Cazaux, 1990; Kirby & Lindley, 2005; Oozeki, 2018). This is also reflected
56 in the extensive, publicly available long-term monitoring dataset of the CPR (Continuous
57 Plankton Recorder, available online: https://data.cprsurvey.org/datacatalog; (Reid et al.,
58 2003a) which records the occurrence of more than 30 copepod taxa on genus or species
59 level, whereas data on meroplanktonic groups is available in less detail (e.g."fish eggs",
60 "polychaete larvae"). Inclusion of the often highly abundant meroplanktonic species,
61 especially in coastal areas (Schwamborn et al., 2001; Kirby & Lindley, 2005; Jansen et al.,
62 2012; Harvey et al., 2018) in zooplankton biodiversity assessments would be beneficial for
63 getting more detailed insights and for better understanding of zooplankton distribution
64 patterns. Molecular techniques like metabarcoding (Taberlet et al., 2012), i.e. the
65 amplification, sequencing and analysis of marker gene fragments ("molecular barcodes",
66 (Ratnasingham & Hebert, 2007)) of whole communities, can help with rapid processing and
67 identification of species in complex samples. The technique has been shown to be an
68 effective tool for identification of species in zooplankton communities (Brown et al., 2015;
69 Casas, Pearman & Irigoien, 2017; Deagle et al., 2018; Zhang et al., 2018) and for
70 identification of larval stages (Kimmerling et al., 2018; Couton et al., 2019). While several
71 studies have shown the benefits of metabarcoding zooplankton, suitable barcoding regions
72 and primers for amplification are still under discussion (Brown et al., 2015; Bucklin et al.,
73 2016; Chain et al., 2016; Clarke et al., 2017), and current DNA reference databases are far
74 from complete (Bucklin et al., 2016). However, the development of highly degenerate
75 primers amplifying a wide range of taxa is an important step towards assessment of complex
76 communities (Leray et al., 2013; Wangensteen et al., 2018) and is therefore especially
77 promising for the assessment of highly diverse zooplankton communities.
78 In this study we use the highly degenerate Leray XT primers (Leray et al., 2013;
79 Wangensteen et al., 2018), which amplify a fragment of the mitochondrial cytochrome c
80 oxidase I gene, to assess the zooplankton community of the North Sea along a transect from

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81 the Dutch coast to the Shetland Islands. The zooplankton of the North Sea, a shelf sea of
82 the Atlantic Ocean, is relatively well known based on morphological analyses (Fransz et al.,
83 1991; Greve et al., 2001; Beare et al., 2002; Lindley & Batten, 2002; Reid et al., 2003b;
84 Alvarez-Fernandez, Lindeboom & Meesters, 2012). Previous studies have shown that the
85 zooplankton community in the northern parts of the North Sea shows a higher abundance of
86 oceanic species, while the community in the southern parts of the North Sea is commonly
87 dominated by more coastal species (Fransz et al., 1991; Krause, M., Dippner, J.W., Beil, J.,
88 1995; Nielsen & Sabatini, 1996; Alvarez-Fernandez, Lindeboom & Meesters, 2012). The
89 community structure is linked to the influx of cold, saline, Atlantic waters entering the North
90 Sea from the north, and flowing south through a corridor of deeper water to the area of the
91 Dogger Bank in the central North Sea (Otto et al., 1990; Fransz et al., 1991; Lindley &
92 Batten, 2002). We hypothesised that metabarcoding of the zooplankton across a latitudinal
93 transect of the North Sea, using highly degenerate COI primers, would allow for the
94 identification of distinct zooplankton communities in the northern and southern parts of the
95 North Sea.
96

97 Material & Methods

98

99 Sampling, DNA extraction and library preparation

100 Samples from nine stations were taken during the North Sea leg 10 of the NICO
101 (Netherlands Initiative Changing Oceans) expedition in May and June 2018 (see
102 supplementary Table S1 for coordinates and Figure 1 for a map). Sampling in UK waters
103 was approved by the Maritime Policy Unit (Legal Directorate) of the Foreign and
104 Commonwealth Office (ref 33/2018). Weather conditions were calm and stable throughout
105 the 12 day cruise. Samples were taken with a plankton MultiNet (Hydro-Bios, Kiel, Germany)
106 with a mesh size of 100 µm. All tows were conducted between 8:00 and 9:00 in the morning.
107 Winch speed was 5 meters per second. Temperature and salinity across the water column

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108 were measured by the on-board CTD prior to sampling. Zooplankton samples were taken
109 from the seafloor to the deepest thermocline, between thermoclines (in case more than one
110 was present), and from the uppermost thermocline to the surface. Zooplankton was removed
111 from the multinet by carefully rinsing the net with seawater into the cod end. Samples were
112 transferred to a Folsom sample splitter (McEwen, Johnson & Folsom, 1954). Half of the
113 sample volume was retained for morphological analyses and half for molecular analyses.
114 The molecular subsample was further split in two halves, which were subsequently
115 processed as separate samples (i.e. extraction replicates) to check for potential biases
116 during processing and sequencing. All samples were transferred to 96% ethanol and stored
117 at -20°C until further processing.
118 Samples were dried under sterile fume hoods and ground to a fine powder using an IKA
119 Ultra Turrax homogenizer (IKA, Staufen, Germany) on full speed for 10 minutes (Macher et
120 al., 2018; Zizka et al., 2019b). DNA was extracted using the Macherey-Nagel (Düren,
121 Germany) NucleoSpin tissue kit on the KingFisher (Waltham, USA) robotic platform,
122 following the manufacturer's protocol. Two negative controls containing ultrapure water were
123 processed together with the samples during all steps. Quantity and size of the extracted
124 DNA was checked on the QIAxcel platform (Qiagen, Hilden, Germany). 15ng of DNA per
125 sample was used for metabarcoding using the Leray-XT primers (313bp product length),
126 which amplify a COI gene fragment of a wide range of marine metazoan taxa (Wangensteen
127 et al., 2018). Samples were amplified using a two-step PCR protocol as commonly used for
128 metabarcoding studies (Andruszkiewicz et al., 2017; Galan et al., 2018; Zizka et al., 2019a).
129 The first PCR was performed in 20 µl PCR reactions containing 10 µl Environmental Master
130 Mix (2x, Thermo Fisher Scientific, Waltham, USA), 7µl ultrapure water, 1ul of each primer
131 (10pMol/ul) and 1 µl (15ng) of DNA template. PCR was conducted with 10 minutes of initial
132 denaturation at 95°C, followed by 30 cycles of 30 seconds denaturation at 95°C, 30 seconds
133 annealing at 50°C, and 20 seconds extension at 72°C. Final extension was set to 7 minutes
134 at 72°C. Amplicons were cleaned with Macherey Nagel NucleoMag beads (Dueren,
135 Germany) according to the manufacturer's protocol and a sample to beads volume of 1:0.9.

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136 The second PCR step was used to tag samples with unique Illumina adapters. Samples
137 were amplified using 7ul of ultrapure water, 10ul of Environmental Master Mix, 1 µl of each
138 primer tagged with Nextera XT adapter (Illumina, San Diego, USA) and 1ul of DNA template.
139 Cycling conditions were the same as described above, but only 10 cycles were used.
140 Amplicon length and concentration was measured on the QIAxcel platform, samples were
141 cleaned and size selected using magnetic beads as described above, and equimolarly
142 pooled using the QIAgility platform (Qiagen, Venlo, Netherlands). Negative controls did not
143 show DNA and were added to the library with 10% of the final volume. Final concentration
144 and fragment length of the library were checked on the Bioanalyzer platform (Agilent
145 Technologies, Santa Clara, USA). The final library was sent for sequencing on the Illumina
146 MiSeq platform (2x300bp read length) at Baseclear (Leiden, Netherlands).
147
148 **Bioinformatic processing**
149 Processing of reads was conducted using the Galaxy platform (Afgan et al., 2018) following
150 the principal steps of (Beentjes et al., 2019). Samples taken from different depths of the
151 same sampling station were combined to allow for studying the zooplankton community of
152 the entire watercolumn. FLASH (Magoč & Salzberg, 2011) was used to merge reads with
153 minimum overlap of 50 and maximum overlap of 300, a maximum mismatch ratio of 0.2, and
154 with non-merged reads discarded. Cutadapt was used to trim primers (settings: both primers
155 need to be present, minimum number of matching bases 10, maximum error rate 0.2).
156 PrinSeq (Schmieder & Edwards, 2011) was used to filter and trim sequences to 310 base
157 pairs to remove reads that contain gaps or indels, which can be present due to amplification
158 of non- eukaryotic taxa (Wangensteen et al., 2018; Macher et al., 2018; Collins et al., 2019).
159 UNOISE (Edgar, 2016) was used for clustering of Operational Taxonomic Units (OTUs). We
160 chose thresholds of alpha = 4 and a minimum number of 10 reads for the denoising
161 approach, which is similar to settings reported in previous studies that found an alpha of 5 to
162 give reliable results (Elbrecht et al., 2018; Turon et al., 2019). We chose an alpha of 4 to be
163 slightly more restrictive and remove more potentially wrong sequence variants from the

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164 dataset, although this approach might also increase the loss of genuine variants. To further
165 reduce the risk of analysing spurious OTUs, only those OTUs with >0.002% relative
166 abundance per sample were retained, which corresponds to >1 read in the sample with the
167 lowest read count. Further, we only retained OTUs that were present in both extraction
168 replicates per sample. Such an approach, i.e. filtering out low abundant OTUs based on
169 relative abundance, is commonly used in metabarcoding studies (Elbrecht et al., 2017;
170 Pereira-da-Conceicoa et al., 2019; Theissinger et al., 2019). After this quality filtering step,
171 the reads of the two technical replicates per sample were summed up to build the final
172 dataset. Quality filtered reads were assigned to species using the BOLD database
173 (Ratnasingham & Hebert, 2007) with the BOLDigger tool (Buchner & Leese, 2020). The
174 following identity thresholds were used for assigning taxonomic ranks: species 98%; genus
175 95%; family 93%; order 90%; class 85%. OTUs that were assigned to the same taxonomic
176 name were subsequently lumped by summing up reads to prevent analyses of intraspecific
177 variability as provided by the UNOISE pipeline. We focussed our analyses on planktonic
178 metazoans (animal zooplankton).
179

180 **Community analyses**

181 We tested which of the parameters: salinity, temperature, bottom depth or latitude, best
182 explained the community composition of zooplankton in the North Sea during the NICO 10
183 expedition. Analyses of community composition were conducted using the R package vegan
184 (Oksanen et al. 2019, https://cran.r-project.org/package=vegan). Averages of the abiotic
185 variables ‘salinity’ and ‘temperature’ across the water column were obtained from the CTD
186 data (Supplementary Table 1). The variables ‘bottom depth at sampling site’ and ‘latitude of
187 sampling site’ were extracted from the ship logbook. The variables were categorized into two
188 classes (<50th percentile, ≥ 50th percentile of the variable range), and sampling sites were
189 assigned to these classes accordingly. The four southernmost sampling sites (south of 56°N)
190 were also the shallowest (shallower than 75m), while the five northern sampling sites (north
191 of 56°N) were all deeper than 75m. Sites were therefore categorized as 'southern/shallow'

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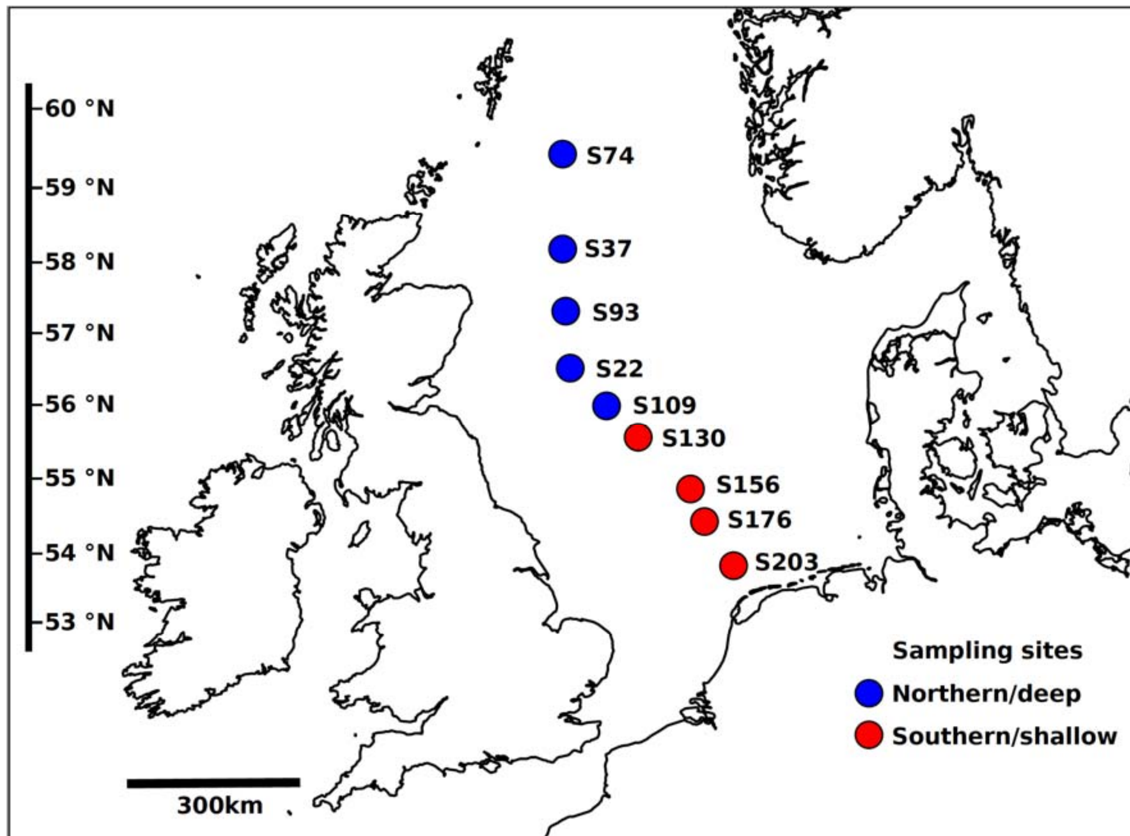
192 and 'northern/deep', respectively (Fig. 1). Mean salinity was lowest in the three southernmost
193 sampling sites. Mean water temperature was lowest in the sampling sites S130 (55.62 °N),
194 S93 (57.36 °N), and S74 (59.42 °N), i.e. did not show a clear latitudinal pattern. Differences
195 in community composition of the entire zooplankton assemblage as a function of the tested
196 variables were analysed based on relative abundance, i.e. read counts transformed to
197 relative abundance per sample. These analyses were conducted on the level of molecularly
198 identified species. The abundant and species-rich classes Actinopterygii (ray-finned fishes),
199 Copepoda and Polychaeta were also analysed separately to test whether similar patterns
200 could be observed for different taxonomic groups.

201



202

203 **Figure 1:** Map showing the location and station names of the nine sampling sites in the
204 North Sea. Blue circles and red circles represent 'northern/deep' and 'southern/shallow'
205 sampling sites, respectively.

206

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207 Bray- Curtis distances were calculated using the vegdist function implemented in the vegan
208 package. Communities were subsequently clustered with an average-linkage algorithm
209 (hclust function) as in (Burdon et al., 2016; Macher et al., 2018). Community composition
210 was analysed using the 'adonis' PERMANOVA function as implemented in vegan. Analyses
211 were run separately with the abiotic variables (depth, latitude, mean salinity, mean
212 temperature) as predictor and the Bray- Curtis distances as response variables. Following
213 (Nakagawa & Cuthill, 2007) and (Cohen, 2013), we regarded significant results with $R^2 >$
214 0.09 (equivalent to $r = 0.30$) as moderate, and $R^2 > 0.25$ ($r = 0.50$) as strong. Species
215 numbers found exclusively in 'northern/deep' or 'southern/shallow' sampling sites, or in both
216 areas were visualised using the Venn diagram creator (available online:
217 https://bioinformatics.psb.ugent.be/webtools/Venn/). Correlation of latitude and relative
218 abundance of species was tested with Pearson correlation analyses using the R package
219 'ggpubr' (Kassambra 2019, https://cran.r-project.org/package=ggpubr). This analysis was
220 conducted for the four most abundant species in the classes Copepoda, Actinopterygii and
221 Polychaeta. For comparison of the copepod metabarcoding data with long-term monitoring
222 data based on morphological identification, the May and June data from 2010 to 2017 (latest
223 available data) of the Continuous Plankton Recorder (CPR) dataset (DOI:
224 10.17031/1628#year=2010-2017;month=5-6) was used. The CPR data was reduced to the
225 277 samples in the area between 0.5°E and 4.5°E and 53.5°N and 59.5°N. This corresponds
226 to the area covered during the NICO leg 10 expedition. We compared the metabarcoding
227 data (relative read abundance) with data from the CPR (abundance/m^3). For the ray- finned
228 fishes and polychaetes using CPR data was not possible, as these taxa are recorded as
229 larvae or eggs without further taxonomic identification. *Oithona similis* in the metabarcoding
230 dataset was compared to the *Oithona* spp. data from the CPR, as *Oithona similis* is not
231 specifically recorded by the CPR, but is by far the most common *Oithona* species in the
232 North Sea (Fransz et al., 1991).
233

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234 ## Results

235 Zooplankton communities from nine sampling sites across the North Sea were analyzed,
236 and 42,798,930 raw reads were obtained. The two negative controls contained a total of
237 1204 reads (0.0028% of all reads). As Illumina platforms commonly show a low percentage
238 of tag switching during sequencing (Schnell, Bohmann & Gilbert, 2015) and no DNA was
239 observed in the negative controls during library preparation, no contamination was
240 suspected. After merging of forward and reverse reads and quality filtering, 18,904,404
241 sequences were retained. Bray- Curtis dissimilarity between extraction replicates of the
242 same sample was low (mean 0.018, standard error of the mean 0.002), and therefore no
243 systematic problem with extraction or laboratory processing was suspected.
244

245 ### Community composition

246 A total of 3315 OTUs were obtained. These belonged to 33 taxonomic classes, to which
247 96.1% of quality filtered reads could be assigned. Of the 33 classes, 26 were identified as
248 animals, while 7 classes (with 0.97% of all reads) were identified as plants and bacteria, and
249 were removed prior to further analysis. The zooplankton classes with the highest abundance
250 (based on read counts) were Copepoda (30.2% of reads, 28 identified species),
251 Actinopterygii (ray- finned fishes; 26.3% of reads, 16 identified species), Sagittoidea (arrow
252 worms, 19.9% of reads, 3 identified species), Branchiopoda (10.1% of reads, 3 identified
253 species), Polychaeta (5.8% of reads, 29 identified species), and Echinoidea (5.7% of reads,
254 5 identified species). All other classes were present with less than 1% of reads. The 26
255 classes were assigned to 59 orders, 103 families, 119 genera, and 127 species, to which
256 75% of all reads could be assigned (see supplementary table S2 for a species list).
257 Community composition of the entire zooplankton assemblage differed significantly and
258 strongly between 'northern/deep' and 'southern/shallow' sampling sites ($R2 = 0.35$, $p =$
259 0.004) as well as between northern and southern sites categorized based on salinity ($R2 =$
260 0.31, $p = 0.018$, Table 1). Water temperature did not explain overall community composition.

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261 Similar results were obtained when focussing only on the copepods and polychaetes. For
262 the ray-finned fishes, 'salinity/latitude' best explained community composition (Table 1).
263 Copepods, ray-finned fishes and polychaetes as the most abundant and species-rich groups
264 are discussed in more detail below. As latitude/depth best explained community
265 composition, we further focus on this factor.

266

267 **Table 1:** Difference in community composition based on Bray- Curtis distance for all species,
268 copepods, ray- finned fishes and polychaetes. Results show the difference in community
269 composition of 'northern/deep' versus 'southern/shallow' sampling sites; sites with higher
270 versus lower salinity; and sites with higher versus lower water temperature. R^2 and p value
271 based on 'adonis' analysis.

Taxon	**Latitude/depth**	**Salinity**	**Temperature**
	R^2 and p value		
All species	R^2 = 0.35 **p = 0.004 ****	R^2 = 0.31 **p = 0.018 ***	R^2 = 0.11 p = 0.596
Copepods	R^2 = 0.48 **p =0.005 ****	R^2 = 0.4 **p =0.011 ***	R^2 = 0.11 p =0.359
Ray-finned fishes	R^2 = 0.3 **p = 0.028 ***	R^2 =0.37 **p = 0.015 ***	R^2 = 0.1 p = 0.602
Polychaetes	R^2= 0.58 **p = 0.008 ***	R^2= 0.41 **p = 0.024 ***	R^2= 0.08 p = 0.536

272

273 Community composition was markedly different between 'northern/deep' and
274 'southern/shallow' sampling sites. We found 51 (39.8%) of all identified species in both
275 'northern/deep' and 'southern/shallow' sites, 48 species (37.5%) were exclusively found in
276 the 'northern/deep', and 29 species (22.7%) were exclusively found in southern/shallow'
277 sampling sites (Figure 2a).

278

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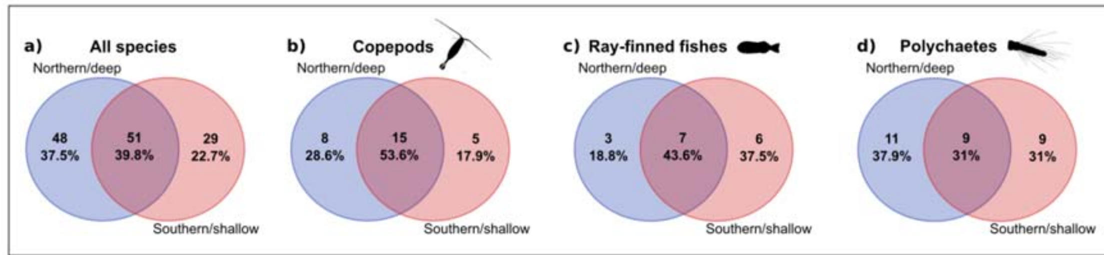

279

280 **Figure 2:** Number and percentage of identified species exclusively found in 'northern/deep'
281 respectively 'southern/shallow' sampling sites, and number of species found in both areas.
282 a) All species, b) Copepods, c) Ray-finned fishes, d) Polychaetes

283

284 **Copepods**

285 We identified 28 copepod species in our dataset. Of these, 8 (28.6%) were found exclusively
286 in the 'northern/deep' sites, 5 (17.9%) exclusively in the 'southern/shallow' sites, and 15
287 (53.6%) were found in both areas (Figure 2 b). The four most common species were *Oithona*
288 *similis* (33.2% of all copepod reads), *Microcalanus pusillus* (13.3%), *Calanus finmarchicus*
289 (12.6%), and *Temora longicornis* (11.4%).
290 We compared the metabarcoding data of these species to the abundance data recorded by
291 the Continuous Plankton Recorder (CPR). For *Oithona similis*, analysis revealed no
292 significant correlation of metabarcoding read abundance with latitude (Figure 3a), which is in
293 congruence with the CPR data (Figure 3e). *Microcalanus pusillus* did not show a significant
294 correlation of read abundance and latitude in the metabarcoding dataset (Figure 3b).
295 However, high read abundances were found in central North Sea sampling sites (Site S109,
296 18.5% of copepod reads; Site S22, 45.4%), whereas abundance of the species did not
297 exceed 6% of reads in all other sampling sites. Metabarcoding and CPR data could not be
298 compared, as *Microcalanus pusillus* is absent from the CPR data due to its distribution in
299 deeper water and small size (Fransz 1991). The CPR samples only the top water layer and
300 the used mesh size does not reliably retain very small organisms. For *Calanus finmarchicus*,
301 a strong, significant increase in read abundance with latitude was observed in the
302 metabarcoding dataset (Figure 3c). Equally, the CPR dataset showed that abundance of the

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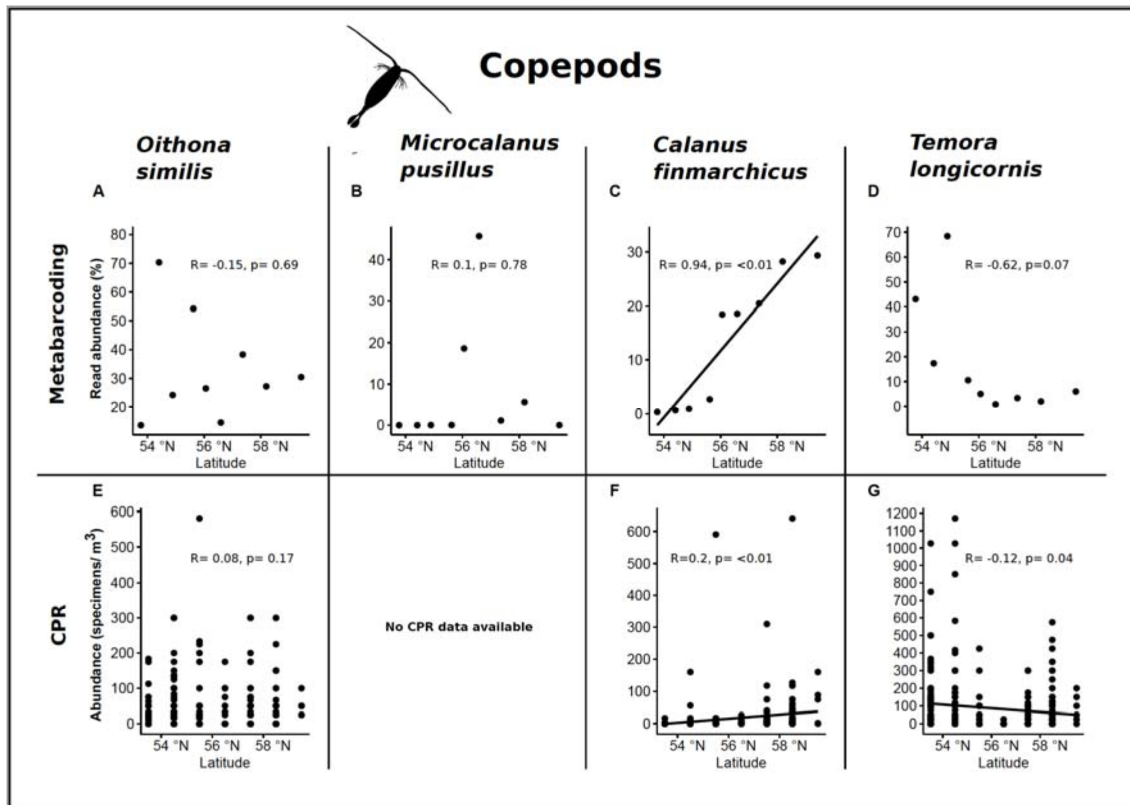
303 species significantly increased with latitude (Figure 3f). *Temora longicornis* showed a
304 negative, but non-significant trend in read abundance from southern to northern sampling
305 sites in the metabarcoding data (Figure 3d). This trend was also found in the CPR data and
306 was significant (Figure 3g) (All statistical results: Tale S3)



307

308 **Figure 3:** Correlations with latitude of the four most abundant copepod taxa in the
309 metabarcoding dataset compared to data of the Continuous Plankton Recorder (CPR).
310 Correlations were based on relative read abundances (metabarcoding data) and
311 specimens/m^3 (CPR).

312

313 **Ray-finned fishes**

314 Out of 16 identified ray-finned fish species, 3 (18.8%) were found exclusively in the
315 'northern/deep' sampling sites, while 6 (37.5%) were only found in the 'southern/shallow'
316 sites, and 7 (43.6%) were found in both areas (Figure 2c). The most abundant species were
317 the common mackerel (*Scomber scombrus*, 48.6% of ray-finned fish reads), common dab

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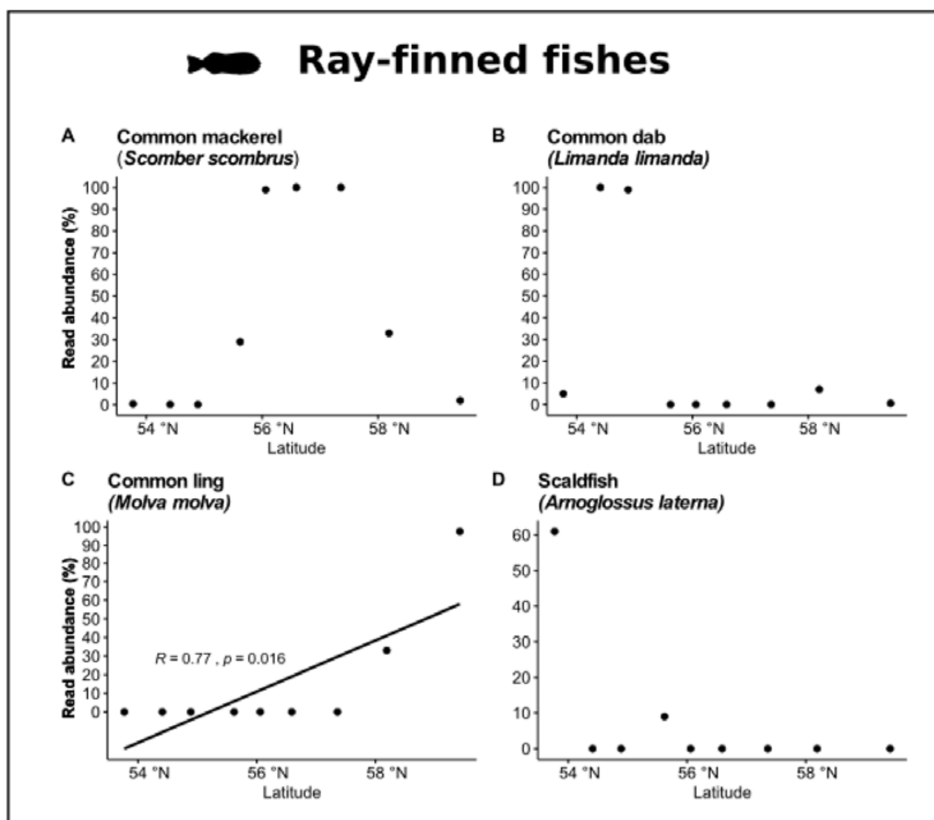
318 (*Limanda limanda*, 19%), common ling (*Molva molva*, 11.8%) and the scaldfish *Arnoglossus*
319 *laterna* (8.3%). As the CPR assesses fish on the level of eggs and larvae without species
320 identification, no comparison of metabarcoding and CPR data was possible.
321 High read abundances of the common mackerel (*Scomber scombrus*) were found in the
322 central and northern part of the North Sea (>90% of ray-finned fish reads; sites S109, 56.06
323 °N; S22, 56.59 °N; S93, 57.36 °N)(figure 4a). The common dab (*Limanda limanda*) was
324 found in high abundance (>90% of fish reads) at sampling sites S176 (54.41°N) and S156
325 (54.89°N) in the southern part of the North Sea (figure 4b). For the common ling (*Molva*
326 *molva*), a significant correlation of latitude and read abundance was found, with read
327 abundance reaching >90% of reads in the northernmost sampling site S74 (59.42 °N, figure
328 4c). The scaldfish (*Arnoglossus laterna*) showed a high read abundance (>60%) in the
329 southernmost sampling site S203 (53.77 °N), but no significant correlation of read
330 abundance and latitude was found (figure 4d) (statistical results: table S4).



331

332 **Figure 4:** Correlations of relative read abundance with latitude of the four most abundant

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333 ray-finned fish species in the metabarcoding dataset (only shown when significant).

334

335 **Polychaetes**

336 Of the 29 identified polychaete species, 11 (37.9%) were exclusively found in the
337 'northern/deep' sampling sites, 9 (31%) exclusively in the 'southern/shallow' sampling sites,
338 and 9 (31%) were found in both areas (Figure 2 d). The most abundant species were
339 *Paramphinome jeffreysii* (28.3% of polychaete reads), *Glycera lapidum* sp. 1 (20.9%),
340 *Pectinaria koreni* (18.4%) and *Magelona johnstoni* (11.8%). As the CPR registers all
341 polychaetes apart from the holoplanktonic Tomopteris, as (unidentified) larvae, no
342 comparison of metabarcoding data and CPR data was possible.
343 *Paramphinome jeffreysii* showed a strong positive correlation of read abundance and latitude
344 (figure 5 a), with read abundances reaching >60% of polychaete reads in all 'northern/deep'
345 sampling sites, while it was only found in one site in the southern North Sea, with a read
346 abundance of <1%. *Pectinaria koreni* did not show a significant correlation of read
347 abundance and latitude, but was found in high abundance in two sampling sites in the
348 'southern/shallow' area of the North Sea (S156, 47%; S130, 29%), and 20% read abundance
349 in the northernmost sampling site (figure 5b). *Glycera lapidum* sp. 1 was commonly found
350 with less than 1% of reads, except in the two southern sampling sites S176 (54.41°N) and
351 S130 (55.62°N), where the species occurred with >50% read abundance. No significant
352 correlation of read abundance and latitude was found (figure 5c). For *Magelona johnstoni*,
353 the highest relative abundances were found in 'southern/shallow' sites (S203, 63%;
354 respectively 32% in S156). Read abundance at all other sampling sites was <1% (figure 5d)
355 (All statistical results: Table S5)

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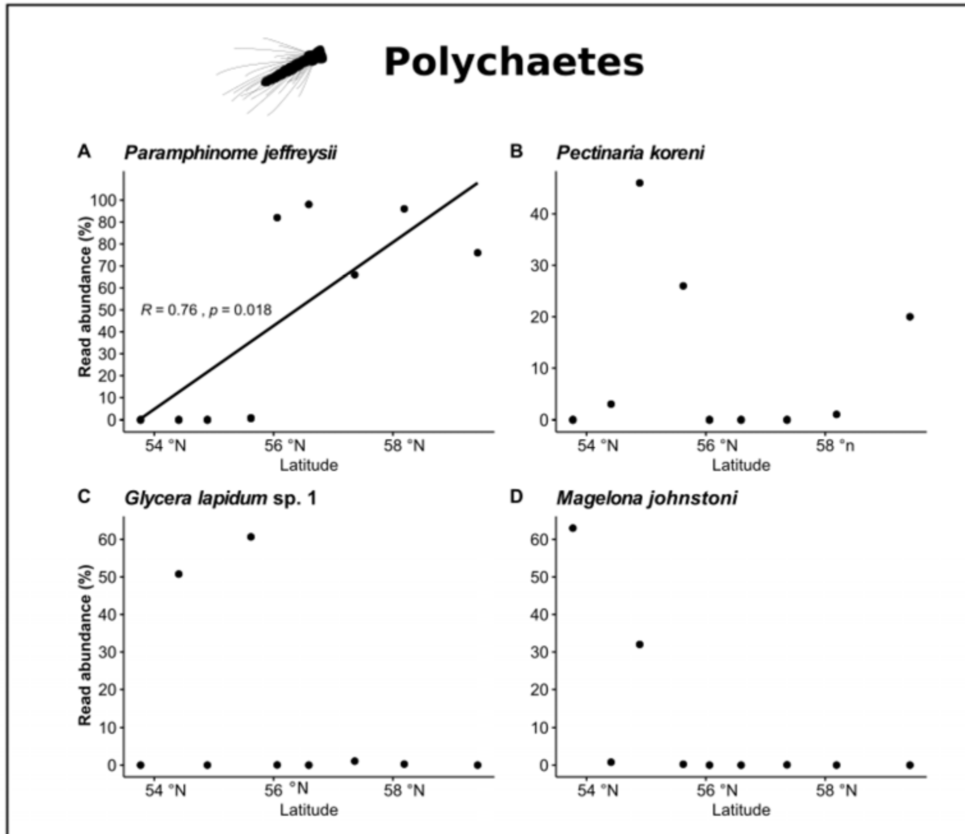

356

357 **Figure 5:** Correlations of relative read abundance with latitude of the four most abundant

358 polychaete species in the metabarcoding dataset (only shown when significant).

359

360 ## Discussion

361 Using metabarcoding we show that the overall community composition of zooplankton differs
362 between northern and southern areas of the North Sea. Differences in community
363 composition are associated with the influx of Atlantic water from the north, which has been
364 documented before based on morphological identifications of zooplankton (Fransz et al.,
365 1991; Krause, M., Dippner, J.W., Beil, J., 1995). However, the eggs and larval stages of
366 common groups such as fish and polychaetes are difficult to identify based on morphology,
367 which is why they are often not recorded by long-term monitoring programs such as the CPR
368 (Reid et al., 2003a). Metabarcoding can help identify those species, potentially allowing
369 better insights into the composition of communities and species distribution patterns

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370 (Kimmerling et al., 2018; Schroeder et al., 2020). Our data show that valuable information on
371 the distribution of zooplankton in the North Sea can be gained through metabarcoding,
372 including information of meroplanktonic species that are still little studied. However, we
373 acknowledge that our study is based on relatively few samping sites. To assess the
374 distribution of zooplankton species in the North Sea more accurately a denser sampling
375 scheme in time and space should be employed. Such sampling is performed by the
376 Continuous Plankton Recorder (Reid et al., 2003a), and future studies should aim at utilising
377 CPR samples for metabarcoding (see e.g. (Kirby, Lindley & Batten, 2006; Stern et al.,
378 2018)). In this way, the long-term CPR dataset could also provide more detailed insights into
379 meroplanktonic groups such as fish and benthic invertebrates. In this study, we could only
380 compare our metabarcoding results to the CPR data on copepods. We point out that
381 comparisons of metabarcoding and CPR data should be interpreted with care, as the CPR
382 samples only the top layers of the water column, and abundance inferred from
383 metabarcoding data can be biased due to amplification and primers bias (Elbrecht & Leese,
384 2015, 2017; Wangensteen et al., 2018; Zizka et al., 2019a). Further, comparing
385 metabarcoding read abundance and number of specimens, as mostly provided by previous
386 morphological studies, is challenging, as reads do not automatically correlate with specimen
387 numbers. However, our results show that the abundance data inferred through
388 metabarcoding mostly matches with the known distribution of species described in previous
389 studies, thus underlining that metabarcoding is a potentially powerful tool for monitoring of
390 zooplankton communities. The highly degenerate Leray XT primers used in this study
391 amplified a wide range of planktonic taxa. A high percentage (96%) of reads could be
392 assigned to a taxonomic class, and only 1% of these reads were assigned to non-metazoan
393 taxa. This is in contrast to previous metabarcoding studies targeting highly diverse
394 communities, which highlighted that degenerate primers commonly co-amplify a high
395 number of non-metazoan taxa (Weigand & Macher, 2018; Wangensteen et al., 2018). We
396 assume that only little non-metazoan biomass was present in our samples, leading to
397 successful amplification of the target planktonic animals. However, only 75% of reads could

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398 be assigned to species level, which highlights the lack of genetic reference data for marine
399 planktonic organisms. This can render molecular identification of species impossible (see
400 e.g. (Porter & Hajibabaei, 2018; Weigand et al., 2019)) and underlines the need for improved
401 reference databases, which can only be otained with the help of taxonomic experts. We
402 discuss the findings on copepods, ray-finned fishes and polychaetes in more detail below.
403

404 **Copepods**

405 Our results show that the copepod community significantly differs between northern and
406 southern areas of the North Sea, which is congruent with previous studies based on
407 morphological identifications (Fransz et al., 1991; Beaugrand et al., 2002). Comparison of
408 the most abundant copepod species in our metabarcoding dataset with CPR data showed a
409 high level of congruence. *Oithona similis* did not show significant differences in read
410 abundance between 'northern/deep' and 'southern/shallow' sampling sites, which
411 corresponds to the *Oithona* spp. abundances recorded by the CPR. We acknowledge that a
412 direct comparison of Oithona similis and *Oithona* spp. data are potentially biased, as other
413 *Oithona* species can be present in the North Sea. Fransz (1991) however pointed out that *O.*
414 *similis* is commonly the most abundant *Oithona* species in the North Sea. Corresponding to
415 that information, the only other *Oithona* species we identified in our metabarcoding dataset
416 was *Oithona atlantica*, which was found in low read abundances (1% of copepod reads). We
417 therefore see the comparison as legitimate. *Microcalanus pusillus* was the second most
418 abundant species in the metabarcoding dataset, but we could not compare our data with
419 CPR data. The species is likely overlooked by CPR sampling due to its distribution in deeper
420 water layers and its small size, which are not sampled by the CPR (Hays & Warner, 1993;
421 Hays, 1994). Previous work identified *M. pusillus* as a mostly Atlantic species (Fransz et al.,
422 1991; Beaugrand et al., 2002) but our data did not show a correlation between abundance of
423 this species and latitude. We found *M. pusillus* in high abundance in the central North Sea.
424 This area, which is located north of the shallow Doggers Bank is known for upwellings
425 bringing nutrient-rich bottom water closer to the surface (Nielsen et al., 1993), which might

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426 explain the high abundance of this species in the area. *Calanus finmarchicus* showed a
427 strong positive correlation of abundance with latitude, and this pattern was also found in the
428 CPR data. Our results are in congruence with previous findings, which found the species to
429 be mostly restricted to northern, Atlantic waters (Beaugrand et al., 2002; Marshall & Orr,
430 2013). For *Temora longicornis*, an indicator species of coastal waters (Beaugrand et al.,
431 2002), high abundances in the coastal regions were found in both the metabarcoding
432 dataset and the CPR data.
433 Copepod species that we found exclusively in the 'northern/deep' area of the North Sea,
434 such as *Candacia armata*, *Scolecithricella minor*, *Anomalocera patersoni*, *Diaixis hibernica*
435 and *Pseudocalanus acuspes*, are all species known predominantly from northern areas of
436 the North Sea (Fransz et al., 1991; Beaugrand et al., 2002; Hovda & Fosshagen, 2003). We
437 further identified *Goniopsyllus rostrata* and *Clausocalanus pergens*, which to the best of our
438 knowledge have not yet been reported from the North Sea. The reference sequences in the
439 BOLD database stem from specimens sampled off northern Spain (BOLD BIN numbers:
440 AAO2968; AAJ1005). We further detected *Pseudocalanus mimus*, which is generally
441 considered a North Pacific species (Frost, 1989; Questel et al., 2016), and that is also where
442 the BOLD references (BIN AAH8134) stem from. However, a few records of the species
443 from between Canada and Greenland exist (http://www.iobis.org/)(Nelson, 2014). The species
444 has not previously been recorded from the North East Atlantic, which could mean that the
445 species was either overlooked, or that its distribution range has recently expanded. Of the
446 copepod species that were exclusively found in the southern sampling sites, all except
447 *Caligus elongatus*, a fish parasite, are known as typical of coastal/shallow waters:
448 *Longipedia* sp. DZMB181 (Khodami et al., 2017), *Haloschizopera pygmaea* (Rossel &
449 Martínez Arbizu, 2019)*, Isias clavipes (Beaugrand et al., 2000),* and *Acartia tonsa* (Fransz et
450 al., 1991; Caudill & Bucklin, 2004).
451

452 **Ray-finned fishes**

453 Fish larvae and eggs are part of the zooplankton for a limited time and their occurrence is

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454 mostly influenced by spawning and nursery areas to which eggs and larvae drift (Knijn,
455 1993; Gibson, 2001; Gibson et al., 2015). We found that the inferred community composition
456 of ray-finned fishes strongly differed between northern and southern sampling sites in the
457 North Sea, and that our data corresponds to known distribution patterns of fish species and
458 their spawning areas. The three species exclusively found in the 'northern/deep' sampling
459 sites were the grey gurnard (*Eutrigla gurnadus*), the argentine (*Argentina sphyraena*) and
460 the slender snipe eel (*Nemichthys scolopaceus*). The grey gurnard and the argentine are
461 known to occur mostly in deeper, northern waters (Knijn, 1993; Wright, Jensen & Tuck,
462 2000), while the slender snipe eel is known from deep sea environments (Feagans-Bartow &
463 Sutton, 2014; Lusher et al., 2016). This corresponds to our results, as we found the slender
464 snipe eel exclusively in the Devil's Hole sampling site (S22) which reaches 230m water
465 depth. Of the species exclusively found in 'southern/shallow' sampling sites, the solenette
466 (*Buglossidium luteum*), European sprat (*Sprattus sprattus*), striped red mullet (*Mullus*
467 *surmuletus*) and common sole (*Solea solea*) are all known to be mainly distributed along the
468 coastlines and in southern regions of the North Sea (Knijn, 1993; Milner, 2016), which is
469 congruent with our findings. The only exception is the sand eel *Ammodytes marinus*, which
470 can be found in shallow, sandy habitats throughout the North Sea, but we found in only one
471 sampling site in the southern North Sea. We assume that we did not find the species in more
472 samples due to low overall abundance and competition with other species during
473 amplification and sequencing of the data. Separate analyses of the four most abundant ray-
474 finned fish taxa correspond to previous findings showing that coastal areas of the North Sea
475 are important spawning and nursing grounds for these species, namely, common dab
476 (*Limanda limanda*) (Bolle et al., 1994) and scaldfish (*Arnoglossus laterna*)(Land & Van der
477 Land, 1991; van Hal, Smits & Rijnsdorp, 2010). Our finding that the common mackerel
478 (*Scomber scombrus*) showed the highest read abundance in the central part of the North
479 Sea corresponds to the known spawning area of this species (Jansen et al., 2012). The
480 common ling (*Molva molva*), found in high abundances in the northernmost sampling sites, is
481 also known to spawn in these areas (Knijn, 1993). Further research will show if

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482 metabarcoding will detect known distribution and spawning areas for a high number of fish
483 species, which will be helpful for monitoring of populations.
484
485 **Polychaetes**
486 With the exception of the holoplanktonic *Tomopteris spp.*, polychaetes in the North Sea are
487 meroplanktonic (Plate & Husemann, 1994; Van Ginderdeuren et al., 2014). Even though
488 polychaetes are a highly diverse and abundant group, their planktonic stages are relatively
489 little known due to difficulties in identification (Williams et al., 1993; Vezzulli & Reid, 2003;
490 Heimeier, Lavery & Sewell, 2010). As for the copepods and ray-finned fishes, we found a
491 strong difference in community composition of polychaetes between northern and southern
492 sampling sites in the North Sea. This corresponds to known patterns of macrobenthos
493 community differences between shallow areas in the southern and northern North Sea
494 (Duineveld et al., 1991). However, information on the distribution of most of the identified
495 species in the North Sea is scarce or not available, rendering a comparison of our
496 metabarcoding data to previous data based on morphological identifications mostly
497 impossible. We assume that the lack of information on many species is due to difficulties in
498 reliable identification and the lack of taxonomic experts, which highlights the need for a
499 combined morphological and molecular approach for future studies and the preparation of
500 reference libraries. Separate analyses of the four most abundant species showed that the
501 polychaete community was dominated by *Paramphinome jeffreysii* in the 'northern/deep'
502 sampling sites. In congruence with our results, this species was previously found in high
503 abundance in northern regions (Kröncke et al., 2011). *Pectinaria koreni* has been recorded
504 in areas of fine sediment, often closer to the coast (Thiébaut et al., 1997; Desroy, 2003) and
505 from areas near the Shetland islands (GBIF dataset: https://doi.org/10.15468/39omei). We
506 also found this species in high abundance in the 'southern/shallow' area of the North Sea, as
507 well as in the northernmost sampling site close to the Shetland Islands. Little information is
508 available on the distribution and larval stages of *Glycera lapidum* and *Magelona johnstoni*.
509 Both species are known from several regions of the North Sea (Kunitzer et al., 1992;

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510 Meißner & Darr, 2009). We consider it possible that the high abundance of these species in
511 a few sampling sites can be explained by local spawning events. Overall, our results show
512 the power of metabarcoding to assess the meroplanktonic polychaete community, but we
513 conclude that more combined molecular and morphological work is required to fully
514 understand distribution patterns of polychaete larvae.
515

516 **Conclusion**

517 We showed that metabarcoding of zooplankton samples from the North Sea, using highly
518 degenerate COI primers, can give valuable insights into the diversity and distribution of
519 planktonic animals. We found clear differences in the overall zooplankton assemblages
520 between northern and southern areas of the North Sea, as well as more specifically for
521 copepods, ray-finned fishes and polychaetes. Our results were largely congruent with
522 previous studies based on morphological identifications, which indicates the robustness of
523 our molecular approach. Nevertheless, we highlight the need for more complete reference
524 databases to be able to make full use of the information gained through metabarcoding. We
525 suggest that metabarcoding should be considered for implementation into future biodiversity
526 assessments, as the ability to quickly assess whole zooplankton samples is valuable for
527 biodiversity studies in times of rapid ocean changes.
528

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532

533 Data availability

534 All raw data are available from figshare: https://doi.org/10.6084/m9.figshare.12698054.v1

535 Declaration of Interests

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536 The authors declare no competing interests.

537 Field Study Permissions

538 The following information was supplied relating to field study approvals (i.e., approving body
539 and any reference numbers): Sampling in UK waters was approved by the Maritime Policy
540 Unit (Legal Directorate) of the Foreign and Commonwealth Office (ref 33/2018).

541

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