

# 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

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;

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

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

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 (Wangenstein  
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.

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 (Wangenstein 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

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-Conceicao 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

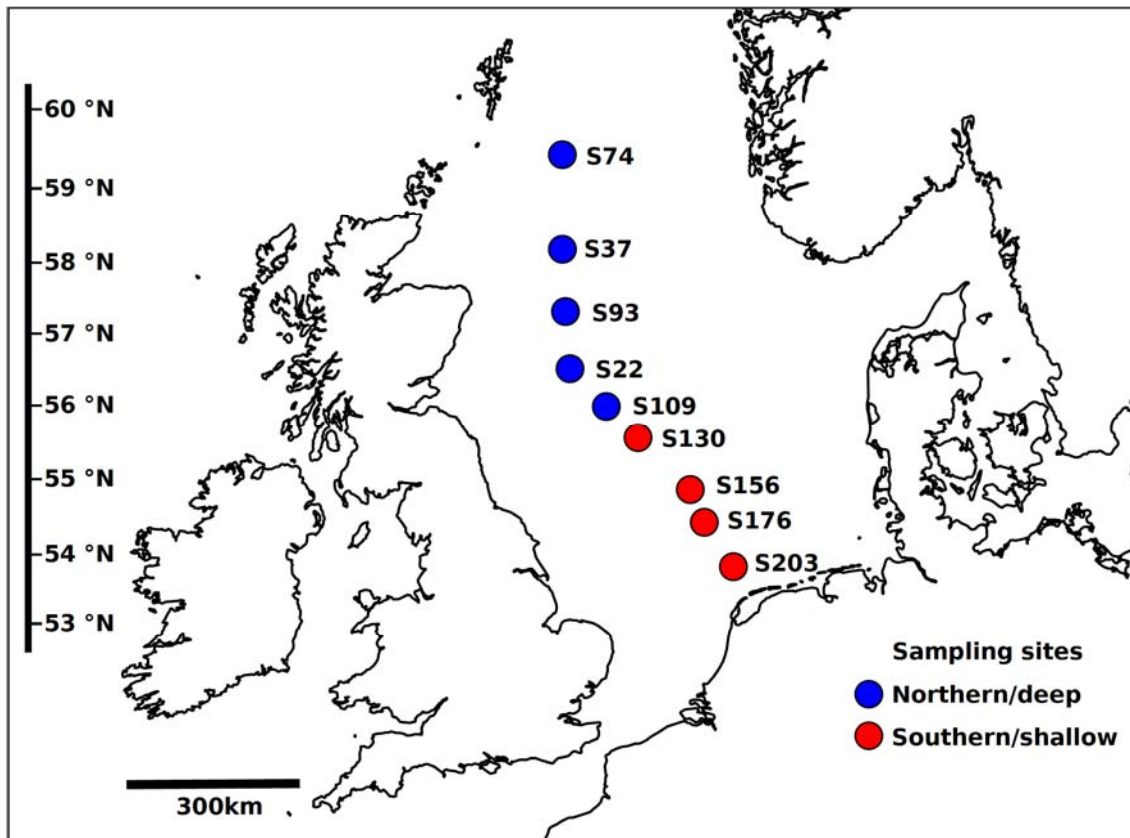
## 180 **Community analyses**

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'



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



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<sup>3</sup>). 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

## 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 ( $R^2 = 0.35$ ,  $p =$   
259  $0.004$ ) as well as between northern and southern sites categorized based on salinity ( $R^2 =$   
260  $0.31$ ,  $p = 0.018$ , Table 1). Water temperature did not explain overall community composition.

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

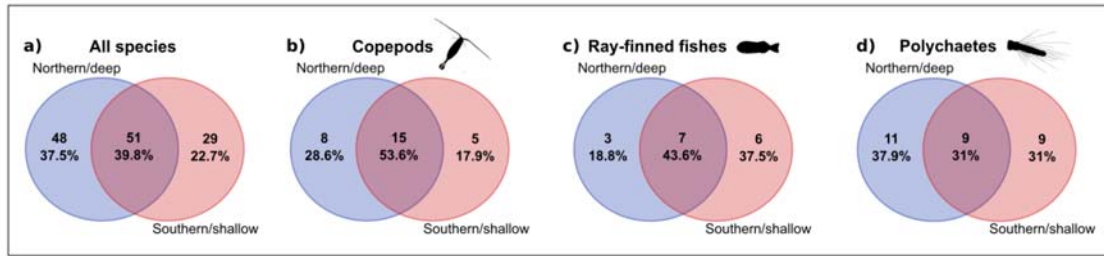
267 **Table 1:** Difference in community composition based on Bray- Curtis distance for all species,  
 268 copepods, ray- finned fishes and polychaetes. Results show the difference in community  
 269 composition of 'northern/deep' versus 'southern/shallow' sampling sites; sites with higher  
 270 versus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

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



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

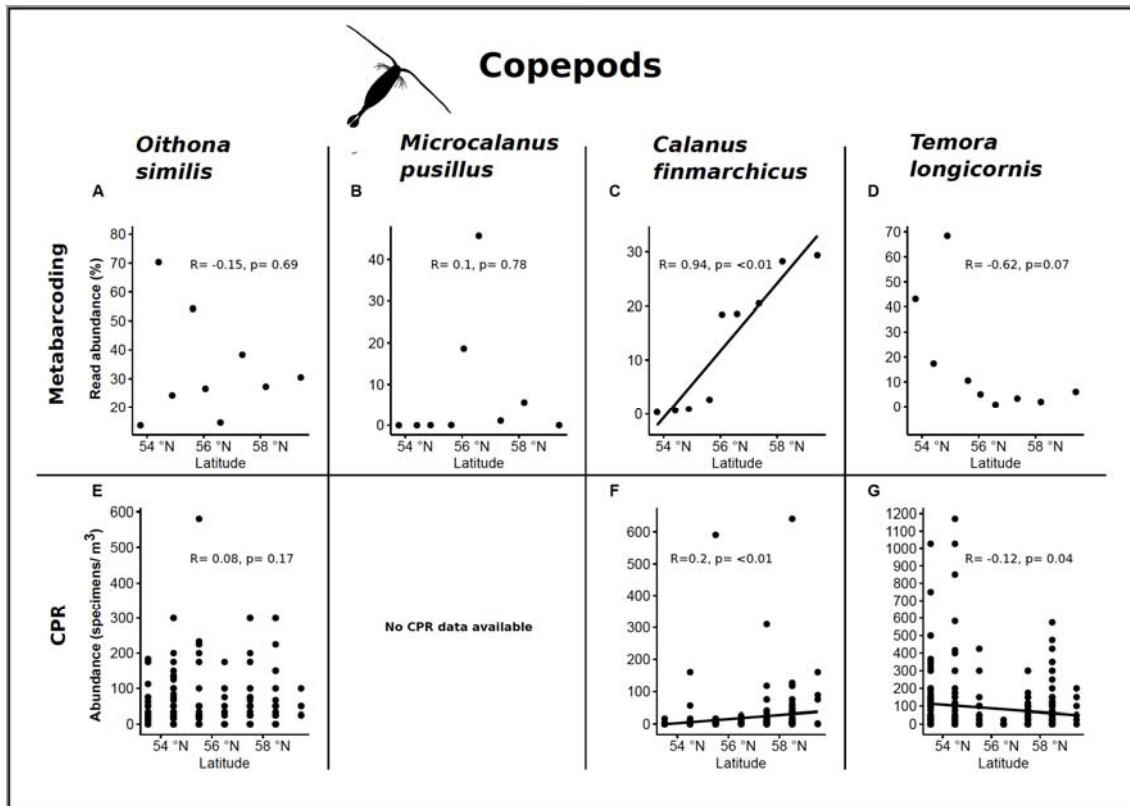
283

### 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

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)



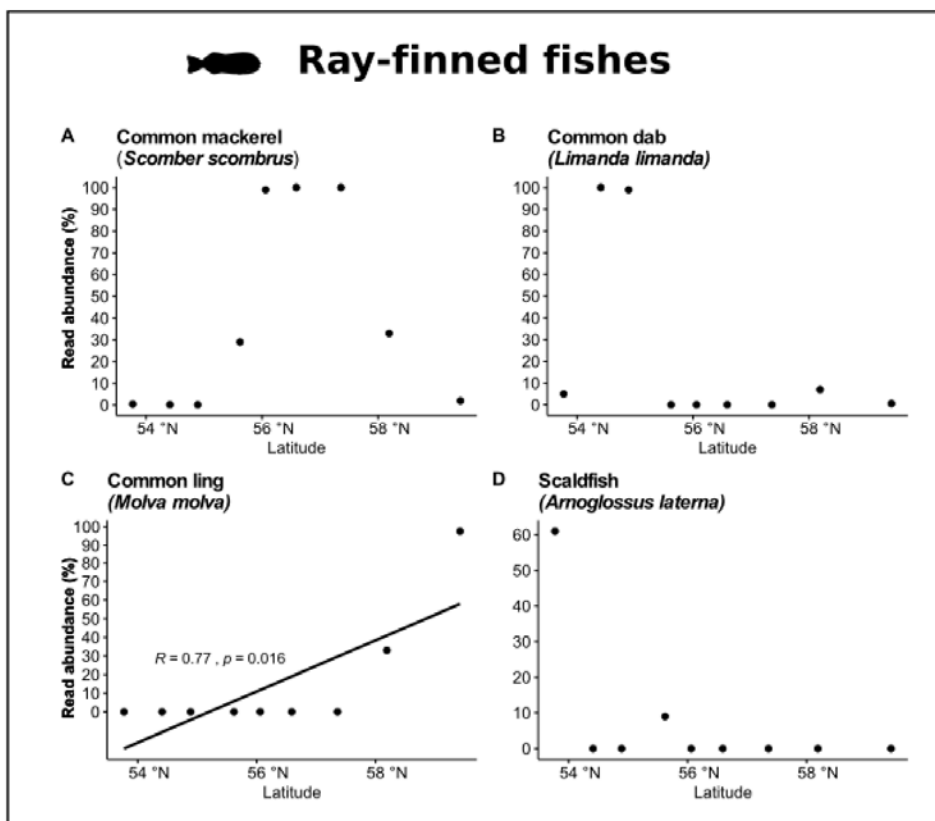
307  
 308 **Figure 3:** Correlations with latitude of the four most abundant copepod taxa in the  
 309 metabarcoding dataset compared to data of the Continuous Plankton Recorder (CPR).  
 310 Correlations were based on relative read abundances (metabarcoding data) and  
 311 specimens/ $m^3$  (CPR).

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

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

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

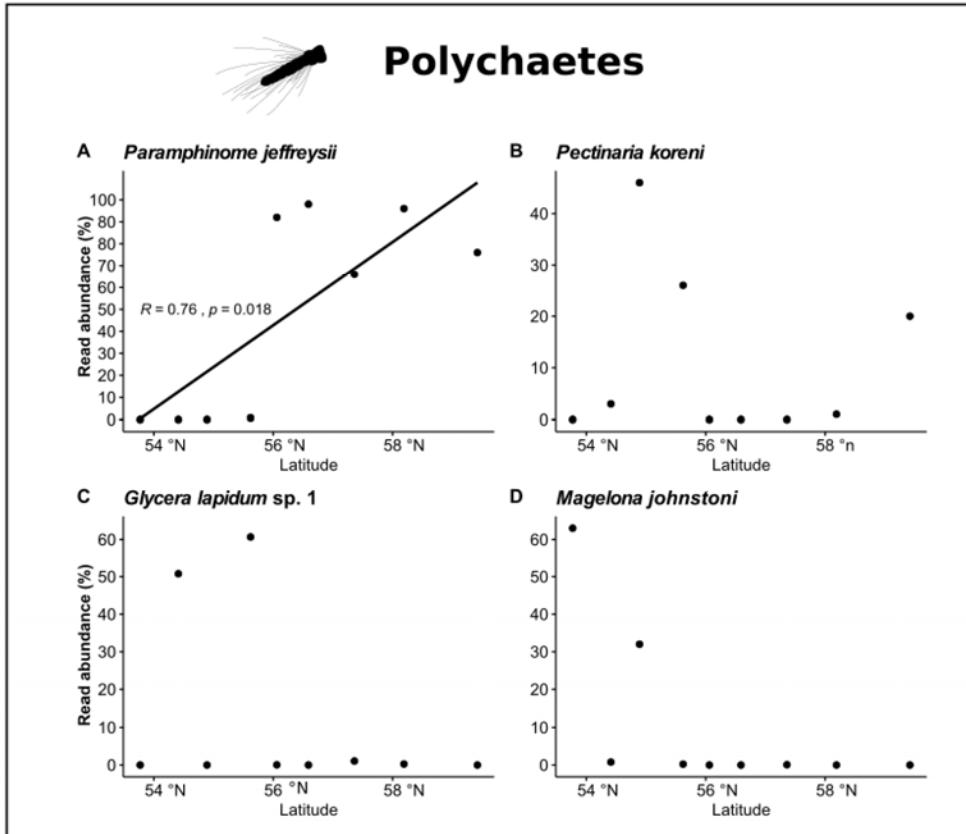
334

### 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)





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

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 sampling 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

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 obtained 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

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

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

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 (Thiébaud 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;

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

## 529 **Acknowledgements**

530 We thank the Pelagia crew for help during the NICO leg 10 cruise and Rob Witbaard for  
531 organising and leading the NICO leg 10. We thank Elza Duijm for support in the lab.

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**



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

## 542 **Reference**

- 543 Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D,  
544 Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltmann S, Jalili V, Rasche H,  
545 Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy  
546 platform for accessible, reproducible and collaborative biomedical analyses: 2018  
547 update. *Nucleic acids research* 46:W537–W544.
- 548 Alvarez-Fernandez S, Lindeboom H, Meesters E. 2012. Temporal changes in plankton of the  
549 North Sea: community shifts and environmental drivers. *Marine Ecology Progress*  
550 *Series* 462:21–38. DOI: 10.3354/meps09817.
- 551 Andruszkiewicz EA, Starks HA, Chavez FP, Sassoubre LM, Block BA, Boehm AB. 2017.  
552 Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS*  
553 *one* 12:e0176343.
- 554 Beare DJ, Batten S, Edwards M, Reid DG. 2002. Prevalence of boreal Atlantic, temperate  
555 Atlantic and neritic zooplankton in the North Sea between 1958 and 1998 in relation to  
556 temperature, salinity, stratification intensity and Atlantic inflow. *Journal of Sea Research*  
557 48:29–49. DOI: 10.1016/s1385-1101(02)00131-4.
- 558 Beaugrand G, Edwards M, Legendre L. 2010. Marine biodiversity, ecosystem functioning,  
559 and carbon cycles. *Proceedings of the National Academy of Sciences of the United*  
560 *States of America* 107:10120–10124.

- 561 Beaugrand G, Ibañez F, Lindley JA, Philip C, Reid PC. 2002. Diversity of calanoid copepods  
562 in the North Atlantic and adjacent seas: species associations and biogeography. *Marine*  
563 *Ecology Progress Series* 232:179–195. DOI: 10.3354/meps232179.
- 564 Beaugrand G, Reid PC, Ibañez F, Planque B. 2000. Biodiversity of North Atlantic and North  
565 Sea calanoid copepods. *Marine Ecology Progress Series* 204:299–303. DOI:  
566 10.3354/meps204299.
- 567 Beentjes KK, Speksnijder AGCL, Schilthuizen M, Hoogeveen M, van der Hoorn BB. 2019.  
568 The effects of spatial and temporal replicate sampling on eDNA metabarcoding. *PeerJ*  
569 7:e7335.
- 570 Bolle LJ, Dapper R, Witte JIJ, Van Der Veer HW. 1994. Nursery grounds of dab (*Limanda*  
571 *limanda* L.) in the southern North Sea. *Netherlands Journal of Sea Research* 32:299–  
572 307. DOI: 10.1016/0077-7579(94)90007-8.
- 573 Brown EA, Chain FJJ, Crease TJ, MacIsaac HJ, Cristescu ME. 2015. Divergence thresholds  
574 and divergent biodiversity estimates: can metabarcoding reliably describe zooplankton  
575 communities? *Ecology and evolution* 5:2234–2251.
- 576 Buchner D, Leese F. 2020. BOLDigger – a Python package to identify and organise  
577 sequences with the Barcode of Life Data systems. *Metabarcoding and Metagenomics* 4.  
578 DOI: 10.3897/mbmg.4.53535.
- 579 Bucklin A, Lindeque PK, Rodriguez-Ezpeleta N, Albaina A, Lehtiniemi M. 2016.  
580 Metabarcoding of marine zooplankton: prospects, progress and pitfalls. *Journal of*  
581 *Plankton Research* 38:393–400. DOI: 10.1093/plankt/fbw023.
- 582 Burdon FJ, Reyes M, Alder AC, Joss A, Ort C, Räsänen K, Jokela J, Eggen RIL, Stamm C.  
583 2016. Environmental context and magnitude of disturbance influence trait-mediated  
584 community responses to wastewater in streams. *Ecology and evolution* 6:3923–3939.
- 585 Casas L, Pearman JK, Irigoien X. 2017. Metabarcoding Reveals Seasonal and  
586 Temperature-Dependent Succession of Zooplankton Communities in the Red Sea.  
587 *Frontiers in Marine Science* 4. DOI: 10.3389/fmars.2017.00241.
- 588 Caudill CC, Bucklin A. 2004. Molecular Phylogeography and Evolutionary History of the

- 589 Estuarine Copepod, *Acartia tonsa*, on the Northwest Atlantic Coast. *Hydrobiologia*  
590 511:91–102. DOI: 10.1023/b:hydr.0000014032.05680.9d.
- 591 Chain FJJ, Brown EA, MacIsaac HJ, Cristescu ME. 2016. Metabarcoding reveals strong  
592 spatial structure and temporal turnover of zooplankton communities among marine and  
593 freshwater ports. *Diversity and Distributions* 22:493–504. DOI: 10.1111/ddi.12427.
- 594 Chiba S, Batten S, Martin CS, Ivory S, Miloslavich P, Weatherdon LV. 2018. Zooplankton  
595 monitoring to contribute towards addressing global biodiversity conservation challenges.  
596 *Journal of plankton research* 40:509–518.
- 597 Clarke LJ, Beard JM, Swadling KM, Deagle BE. 2017. Effect of marker choice and thermal  
598 cycling protocol on zooplankton DNA metabarcoding studies. *Ecology and evolution*  
599 7:873–883.
- 600 Cohen J. 2013. *Statistical Power Analysis for the Behavioral Sciences*. Routledge.
- 601 Collins RA, Bakker J, Wangenstein OS, Soto AZ, Corrigan L, Sims DW, Genner MJ, Mariani  
602 S. 2019. Non-specific amplification compromises environmental DNA metabarcoding  
603 with COI. *Methods in Ecology and Evolution*. DOI: 10.1111/2041-210x.13276.
- 604 Cornils A, Held C. 2014. Evidence of cryptic and pseudocryptic speciation in the  
605 *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Frontiers in*  
606 *zoology* 11:19.
- 607 Couton M, Comtet T, Le Cam S, Corre E, Viard F. 2019. Metabarcoding on planktonic larval  
608 stages: an efficient approach for detecting and investigating life cycle dynamics of  
609 benthic aliens. *Management of Biological Invasions* 10:657–689. DOI:  
610 10.3391/mbi.2019.10.4.06.
- 611 Deagle BE, Clarke LJ, Kitchener JA, Polanowski AM, Davidson AT. 2018. Genetic  
612 monitoring of open ocean biodiversity: An evaluation of DNA metabarcoding for  
613 processing continuous plankton recorder samples. *Molecular ecology resources*  
614 18:391–406.
- 615 Desroy N. 2003. Macrobenthic resources of the shallow soft-bottom sediments in the eastern  
616 English Channel and southern North Sea. *ICES Journal of Marine Science* 60:120–131.

- 617 DOI: 10.1006/jmsc.2002.1333.
- 618 Duineveld GCA, Kunitzer A, Niermann U, De Wilde PAWJ, Gray JS. 1991. The  
619 macrobenthos of the north sea. *Netherlands Journal of Sea Research* 28:53–65. DOI:  
620 10.1016/0077-7579(91)90004-k.
- 621 Edgar RC. 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon  
622 sequencing. DOI: 10.1101/081257.
- 623 Elbrecht V, Leese F. 2015. Can DNA-based ecosystem assessments quantify species  
624 abundance? Testing primer bias and biomass - sequence relationships with an  
625 innovative metabarcoding protocol. *PloS one*, 10(7), e0130324.
- 626 Elbrecht V, Leese F. 2017. Validation and development of COI metabarcoding primers for  
627 freshwater macroinvertebrate bioassessment. *Frontiers in Environmental Science* 5  
628 (2017): 11.
- 629 Elbrecht V, Vamos EE, Meissner K, Aroviita J, Leese F. 2017. Assessing strengths and  
630 weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine  
631 stream monitoring. *Methods in Ecology and Evolution* 8:1265–1275. DOI:  
632 10.1111/2041-210x.12789.
- 633 Elbrecht V, Vamos EE, Steinke D, Leese F. 2018. Estimating intraspecific genetic diversity  
634 from community DNA metabarcoding data. *PeerJ* 6:e4644.
- 635 Feagans-Bartow JN, Sutton TT. 2014. Ecology of the oceanic rim: pelagic eels as key  
636 ecosystem components. *Marine Ecology Progress Series* 502:257–266. DOI:  
637 10.3354/meps10707.
- 638 Franz HG, Colebrook JM, Gamble JC, Krause M. 1991. The zooplankton of the north sea.  
639 *Netherlands Journal of Sea Research* 28:1–52. DOI: 10.1016/0077-7579(91)90003-j.
- 640 Frost BW. 1989. A taxonomy of the marine calanoid copepod genus *Pseudocalanus*.  
641 *Canadian Journal of Zoology* 67:525–551. DOI: 10.1139/z89-077.
- 642 Galan M, Pons J-B, Tournayre O, Pierre É, Leuchtman M, Pontier D, Charbonnel N. 2018.  
643 Metabarcoding for the parallel identification of several hundred predators and their prey:  
644 Application to bat species diet analysis. *Molecular ecology resources* 18:474–489.

- 645 Gibson RN. 2001. *Oceanography and Marine Biology, An Annual Review, Volume 39: An*  
646 *Annual Review*: CRC Press.
- 647 Gibson RN, Nash RDM, Geffen AJ, Van der Veer HW. 2015. *Flatfishes: Biology and*  
648 *Exploitation*. John Wiley & Sons.
- 649 Greve W, Lange U, Reiners F, Nast J. 2001. Predicting the seasonality of North Sea  
650 zooplankton. *Senckenbergiana maritima* 31:263–268. DOI: 10.1007/bf03043035.
- 651 van Hal R, Smits K, Rijnsdorp AD. 2010. How climate warming impacts the distribution and  
652 abundance of two small flatfish species in the North Sea. *Journal of Sea Research*  
653 64:76–84. DOI: 10.1016/j.seares.2009.10.008.
- 654 Harvey JBJ, Fisher JL, Ryan JP, Johnson SB, Peterson WT, Vrijenhoek RC. 2018. Changes  
655 in zooplankton assemblages in northern Monterey Bay, California, during a fall  
656 transition. *Marine Ecology Progress Series* 604:99–120. DOI: 10.3354/meps12742.
- 657 Hays GC. 1994. Mesh selection and filtration efficiency of the Continuous Plankton  
658 Recorder. *Journal of Plankton Research* 16:403–412. DOI: 10.1093/plankt/16.4.403.
- 659 Hays GC, Warner AJ. 1993. Consistency of Towing Speed and Sampling Depth for the  
660 Continuous Plankton Recorder. *Journal of the Marine Biological Association of the*  
661 *United Kingdom* 73:967–970. DOI: 10.1017/s0025315400034846.
- 662 Heimeier D, Lavery S, Sewell MA. 2010. Using DNA barcoding and phylogenetics to identify  
663 Antarctic invertebrate larvae: Lessons from a large scale study. *Marine genomics*  
664 3:165–177.
- 665 Hovda JI, Fosshagen A. 2003. Hyperbenthic calanoids and *Thespesiopsyllus paradoxus*  
666 (Sars) collected with a light trap in western Norway. *Sarsia* 88:89–94. DOI:  
667 10.1080/00364820308467.
- 668 Jansen T, Kristensen K, Payne M, Edwards M, Schrum C, Pitois S. 2012. Long-term  
669 retrospective analysis of mackerel spawning in the North Sea: a new time series and  
670 modeling approach to CPR data. *PloS one* 7:e38758.
- 671 Khodami S, McArthur JV, Blanco-Bercial L, Martinez Arbizu P. 2017. Molecular Phylogeny  
672 and Revision of Copepod Orders (Crustacea: Copepoda). *Scientific reports* 7:9164.

- 673 Kimmerling N, Zuerqert O, Amitai G, Gurevich T, Armoza-Zvuloni R, Kolesnikov I, Berenshtein  
674 I, Melamed S, Gilad S, Benjamin S, Rivlin A, Ohavia M, Paris CB, Holzman R, Kiflawi M,  
675 Sorek R. 2018. Quantitative species-level ecology of reef fish larvae via metabarcoding.  
676 *Nature ecology & evolution* 2:306–316.
- 677 Kirby RR, Lindley JA. 2005. Molecular analysis of Continuous Plankton Recorder samples,  
678 an examination of echinoderm larvae in the North Sea. *Journal of the Marine Biological*  
679 *Association of the United Kingdom* 85:451–459. DOI: 10.1017/s0025315405011392.
- 680 Kirby RR, Lindley JA, Batten SD. 2006. Spatial heterogeneity and genetic variation in the  
681 copepod *Neocalanus cristatus* along two transects in the North Pacific sampled by the  
682 Continuous Plankton Recorder. *Journal of Plankton Research* 29:97–106. DOI:  
683 10.1093/plankt/fbl074.
- 684 Knijn RJ. 1993. *Atlas of North Sea Fishes: Based on Bottom-trawl Survey Data for the Years*  
685 *1985-1987*.
- 686 Krause, M., Dippner, J.W., Beil, J. 1995. A review of hydrographic controls on the distribution  
687 of zooplankton biomass and species in the North Sea with particular reference to a  
688 survey conducted in January–March 1987. *Progress in oceanography* 35:81–152.
- 689 Kröncke I, Reiss H, Eggleton JD, Aldridge J, Bergman MJN, Cochrane S, Craeymeersch JA,  
690 Degraer S, Desroy N, Dewarumez J-M, Duineveld GCA, Essink K, Hillewaert H,  
691 Lavaleye MSS, Moll A, Nehring S, Newell R, Oug E, Pohlmann T, Rachor E, Robertson  
692 M, Rumohr H, Schratzberger M, Smith R, Vanden Berghe E, van Dalfsen J, van Hoey  
693 G, Vincx M, Willems W, Rees HL. 2011. Changes in North Sea macrofauna  
694 communities and species distribution between 1986 and 2000. *Estuarine, Coastal and*  
695 *Shelf Science* 94:1–15. DOI: 10.1016/j.ecss.2011.04.008.
- 696 Kunitzer A, Basford D, Craeymeersch JA, Dewarumez JM, Dorjes J, Duineveld GCA,  
697 Eleftheriou A, Heip C, Herman P, Kingston P, Niermann U, Rachor E, Rumohr H, de  
698 Wilde PAJ. 1992. The benthic infauna of the North Sea: species distribution and  
699 assemblages. *ICES Journal of Marine Science* 49:127–143. DOI:  
700 10.1093/icesjms/49.2.127.

- 701 Land MAV der, Van der Land MA. 1991. Distribution of flatfish eggs in the 1989 egg surveys  
702 in the southeastern North Sea, and mortality of plaice and sole eggs. *Netherlands*  
703 *Journal of Sea Research* 27:277–286. DOI: 10.1016/0077-7579(91)90030-5.
- 704 Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ.  
705 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI  
706 region for metabarcoding metazoan diversity: application for characterizing coral reef  
707 fish gut contents. *Frontiers in Zoology* 10:34. DOI: 10.1186/1742-9994-10-34.
- 708 Lindley JA, Batten SD. 2002. Long-term variability in the diversity of North Sea zooplankton.  
709 *Journal of the Marine Biological Association of the United Kingdom* 82:31–40. DOI:  
710 10.1017/s0025315402005155.
- 711 Lusher AL, O'Donnell C, Officer R, O'Connor I. 2016. Microplastic interactions with North  
712 Atlantic mesopelagic fish. *ICES Journal of Marine Science: Journal du Conseil* 73:1214–  
713 1225. DOI: 10.1093/icesjms/fsv241.
- 714 Macher J-N, Vivancos A, Piggott JJ, Centeno FC, Matthaei CD, Leese F. 2018. Comparison  
715 of environmental DNA and bulk-sample metabarcoding using highly degenerate  
716 cytochrome c oxidase I primers. *Molecular ecology resources* 18:1456–1468.
- 717 Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve  
718 genome assemblies. *Bioinformatics* 27:2957–2963.
- 719 Marshall SM, Orr AP. 2013. *The Biology of a Marine Copepod: Calanus finmarchicus*  
720 *(Gunnerus)*. Springer Science & Business Media.
- 721 Mathivat-Lallier M-H, Cazaux C. 1990. Larval exchange and dispersion of polychaetes  
722 between a bay and the ocean. *Journal of Plankton Research* 12:1163–1172. DOI:  
723 10.1093/plankt/12.6.1163.
- 724 McEwen GF, Johnson MW, Folsom TR. 1954. A statistical analysis of the performance of  
725 the folsom plankton sample splitter, based upon test observations. *Archiv für*  
726 *Meteorologie, Geophysik und Bioklimatologie Serie A* 7:502–527. DOI:  
727 10.1007/bf02277939.
- 728 McManus GB, Katz LA. 2009. Molecular and morphological methods for identifying plankton:



- 729 what makes a successful marriage? *Journal of Plankton Research* 31:1119–1129. DOI:  
730 10.1093/plankt/fbp061.
- 731 Meißner K, Darr A. 2009. Distribution of Magelona species (Polychaeta: Magelonidae) in the  
732 German Bight (North Sea): a modeling approach. *Zoosymposia* 2:567–586. DOI:  
733 10.11646/zoosymposia.2.1.39.
- 734 Milner N. 2016. FISH ATLAS OF THE CELTIC SEA, NORTH SEA, AND BALTIC SEA.  
735 *Journal of Fish Biology* 89:1511–1512. DOI: 10.1111/jfb.13072.
- 736 Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a  
737 practical guide for biologists. *Biological reviews of the Cambridge Philosophical Society*  
738 82:591–605.
- 739 Nielsen TG, Løkkegaard B, Richardson K, Bo Pedersen R, Hansen L. 1993. Structure of  
740 plankton communities in the Dogger Bank area (North Sea) during a stratified situation.  
741 *Marine Ecology Progress Series* 95:115–131. DOI: 10.3354/meps095115.
- 742 Nielsen TG, Sabatini M. 1996. Role of cyclopoid copepods *Oithona* spp. in North Sea  
743 plankton communities. *Marine Ecology Progress Series* 139:79–93. DOI:  
744 10.3354/meps139079.
- 745 Oozeki Y. 2018. Biological Monitoring: Fish Eggs, Fish Larvae, and Zooplankton. *Fish*  
746 *Population Dynamics, Monitoring, and Management*:111–138. DOI: 10.1007/978-4-431-  
747 56621-2\_7.
- 748 Otto L, Zimmerman JTF, Furnes GK, Mork M, Saetre R, Becker G. 1990. Review of the  
749 physical oceanography of the North Sea. *Netherlands Journal of Sea Research* 26:161–  
750 238. DOI: 10.1016/0077-7579(90)90091-t.
- 751 Pereira-da-Conceicao L, Elbrecht V, Hall A, Briscoe A, Barber-James H, Price B. 2019.  
752 Metabarcoding unsorted kick-samples facilitates macroinvertebrate-based biomonitoring  
753 with increased taxonomic resolution, while outperforming environmental DNA. *bioRxiv*.  
754 DOI: 10.1101/792333.
- 755 Plate S, Husemann E. 1994. Identification guide to the planktonic polychaete larvae around  
756 the island of Helgoland (German Bight). *Helgoländer Meeresuntersuchungen* 48:1–58.

- 757 DOI: 10.1007/bf02366201.
- 758 Porter TM, Hajibabaei M. 2018. Over 2.5 million COI sequences in GenBank and growing.  
759 *PloS one* 13:e0200177.
- 760 Questel JM, Blanco-Bercial L, Hopcroft RR, Bucklin A. 2016. Phylogeography and  
761 connectivity of the (Copepoda: Calanoida) species complex in the eastern North Pacific  
762 and the Pacific Arctic Region. *Journal of plankton research* 38:610–623.
- 763 Ratnasingham S, Hebert PDN. 2007. bold: The Barcode of Life Data System  
764 (<http://www.barcodinglife.org>). *Molecular ecology notes* 7:355–364.
- 765 Reid PC, Colebrook JM, Matthews JBL, Aiken J. 2003a. The Continuous Plankton Recorder:  
766 concepts and history, from Plankton Indicator to undulating recorders. *Progress in*  
767 *Oceanography* 58:117–173. DOI: 10.1016/j.pocean.2003.08.002.
- 768 Reid PC, Edwards M, Beaugrand G, Skogen M, Stevens D. 2003b. Periodic changes in the  
769 zooplankton of the North Sea during the twentieth century linked to oceanic inflow.  
770 *Fisheries Oceanography* 12:260–269. DOI: 10.1046/j.1365-2419.2003.00252.x.
- 771 Rossel S, Martínez Arbizu P. 2019. Revealing higher than expected diversity of  
772 Harpacticoida (Crustacea:Copepoda) in the North Sea using MALDI-TOF MS and  
773 molecular barcoding. *Scientific reports* 9:9182.
- 774 Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic  
775 datasets. *Bioinformatics* 27:863–864.
- 776 Schminke HK. 2007. Entomology for the copepodologist. *Journal of Plankton Research*  
777 29:i149–i162. DOI: 10.1093/plankt/fbl073.
- 778 Schnell IB, Bohmann K, Gilbert MTP. 2015. Tag jumps illuminated--reducing sequence-to-  
779 sample misidentifications in metabarcoding studies. *Molecular ecology resources*  
780 15:1289–1303.
- 781 Schroeder A, Stanković D, Pallavicini A, Gionechetti F, Pansera M, Camatti E. 2020. DNA  
782 metabarcoding and morphological analysis - Assessment of zooplankton biodiversity in  
783 transitional waters. *Marine Environmental Research* 160:104946. DOI:  
784 10.1016/j.marenvres.2020.104946.

- 785 Schwamborn R, Neumann-Leitão S, De Almeida e SILVA T, Silva AP, Ekau W, Saint-Paul  
786 U. 2001. Distribution And Dispersal of Decapod Crustacean Larvae and Other  
787 Zooplankton In The Itamaracá Estuarine System, Brazil. *Tropical Oceanography* 29.  
788 DOI: 10.5914/tropocean.v29i1.2834.
- 789 Steinberg DK, Landry MR. 2017. Zooplankton and the Ocean Carbon Cycle. *Annual review*  
790 *of marine science* 9:413–444.
- 791 Stern R, Kraberg A, Bresnan E, Wiebe H C, Lovejoy C, Montresor M, Morán XAG, Not F,  
792 Salas R, Siano R, Vulot D, Amaral-Zettler L, Zingone A, Metfies K. 2018. Molecular  
793 analyses of protists in long-term observation programmes—current status and future  
794 perspectives. *Journal of Plankton Research* 40:519–536. DOI: 10.1093/plankt/fby035.
- 795 Nelson, John. (2014). DFO Pacific IOS zooplankton database - Zooplankton samples  
796 collected during cruises to the Canadian Arctic, 2006-2009. Version 1 In OBIS Canada  
797 Digital Collections. Bedford Institute of Oceanography, Dartmouth, NS, Canada.  
798 Published by OBIS, Digital <http://www.iobis.org/>. Accessed on 20-09-2019
- 799 Suthers IM, Rissik D. 2009. *Plankton: A Guide to Their Ecology and Monitoring for Water*  
800 *Quality*. CSIRO PUBLISHING.
- 801 Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E. 2012. Towards next-  
802 generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*  
803 21:2045–2050. DOI: 10.1111/j.1365-294x.2012.05470.x.
- 804 Theissinger K, Röder N, Allgeier S, Beermann AJ, Brühl CA, Friedrich A, Michiels S,  
805 Schwenk K. 2019. Mosquito control actions affect chironomid diversity in temporary  
806 wetlands of the Upper Rhine Valley. *Molecular ecology* 28:4300–4316.
- 807 Thiébaud E, Cabioch L, -C. Dauvin J, Retiere C, Gentil F. 1997. Spatio-Temporal Persistence  
808 of the *Abra Alba-Pectinaria Koreni* Muddy-Fine Sand Community of the Eastern Bay of  
809 Seine. *Journal of the Marine Biological Association of the United Kingdom* 77:1165–  
810 1185. DOI: 10.1017/s0025315400038698.
- 811 Turner JT. 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's  
812 biological pump. *Progress in Oceanography* 130:205–248. DOI:

- 813 10.1016/j.pocean.2014.08.005.
- 814 Turon X, Antich A, Palacín C, Præbel K, Wangensteen OS. 2019. From metabarcoding to  
815 metaphylogeography: separating the wheat from the chaff. DOI: 10.1101/629535.
- 816 Van Ginderdeuren K, Van Hoey G, Vincx M, Hostens K. 2014. The mesozooplankton  
817 community of the Belgian shelf (North Sea). *Journal of Sea Research* 85:48–58. DOI:  
818 10.1016/j.seares.2013.10.003.
- 819 Vezzulli L, Reid PC. 2003. The CPR survey (1948–1997): a gridded database browser of  
820 plankton abundance in the North Sea. *Progress in Oceanography* 58:327–336. DOI:  
821 10.1016/j.pocean.2003.08.011.
- 822 Wangensteen OS, Palacín C, Guardiola M, Turon X. 2018. DNA metabarcoding of littoral  
823 hard-bottom communities: high diversity and database gaps revealed by two molecular  
824 markers. *PeerJ* 6:e4705.
- 825 Weigand H, Beermann AJ, Čiampor F, Costa FO, Csabai Z, Duarte S, Geiger MF,  
826 Grabowski M, Rimet F, Rulik B, Strand M, Szucsich N, Weigand AM, Willassen E, Wyler  
827 SA, Bouchez A, Borja A, Čiamporová-Zaťovičová Z, Ferreira S, Dijkstra K-DB, Eisendle  
828 U, Freyhof J, Gadawski P, Graf W, Haegerbaeumer A, van der Hoorn BB, Japoshvili B,  
829 Keresztes L, Keskin E, Leese F, Macher JN, Mamos T, Paz G, Pešić V, Pfannkuchen  
830 DM, Pfannkuchen MA, Price BW, Rinkevich B, Teixeira MAL, Várbíró G, Ekrem T. 2019.  
831 DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-  
832 analysis and recommendations for future work. *The Science of the total environment*  
833 678:499–524.
- 834 Weigand AM, Macher J-N. 2018. A DNA metabarcoding protocol for hyporheic freshwater  
835 meiofauna: Evaluating highly degenerate COI primers and replication strategy.  
836 *Metabarcoding and Metagenomics* 2. DOI: 10.3897/mbmg.2.26869.
- 837 Williams R, Lindley JA, Hunt HG, Collins NR. 1993. Plankton community structure and  
838 geographical distribution in the North Sea. *Journal of Experimental Marine Biology and*  
839 *Ecology* 172:143–156. DOI: 10.1016/0022-0981(93)90094-5.
- 840 Wright PJ, Jensen H, Tuck I. 2000. The influence of sediment type on the distribution of the

- 841 lesser sandeel, *Ammodytes marinus*. *Journal of Sea Research* 44:243–256. DOI:  
842 10.1016/s1385-1101(00)00050-2.
- 843 Zhang GK, Chain FJJ, Abbott CL, Cristescu ME. 2018. Metabarcoding using multiplexed  
844 markers increases species detection in complex zooplankton communities. *Evolutionary*  
845 *applications* 11:1901–1914.
- 846 Zizka VMA, Elbrecht V, Macher J-N, Leese F. 2019a. Assessing the influence of sample  
847 tagging and library preparation on DNA metabarcoding. *Molecular ecology resources*  
848 19:893–899.
- 849 Zizka VMA, Leese F, Peinert B, Geiger MF. 2019b. DNA metabarcoding from sample fixative  
850 as a quick and voucher-preserving biodiversity assessment method. *Genome* 62:122–  
851 136. DOI: 10.1139/gen-2018-0048.