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1 **Changes in marine phytoplankton diversity: assessment under the Marine Strategy**

2 **Framework Directive**

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46 **Abstract**

47 The Marine Strategy Framework Directive requires EU Member States to assess the Good  
48 Environmental Status (GES) of their marine waters in a coherent and strategic manner. For  
49 the regional assessment of biodiversity, the OSPAR Intersessional Coordination Group of  
50 Biodiversity Assessment and Monitoring (ICG-COBAM) provides substantial advice.  
51 Through expert working groups, phytoplankton indicators are currently being developed to  
52 measure the state and the change in pelagic diversity, to quantify food web dynamics and to  
53 measure the extent of eutrophication impacts. We developed a multi-metric indicator that is  
54 compliant with the common OSPAR indicator “Changes in plankton diversity” (PH3). The  
55 aim was to describe the structure of the phytoplankton community (alpha diversity) and to  
56 detect significant temporal changes (beta diversity) to evaluate the health of pelagic habitats.  
57 In this pilot study, we used three coastal time-series in the Western Channel and the north of  
58 the Bay of Biscay (North Atlantic, France) to test the efficiency and the performance of  
59 several existing diversity indices. We validated two alpha diversity indices, namely the  
60 Menhinick Index ( $D$ ) and the Hulburt Index ( $\delta$ ), based on their complementary ecological  
61 information, their strong relationship with habitat characteristics, and their relative ease of  
62 interpretation for stakeholders. Temporal shifts or rate of change in community structure  
63 were detected by the Local Contributions to Beta Diversity index (LCBD; a beta diversity  
64 measure). For the years where significantly high LCBD values were found, the Importance  
65 Value Index (IVI) was calculated to potentially identify the taxa (genus) responsible for the  
66 “unusual” community structure. For example, at the Ouest Loscolo site in 2008, an elevated  
67 LCBD (0.45) coincided with a high dominance value (Hulburt’s Index) caused by the  
68 occurrence of a monospecific bloom of *Leptocylindrus* spp. (IVI = 73%) in July ( $2.2 \times 10^6$  cells L<sup>-1</sup>)

69 and October ( $8 \times 10^6$  cells  $L^{-1}$ ). In this way, PH3 informs on different aspects of phytoplankton  
70 diversity from a community to a genus level. At the current stage of development, however,  
71 PH3 acts as a “surveillance” rather than an operational indicator since the relationship to  
72 GES is not directly tracked. In the future, by additional testing of PH3 and extending the  
73 geographical scope, the robustness of the assessment could be further determined across the  
74 OSPAR Maritime Area.

75

## 76 Introduction

77 The Marine Strategy Framework Directive (MSFD) requires that European Member States  
78 that share a marine region or sub-region cooperate when developing their marine strategies  
79 (CEC 2008). In this respect, Regional Sea Conventions, like OSPAR (Convention for the  
80 Protection of the Marine Environment of the North-East Atlantic), take a key role as a  
81 platform for EU Member States to coordinate their approaches in implementing the MSFD at  
82 a regional scale. For the ‘biodiversity’ descriptors of the Directive (i.e. D1 Biodiversity, D2  
83 Non-indigenous species, D4 Food webs and D6 Seafloor integrity), the OSPAR Intersessional  
84 Coordination Group of Biodiversity Assessment and Monitoring (ICG-COBAM) provides  
85 substantial regional advices for the North East Atlantic, on the basis of its intersessional  
86 work and its seven dedicated working groups each covering an ecosystem component  
87 (marine mammals, seabirds, fish and cephalopods, benthic habitats, pelagic habitats, non-  
88 indigenous species and food webs). The main tasks of the working groups are to identify a  
89 set of common indicators and to coordinate the development of these indicators for their use  
90 in regional assessments. To date, common indicators based on plankton communities have

91 been adopted by OSPAR to assess Good Environmental Status (GES) of pelagic habitats at  
92 the regional scale of the North East Atlantic ([https://oap.ospar.org/en/ospar-](https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/habitats/)  
93 [assessments/intermediate-assessment-2017/biodiversity-status/habitats/](https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/habitats/)).

94

95 Coastal ecosystems face increasing human disturbances such as pollution and/or  
96 eutrophication (i.e. excessive nutrients or organic enrichments) that can drive marked  
97 changes in the plankton community dynamics and thus in its structural attributes, such as  
98 diversity, dominance or size structure. Phytoplankton, for example, show rapid responses to  
99 altered nutrient levels through changes in biomass and composition (Reynolds, 2006).

100 Whereas the use of phytoplankton biomass for water quality assessment has a long history  
101 (Paształeniec, 2016), the evaluation of community composition has gained a more recent  
102 interest through the implementation of the Water Framework Directive (WFD) (Devlin et al.,  
103 2009; Uusitalo et al., 2013). In the WFD, three metrics, namely ‘phytoplankton abundance’,  
104 ‘phytoplankton biomass’ and ‘phytoplankton taxonomic composition’, are part of the  
105 biological quality elements (BQEs), i.e. organism groups which integrate the effects of  
106 various stressors such as nutrient enrichment, acidification, and, to some extent, hypoxia or  
107 habitat degradation (Lyche-Solheim et al., 2013). In contrast to measurements for chlorophyll  
108 *a* as a proxy for biomass, the assessment of the taxonomic composition of the phytoplankton  
109 assemblage could provide information about the whole community, including the  
110 importance of the different size-groups such as the pico- and nano-phytoplankton  
111 (Domingues et al., 2008).

112

113 Diversity indices summarise the abundance data for multiple species in an assemblage into a  
114 single number to describe the state of the community (Kwak and Peterson, 2007). A plethora

115 of indices exist in the scientific literature that focus on different aspects of biodiversity  
116 (richness, dominance, evenness) and are usually weighted in different ways, for example, the  
117 Simpson's index is more weighted on dominant species compared to the Shannon index  
118 (Magurran, 1988). The choice of the most appropriate indices depends on the type of  
119 assemblage considered, the objectives of the study and the data availability (e.g. Chiarucci et  
120 al., 2011; Morris et al., 2014). In terms of community structure, many natural biotic  
121 communities, such as phytoplankton, are characterized by the presence of a few common  
122 species with high abundances and many rare species (Wilhm and Dorris, 1968). Over time,  
123 abundances of phytoplankton can vary by several orders of magnitude at the seasonal,  
124 interannual and interdecadal time scales as a result of variations in natural environmental  
125 conditions and/or from anthropogenic pressures (e.g. Zingone et al., 2010; Muñiz et al., 2018).  
126 On a seasonal basis, phytoplankton exhibit a distinct succession in species composition, i.e.  
127 an ordered sequence of substitutions of species (Margalef, 1978; Reynolds, 2006), and these  
128 variations are sometimes even more significant than inter-annual trends in phytoplankton  
129 community structure. The causes of succession are complex and have not been totally  
130 elucidated (Sommer et al., 2012). Succession can depend on species-interactions and, more  
131 importantly, the reactivity to favourable environmental conditions throughout the year, such  
132 as seasonal changes in temperature, water column mixing/stratification, nutrient loadings  
133 and light availability (Chalar, 2009). Other processes act on time periods of days to weeks,  
134 like meteorological (wind, rain and cloudiness) and hydrological events  
135 (upwelling/downwelling events). Finally, marked changes in the relative abundances of  
136 species can also be a result of environmental perturbations such as pollution or  
137 eutrophication (Bužančić et al., 2016; Domingues et al., 2017). In these cases, an increase in

138 dominance occurs because only a subset of species can actively benefit from the new  
139 conditions (Ben Othman et al., 2018; Coclet et al., 2018).

140

141 Biodiversity measures can be useful for conservation practice and management purposes  
142 (Chiarucci et al., 2011; Scheiner et al., 2017). In this respect, "species richness" was identified  
143 as an Essential Biodiversity Variable (EBV), a measurement required for studying, reporting  
144 and managing biodiversity change (Pereira et al., 2013; Kissling et al., 2018). Whilst  
145 taxonomic richness is a useful biodiversity metric, its applicability to assess the state of  
146 pelagic habitats in water quality assessment is debatable and to date no consensus has been  
147 achieved about which indices are more appropriate and informative for assessing the state  
148 and change in phytoplankton communities. One of the main problems is that the response of  
149 phytoplankton communities to anthropogenic pressures is often non-linear, making clear  
150 state-pressure relationships difficult to identify (Garmendia et al., 2013; Ninčević-Gladan et  
151 al., 2015). As an example, Shannon and Simpson indices are widely used in descriptive  
152 studies to quantify community diversity but were found inappropriate as tools for water  
153 quality assessment due to their erratic behaviour along a eutrophication gradient (Spatharis  
154 et al., 2011). To increase the robustness of assessment using diversity indices, several studies  
155 have proposed to modify already existing diversity metrics, for example the Shannon95  
156 (Uusitalo et al., 2013), and/or the use of composite indices (Spatharis and Tsirtsis, 2010;  
157 Vadrucci et al., 2013; Laplace-Treytoure and Feret, 2016), to date mainly developed for  
158 freshwater systems and transitional waters. Whilst these studies agree on the use of  
159 phytoplankton community structure as an essential component for water quality assessment  
160 (Devlin et al., 2009; Facca et al., 2014), further work is needed in this respect (Caroppo et al.,  
161 2013; Garmendia et al., 2013; Varkitzi et al., 2018).



162

163 Within the OSPAR Regional Sea Convention, marine phytoplankton and zooplankton  
164 community indicators are currently under development to assess the Environmental Status  
165 of Pelagic Habitats (OSPAR 2017a). Pelagic Habitat indicator 1 (PH1) "Changes in  
166 phytoplankton and zooplankton communities" uses the relative changes in abundances of  
167 lifeform pairs based on functional traits to indicate ecological change ( Tett et al., 2008;  
168 McQuatters-Gollop et al., 2015; OSPAR, 2017b). For example, in the pairing of diatoms and  
169 dinoflagellates, the dominance of the latter could indicate eutrophication resulting in less  
170 desirable food webs. Pelagic Habitat indicator 2 (PH2) "Changes in Phytoplankton Biomass  
171 and Zooplankton Abundance" provides an indication of deviations in total biomass or  
172 abundance of plankton from the assumed natural variability in time-series (OSPAR, 2017c).  
173 Finally, Pelagic Habitat indicator 3 (PH3) identifies changes in the community structure  
174 using taxonomic diversity indices (OSPAR, 2017d). These three common indicators consider  
175 plankton communities at different organizational levels: PH2 at the broadest organizational  
176 level since it considers total phytoplankton biomass and total copepod abundance, PH1 at an  
177 intermediate level since it considers lifeform pairs, and PH3 at the finest level of  
178 organization, if possible down to the species level.

179 This paper summarises the development of the OSPAR common indicator "Changes in  
180 plankton diversity" (PH3) for phytoplankton communities. The aim of PH3 is to characterise  
181 the phytoplankton community structure and to detect potential temporal shifts, preferably in  
182 relation to the environment. Frequently used diversity indices, mainly developed in the  
183 context of the Water Framework Directive, were preselected. Microphytoplankton counts  
184 obtained from three coastal time-series in the Western Channel and the north of the Bay of  
185 Biscay (fig. 1) were used here to test the efficiency and the performance of several diversity

186 indices for assessing GES of pelagic habitats under the MFSD. More specifically, we tested  
187 these diversity indices for their ecological relevance, mathematical consistency and link to  
188 marine hydrological factors.

189

## 190 Materials and methods

### 191 1. Phytoplankton and environmental datasets

192 Microscopic counts of phytoplankton data from the Western Channel and the north of the  
193 Bay of Biscay, France, were collated from two sources, namely RESOMAR-Pelagos (Pelagic  
194 database of the Réseau National des Stations et Observatoires Marins;  
195 <http://resomar.cnrs.fr/Base-de-donnee-Pelagos>) and REPHY (Réseau d'Observation et de  
196 Surveillance du Phytoplancton et des  
197 Phycotoxines;[http://envlit.ifremer.fr/surveillance/phytoplancton\\_phycotoxines/presentation](http://envlit.ifremer.fr/surveillance/phytoplancton_phycotoxines/presentation)).

198 The REPHY is implemented and managed by the French Research Institute for the  
199 Exploitation of the Sea (IFREMER). The database of RESOMAR-Pelagos hosts plankton data  
200 collected from most of the French coastal marine stations and observatories. From the  
201 RESOMAR-Pelagos database, we filtered for stations where samples were collected and  
202 analysed using consistent methodology, were sampled at a minimum monthly frequency,  
203 which contained minimal gaps in the sampling, and which simultaneously sampled  
204 nutrients and hydrological factors. This selection resulted in the station of SOMLIT-Astan  
205 (2007-2013, fig. 1), a coastal long-term monitoring station situated 4.6 km from the coast that  
206 is characterized by permanently mixed waters with limited continental influence. Twice a  
207 month, seawater samples are collected at 1 m depth using a 5 liters Niskin bottle for

208 phytoplankton analysis. Samples are fixed with acid Lugol's iodine solution and then stored  
209 according to the methods described in Sournia (1978). Cell counts are made under an  
210 inverted light microscope at 200-400x magnification. Further details on phytoplankton  
211 quantification and identification protocols for SOMLIT-Astan can be found in Guilloux et al.  
212 (2013). Environmental data from the site are collected by the Station Biologique de Roscoff  
213 and hosted by the SOMLIT (Service d'Observation en Milieu LITtoral, INSU-CNRS)  
214 database; they were retrieved from their online platform (<http://somlit.epoc.u->  
215 [bordeaux1.fr/fr/](http://somlit.epoc.u-bordeaux1.fr/fr/)). Data on salinity (psu), temperature (°C), inorganic nutrients (ammonia,  
216 nitrate, nitrites, silicate, phosphates; in  $\mu\text{mol L}^{-1}$ ) and oxygen ( $\text{ml L}^{-1}$ ) were used in the  
217 analysis.

218 In the Bay of Biscay, data from two REPHY sites, Ouest Loscolo and Le Croisic, were made  
219 available for analyses (Catherine Belin, pers. comm.). These sites are shallow, meso- to  
220 macrotidal, with a moderate wave exposure at 2.9 km from the coast for the Ouest Loscolo  
221 station and 0.2 km from the coast for Le Croisic station. They are both under the influence of  
222 riverine output, namely from the Loscolo and the Loire River. Water samples are collected  
223 on a bi-monthly basis at the surface in order to determine phytoplankton cell abundance and  
224 taxonomic composition. Phytoplankton samples are fixed with Lugol's solution (neutral or  
225 acidic) and counted according to the Utermöhl method (Utermöhl, 1958). Further details  
226 about sampling and processing of phytoplankton and physico-chemical parameters are  
227 available in the literature (Neaud-Masson, 2015). The level of taxonomic identification  
228 depends on the analytical method used and the experience of the phytoplankton analyst.  
229 Changes in the taxonomic analyst may lead to heterogeneous data regarding taxonomic  
230 classification and hence to a misinterpretation of phytoplankton time-series (Hernández-  
231 Fariñas et al., 2013); this is true of many multidecadal datasets. Consequently, although

232 phytoplankton data in SOMLIT-Astan has been collected from the year 2000 onwards, only  
233 the period 2007-2014 was considered for analysis since the same two operators worked  
234 closely for the analyses of the samples during this time-period. Across datasets, most taxa  
235 were identified to the species level but for consistency and again to reduce bias from  
236 misidentification, abundance data (expressed as number of cells per liter) of the taxonomic  
237 units were grouped to the genus level and pooled monthly. If the identification was at a  
238 lower taxonomic level (Class, Phylum, as is the case for the smaller species), then these were  
239 also taken into account but cells that were classified as “non-identified” were not used in  
240 the analysis.

241

## 242 2. Data analysis

243 To select the most appropriate indices for the assessment of GES for pelagic habitats,  
244 diversity indices were tested on the three sites in a range of simple and multivariate  
245 analyses. After pre-selecting diversity indices from the literature, we have adopted some  
246 criteria that biodiversity measures should satisfy for their use in quality assessment (van  
247 Strien et al., 2012; Buckland et al., 2005). The final indicator should (1) provide ecological  
248 information on the state condition of phytoplankton communities using several aspects of  
249 biodiversity: richness, dominance, and evenness; and detect significant temporal changes in  
250 the structure of the phytoplankton community (2) be mathematically consistent, (3) have a  
251 link with environmental conditions.

252

### 253 2.1. Selection of diversity indices for the quantification of alpha diversity

#### 254 2.1.1. Ecological relevance

255 In terms of ecological information, three aspects of diversity indices, i.e. the number of taxa,  
256 their overall abundance and their evenness in the community, are of primary interest to  
257 describe community structure and change, and have received an increased interest in  
258 environmental management, especially in combination with each other (Buckland et al.,  
259 2011). The aim was to select an index from each group so as to describe different aspects of a  
260 phytoplankton community. Monthly and annual means in diversity indices were then  
261 calculated for the three time-series so as to identify seasonal and annual trends in  
262 community structure in terms of abundance of taxa.

263

#### 264 2.1.1.1. Indices based on richness (number of taxa)

265 In phytoplankton studies, the most commonly used indices to describe the number of taxa in  
266 the community includes species richness ( $S$ ), the Margalef ( $d$ ) Index and the Menhinick ( $D$ )  
267 Index (Varkitzi et al., 2018). The latter index, in particular, has been found suitable as an  
268 indicator of eutrophication in transitional (Facca et al., 2014) and coastal waters (Spatharis  
269 and Tsirtsis, 2010; Buzançıç et al., 2016). The Menhinick index ( $D$ ; Whittaker, 1977) is a  
270 measure of taxonomic richness where  $S$  represents the number of taxa, and  $N$ , the number of  
271 individuals.

$$272 \quad D = \frac{S}{\sqrt{N}} \quad (1)$$

273

274 Whilst species richness ( $S$ ) is the simplest and most straightforward index to calculate, this  
275 estimate is strongly influenced by the sampling process (Peet, 1974; Rodriguez-Samos et al.,  
276 2014). To investigate the effect of sampling effort on our estimates of richness, the cumulative  
277 number of species as a function of the consecutive number of samples in time, were drawn.

278

279 2.1.1.2. Indices based on dominance and evenness (relative abundance)

280 As mentioned previously, phytoplankton communities are characterized by complex  
281 dynamics with a strong seasonal cycle. Hence, indices that provide information on the  
282 temporary dominance of species are of particular interest for the development of the  
283 indicator, PH3, described here. For this purpose, diversity measures that include a richness  
284 and an evenness component were used to express a relative concentration of dominance. In  
285 this respect, the Shannon-Wiener and the Simpson's index are frequently used for describing  
286 diversity in ecological assessment (Heip, 1998; Kabuta and Duijts, 2000). Additionally,  
287 another dominance measure, the Hulburt index ( $\delta$ ; Hulburt, 1963) has been developed to  
288 describe phytoplankton communities in particular and was recently proposed as a suitable  
289 indicator of eutrophication in the context of the WFD (Facca et al., 2014). Since this index is  
290 expressed as a percentage, it is relatively easy to interpret.

$$291 \quad \delta = 100 (n_1 + n_2) / N \quad (2)$$

292

293 where  $n_1$  is the abundance of the dominant genus;  $n_2$  is the abundance of the second most  
294 abundant genus; and  $N$  is the total abundance.

295 Classical measures such as Shannon and Simpson's are based on species proportions and fail  
296 to measure changes in abundance if all species in a community are declining at the same rate  
297 (Buckland et al., 2011). To overcome this issue, the geometric mean index  $G_j$ , for example,  
298 quantifies the average trend in relative abundance across species in the community  
299 (Buckland et al., 2011). Finally, evenness indices express the equitability of species

300 abundance in the sample or the community (Washington, 1984). Here, we applied the  
301 Pielou's index ( $J'$ ; Pielou, 1975).

302

### 303 2.1.2. Mathematical consistency

304 Within each index group, however, indices can be mathematically related since they are  
305 either using common metrics and/or are derived from similar equations. With these  
306 potentially competing indices, it is important to examine their mathematical convergence so  
307 as to reduce redundancy in the information and to select only an optimal subset of indices  
308 (Lyashevskaya and Farnsworth, 2012; van Strien et al., 2012; Bandeira et al., 2013). To do so,  
309 simple statistical correlations (Bravais-Pearson) between all selected diversity indices (based  
310 on monthly abundances) were calculated for each sampling site separately to investigate the  
311 mathematical redundancies within each group.

312

### 313 2.1.3. Link with environmental conditions

314 Biodiversity metrics that respond differently to environmental factors can be considered  
315 complementary (Gascon et al., 2009; Gallardo et al., 2011). Hence, we investigated to what  
316 extent the selected biodiversity measures reflected changes in the environmental conditions  
317 and if certain indices are interrelated.

318 A standardized Principal Components Analysis (PCA; Jolliffe, 1986) was applied to the  
319 potential environmental correlates of phytoplankton diversity to determine: (1) the  
320 environmental variables that explained the largest variation in the data set, (2) the  
321 relationships among these potential environmental predictors, and (3)  
322 how the scores of the principal components were related to the phytoplankton diversity  
323 metrics. The procedure was applied to each single time-series separately. For each

324 environmental variable, the annual mean and the coefficient of variation (COV), used here as  
325 an index of seasonal variation, were calculated. The environmental data were first  
326 normalized using the omnibus procedure (Legendre and Legendre, 1998). The correlation  
327 matrix of all standardized variables was used to calculate the eigenvectors and the Principal  
328 Components (PCs). The PCs were then ranked in order of significance and the contribution  
329 of each variable to each PC was calculated. To check for nonlinearity among environmental  
330 descriptors, the multinormality of the PCs was tested. The outcome of the PCA was used to  
331 investigate the relationships of phytoplankton diversity with a combination of  
332 environmental factors instead of computing a suite of correlation coefficients of diversity  
333 with single factors. Linear Bravais–Pearson’s correlations were calculated to assess the  
334 relationship between each PC and the phytoplankton diversity indices.

335

## 336 2.2. Measuring beta diversity

337 Since considerable community changes can occur without being reflected in alpha diversity,  
338 we also used measures of directional turnover to investigate the rate of change in community  
339 structure. Here, we applied a beta diversity measure to assess the change in community  
340 structure from one sampling unit to another along a temporal gradient (from year to year)  
341 (see Andersen et al., 2011 for definitions on beta diversity). According to Legendre and De  
342 Cáceres (2013), total beta diversity can be partitioned into Species Contributions (SCBD:  
343 degree of variation of individual species across the study area) and Local Contributions  
344 (LCBD: comparative indicators of the ecological uniqueness of the sites) to Beta Diversity.  
345 For the objective of the study, we were interested in the LCBD indices that indicate how  
346 much each observation contributes to the total community variance in time. Where a year



347 with an average species composition would have an LCBD value of 0, large LCBD values  
348 may indicate degraded and species-poor sites that are in need of restoration (Legendre and  
349 De Cáceres, 2013). High values may also correspond to special ecological conditions, or may  
350 result from the disturbance effect of invasive species on communities. Here, temporal beta  
351 diversity was computed as the method described in detail by Legendre and De Cáceres  
352 (2013). Firstly, the raw abundance data were transformed using the Chord method (Legendre  
353 and Galagher, 2001). Secondly, the total variance of the transformed community composition  
354 was calculated by taking the squared deviations from the column means. The relative  
355 contribution of the sampling unit  $j$  to beta or LCBD is the sum of squares for each sampling  
356 unit divided by the total sum of squares. The statistical significance of the LCBD values was  
357 also calculated. For the years where significant LCBD values were found, the Importance  
358 Value Index (IVI; Curtis, 1959) was calculated. In addition to diversity indices, the IVI can be  
359 used to indicate the overall importance of a species in a community (Jose, 2012) and here, to  
360 potentially identify the taxa (genus) responsible for the “unusual” community structure. For  
361 the genera where only one species was identified, the species instead of the genus name was  
362 retained. The IVI (Eq. 3) was calculated as the sum of the relative density (RD; Eq. 4) and the  
363 relative frequency (RF; Eq. 5) of the taxonomic units in the community.

$$364 \quad \text{IVI} = \text{RD}_i + \text{RF}_i \quad (3)$$

365 Here, the RD reflected the numerical strength of a genus in relation to the total number of  
366 individuals of all the genera and can be calculated as:

$$367 \quad \text{RD}_i = (n_i / N) * 100 \quad (4)$$

368 where  $n_i$  is the number of individuals of the genus  $i$  and  $N$  is the total number of individuals  
369 of all the genera. The RF is the degree of dispersion of individual genera over time in relation  
370 to the number of all the genera which occurred in the time-series.

371 
$$RF_i = (f_i/F)*100 \quad (5)$$

372 where  $f_i$  is the number of occurrence of the genus  $i$  and  $F$  is the total number of occurrence of  
373 all the genera.

374 For these analyses, only monthly abundance time-series data (at the genus level) from the  
375 Ouest Loscolo and Le Croisic site (Bay of Biscay) were considered, as these long time-series  
376 (>25 years) provided the most robust analyses compared to the shorter available data set of  
377 SOMLIT-Astan. In the graphical representations, only the top 5 genera with the highest IVI  
378 values are shown.

379 All analyses were carried out using the software package MATLAB R2015a.

380

## 381 2. Results

382 Species accumulation curves showed that our observed richness values likely  
383 underestimated the total richness of the phytoplankton communities (Figure S1). For the  
384 three datasets, there is an increasing trend in the number of species along the time-series and  
385 the curves did not reach saturation level indicating that the total community has not been  
386 sampled yet.

387

388 Using all nine indices, correlation analyses investigated the likely redundancy between  
389 indices from a mathematical perspective. Similar results were obtained for all sampling sites  
390 but only the results for SOMLIT-Astan are presented here (Table 1). As expected, strong  
391 correlations between diversity measures were found. This is not surprising as they represent  
392 aspects of the same phenomenon (Morris et al., 2014). For the richness group, the Margalef's  
393 index ( $d$ ) and the number of genera ( $S$ ) were highly and positively correlated ( $r^2=0.87$ ). The

394 Menhinick's index ( $D$ ) was not related to the other indices within the group suggesting that  
395 its information is complementary to the two others. For the dominance indices, the Hulburt's  
396 index ( $\delta$ ), the Simpson's index ( $\lambda$ ), the Shannon index ( $H'$ ) and the Berger Parker's index ( $BP$ )  
397 were all strongly related ( $r^2 > 0.90$ ). Between categories,  $D$  was strongly and negatively related  
398 ( $r^2 \geq -0.90$ ) to the Brillouin's index ( $H_B$ ) and this could suggest that these metrics carry similar  
399 information despite not being related mathematically. The Pielou's index ( $J'$ ) was not  
400 significantly related to any of the other indices. The behaviour of geometric means ( $G_j$ ) could  
401 not be investigated since it requires that each species is recorded in every year.  
402 Unfortunately, relative abundance estimates of many phytoplankton species were equal to  
403 zero and thus  $G_j$  could not be calculated.

404

405 The Principal Components Analysis (PCA) investigated the relationships among the mean  
406 and seasonal variations in physico-chemical factors (Fig. 2), and the relationships of the PC  
407 with phytoplankton diversity indices (Table 2). Similar correlations were found for the  
408 different test sites, suggesting that the analyses explain the general behaviour of the index  
409 and that the responses are not only a function of the prevailing local environmental  
410 conditions. In SOMLIT-Astan, for example, the first Principal Component (PC1) explained  
411 43% of the variation in the data where temperature, nitrate, phosphate and silicate  
412 contributed mostly (Fig. 2a). The PC2 was explained by salinity, oxygen and nitrite and  
413 accounted for 26% in the variation. For the seasonal variations in the environmental factors  
414 (Fig. 2b), the PC1 explained 28% and the PC2 explained 26%. However, in terms of the  
415 correlations with the PC and diversity indices, the seasonal variations in environmental  
416 factors are more strongly related to diversity than annual mean conditions (Table 2). For the

417 richness group,  $D$  was the metric best explained by the seasonal variations in environmental  
418 factors for SOMLIT-Astan ( $r^2 = 0.76$ ;  $p < 0.001$ ).

419 For the dominance metrics,  $H_B$  best reflected the seasonal variations in the environment ( $r^2 =$   
420  $0.74$ ;  $p < 0.001$ ). This common sensitivity of  $D$  and the  $H_B$  in relation to changes in the  
421 environment might explain the strong interrelationships previously detected (Table 1).

422

423 A summary table describes the performance for each  $\alpha$  diversity index in relation to the  
424 previously described criteria: ecological relevance, mathematical consistency and link with  
425 hydrological conditions (Table 3). The final selection for the indices included  $D$  to describe  
426 genus richness and  $\delta$  to describe genus dominance since they have the best scores for the  
427 three criteria. Whilst  $J'$  described a different aspect of diversity, this measure was not  
428 retained for the PH3 indicator since it contained little complementary information for the  
429 assessment.

430

431 To investigate the seasonal and annual variations in the three aspects of diversity  
432 simultaneously, contour plots of genus richness (expressed here as  $D$ ), dominance (expressed  
433 here as  $\delta$ ) and evenness ( $J'$ ) per sampling site are shown (Fig. 3). Since similar trends in  
434 biodiversity change were found for those indices that are strongly interrelated, only the  
435 contour plots of the three previously selected indices (indicated in bold in Table 3) are  
436 presented here. Here, both richness and dominance were highly variable between years and  
437 variations were site-specific. In contrast, the evenness was comparatively less variable and  
438 showed trends that were more similar than the ones encountered for dominance. For the  
439 longer time-series of Le Croisic and Ouest Loscolo, there was an increase in the number and  
440 duration of high dominance events along the period. For Le Croisic, for example, there

441 seemed to be a trend where the start of the dominance period occurred earlier in the year  
442 from 2001 onwards. For Ouest Loscolo, the dominance period was nearly extended across all  
443 seasons with longer peak periods (from 2007) compared to earlier years in the time-series  
444 where the dominance periods were confined to spring and autumn times. This seasonal  
445 expansion of high dominance correlated with increased periods of low richness and  
446 evenness.

447 For SOMLIT-Astan, a short but high dominance event was recorded in May 2008 with an  
448 unusually low dominance in September of the same year (Fig. 3; Fig. S2a). The next year, the  
449 dominance period was more spread out from mid-April to October with two peaks in May  
450 and September.

451 Whilst the contour plots for  $\alpha$  diversity indices informed on the state of the community, the  $\beta$   
452 index was able to detect significant temporal changes at the community (LCBD) and the  
453 genus level (IVI) on an annual basis. For Le Croisic, a year of relatively low richness and high  
454 dominance (2007) was followed by a year of high richness, with peaks in June-July and  
455 September (2008) (Fig. 3, Fig. S2b). The events in 2007 were marked by a relatively elevated  
456 value of the LCBD (0.26) indicating a significant shift in the phytoplankton community  
457 structure (Fig.4). Upon visual inspection of the IVI for the same year (Fig. 5a), the peak in  
458 dominance was due to the blooming of the species *Lepidodinium chlorophorum* (47%) with an  
459 abundance of  $3.9 \times 10^6$  cells  $L^{-1}$  in July and to a lesser extent to the genera *Skeletonema* spp. ( $1.5$   
460  $\times 10^6$  cells  $L^{-1}$ ) in April and *Leptocylindrus* spp. in Mai ( $5.4 \times 10^5$  cells  $L^{-1}$ ) and September ( $6.13$   
461  $\times 10^5$  cells  $L^{-1}$ ). The previous year at the same site was characterised by a community  
462 dominated by *Chaetoceros* spp. (32%) and *Gymnodinium* spp. (18%) with lower abundances  
463 ( $<8 \times 10^5$  cells  $L^{-1}$ ). In 2014, a value of the LCBD (0.25) similar to that of 2007 was found, that  
464 also coincided with a bloom of *Lepidodinium chlorophorum* (77%), with an abundance of

465 1.15x10<sup>7</sup> cells L<sup>-1</sup>(Fig. 5b). Before and after the bloom, *Leptocylindrus* spp. (13%) was also  
466 abundant (>8x10<sup>5</sup> cells L<sup>-1</sup>). Similarly, in the Ouest Loscolo site, high LCBD (0.45) and  
467 dominance values were recorded in 2008 (Fig. 3). In this case, a monospecific bloom of  
468 *Leptocylindrus* spp. (73%) that peaked in July (2.2x10<sup>6</sup> cells L<sup>-1</sup>) and October (8x10<sup>6</sup> cells L<sup>-1</sup>)  
469 was responsible (Fig. 5c). Earlier in the year, smaller blooms were recorded in April for the  
470 genus *Skeletonema* spp. (1.17 x10<sup>6</sup> cells L<sup>-1</sup>) and in June for the Chaetocerotaceae (1.8x10<sup>6</sup> cells  
471 L<sup>-1</sup>). In 2011, an unusually high richness and relatively low dominance was recorded at Ouest  
472 Loscolo but this marked change in community structure was not reflected in the LCBD's.  
473 This shows the importance to consider both  $\alpha$  and  $\beta$  diversity indices together to detect and  
474 interpret potential changes in the phytoplankton community structure.

475

## 476 Discussion

477 Ecological indicators based on key functional groups, such as phytoplankton, can provide  
478 sensitive and quantifiable indications of ecological changes and environmental perturbations  
479 in marine surface waters (Paerl et al., 2003; Rombouts et al, 2013). The common OSPAR  
480 Pelagic Habitat indicator “Changes in plankton diversity” was developed as a surveillance  
481 indicator to describe the phytoplankton community structure and to identify temporal  
482 changes or “events” within the assessment period. Since biodiversity is multi-dimensional,  
483 no single measure can meet all needs for assessing change (Buckland et al., 2017). It is,  
484 therefore, important to use PH3 as a composite indicator where the alpha diversity, i.e. the  
485 diversity within a site or sample, and the beta diversity that focuses on the rate of change, or  
486 turnover, in species composition are being considered. For this purpose, four indices were  
487 identified that focus on different aspects of plankton biodiversity from a community to

488 genus level namely the taxon (genus) richness (Menhinick's index,  $D$ ), dominance (Hulburt  
489 index,  $\delta$ ), temporal variation (Local Contributions to Biodiversity, LCBD) and taxa  
490 identification (Important Value Index, IVI). Whilst the richness and dominance indices are  
491 evaluated on a monthly basis, the temporal variation and taxa identification are assessed on  
492 an annual level.

493

494 The final selection of one richness and one dominance index was based on a comparative  
495 analysis of the metrics' performances. The performances were mainly evaluated from an  
496 ecological perspective and from the sensitivity of the metrics but ultimately, the selected  
497 indices were retained on their ability to synthesise relevant information in an understandable  
498 and unambiguous manner to stakeholders. The Menhinick's diversity index ( $D$ ) was selected  
499 as the most appropriate metric to describe the number of taxa in the community. In this  
500 study, it was found to be the most sensitive to changes in environmental conditions that  
501 could be either from a natural or an anthropogenic source. Similar studies agree that  $D$  is one  
502 of the most efficient tools for the assessment of water quality (e.g. Facca et al., 2014; Spatharis  
503 and Tsirtsis, 2010; Buzançıç et al., 2016; Varkitzi et al., 2018). However, caution must be taken  
504 when interpreting any index based on estimates of the number of species in the community  
505 since these are biased (Heip et al., 1998). An observed increase in the counts of  
506 phytoplankton taxa and thus an increase in the biodiversity index can have numerous  
507 causes: sampling methods (Rodriguez-Ramos et al., 2014) and effort (Cozzoli et al., 2017),  
508 advection of new taxa (Lévy et al., 2014; Sun and Xue, 2016), increased knowledge of the  
509 taxonomic analyst (Dromph et al., 2013), etc. Whilst these factors likely underestimate the  
510 true taxonomic diversity in the phytoplankton community, here, we are more interested in  
511 the overall state and the relative changes in the community composition on a seasonal and

512 annual basis. In any case, considering the highly intra-annual variability of taxa and  
513 abundances, consistent monthly monitoring is essential when quantifying phytoplankton  
514 community diversity. Also, any taxonomic richness index should be interpreted in  
515 conjunction with a dominance index to better understand the overall structure of the  
516 phytoplankton community. Here, visual inspection suggests a seasonal expansion of the low  
517 diversity in conjunction with high dominance periods over years, especially notable for the  
518 longer time-series, Ouest Loscolo and Le Croisic.

519

520 Dominance phenomena and significant changes in phytoplankton community structure can  
521 occur in impacted areas (e.g. Buzançıç et al., 2016). Here, as a dominance measure, the  
522 Hulburt index ( $\delta$ ) was mainly selected for its ease of interpretation (as a percentage, where a  
523 high value indicates high dominance) but also for its recent applications in water quality  
524 assessments (Facca et al., 2014). Using the Principal Component Analysis, the Brillouin index  
525 ( $H_B$ ) was found to be the only dominance measure that explained the variations in the  
526 environment but since this metric was interrelated with  $D$  and thus likely to be redundant,  
527 the former was not retained. Periods of relatively high dominance were also identified by the  
528 LCBDs as a general period of significant change or turnover. For the stations Ouest Loscolo  
529 and Le Croisic in the Bay of Biscay, 2007 and 2008, respectively, were identified as years with  
530 a temporary shift to relatively high community variation. The analysis of the Important  
531 Value Index (IVI) showed that these observed temporal shifts in community structure were  
532 marked by a monospecific bloom from *Leptocylindrus* spp. (a diatom - at Ouest Loscolo, > 8  
533 million cells L<sup>-1</sup>) and *Lepidodinium* spp. (a dinoflagellate - at Le Croisic, > 4 million cells L<sup>-1</sup>). A  
534 high increase of biomass, so called bloom events if the number of cells > 1 million cells L<sup>-1</sup>,  
535 can be a result of nutrient inputs such as nitrate and phosphate (Alves-de-Souza et al., 2006),



536 but also of changing environmental conditions, for example temperature and salinity  
537 (Pizarra et al., 1997). *Lepidodinium chlorophorum*, for example, is known to form regular  
538 “green” blooms over the French Atlantic Shelf (Sourisseau et al., 2016), but in the year 2007 a  
539 unusual high number of events was observed (Chauvin, 2012). In terms of ecological  
540 impacts, their blooms can cause anoxia and bright-green coloured waters. For the genus  
541 *Leptocylindrus* spp, the unusual high temperatures recorded in 2007 could explain the  
542 observed bloom since the genus has an ecological niche of relatively warm temperatures and  
543 high light conditions (Hernández-Fariñas et al., 2013). Whilst *Leptocylindrus* spp. has been  
544 identified as an indicator of eutrophication (Ninčević-Gladan et al., 2015), there are no  
545 records of a similar application in our study area. In this specific case, taxa identification  
546 using the IVI index helped to understand the ecological behaviour of the taxa (for example,  
547 as a response to environmental conditions). Also, in case a genus would develop into a  
548 Harmful Algal Bloom (HAB), the potential effects of blooming taxa on the ecosystem could  
549 be investigated. Further analyses of the effects of natural and anthropogenic pressures on  
550 phytoplankton communities will help to identify the most effective mechanisms and the  
551 actions needed to maintain or to restore GES conditions (Crise et al., 2015).

552

553 Volume indices, such as the geometric mean of relative abundance (G), are increasingly  
554 being used to examine trends in biological diversity and to assess whether biodiversity  
555 targets are being met (Buckland et al., 2011). In contrast to the Shannon’s and Simpson’s  
556 indices, G will decline if all species are declining at the same rate even if there is no trend in  
557 evenness. Whilst the concept of this volume index is interesting, the geometric mean has also  
558 a number of drawbacks that unfortunately make the index unsuitable for assessing  
559 phytoplankton communities. Most importantly, the index is based on within-taxon trends

560 and requires a robust calculation where each taxon is recorded in every year. Since  
561 phytoplankton datasets are generally characterized by a small number of abundant species  
562 and many rare species, the index is likely to exhibit high variance and unstable behaviour  
563 when species are not consistently present in the community. A potential solution would be  
564 to calculate the index on only those taxa that are present in every sample but then the index  
565 would reflect trends of the subset of taxa and not the whole community, and as such, the  
566 index has limited use as a community diversity measure to assess GES of pelagic habitats.

567

568 Compared to phytoplankton biomass indicators, the development of community  
569 composition indicators for water quality assessment is in its early stages. Firstly, the  
570 responses of phytoplankton community composition to a combination of nutrients is  
571 relatively unpredictable and so, establishing significant pressure-state relationships can  
572 become difficult (Garmendia et al., 2013; Ochocka and Pasztaleniec, 2016), especially in  
573 marine open water systems. Studies of phytoplankton communities in relation to pressure  
574 gradients confirmed the intermediate disturbance level hypothesis, which predicts high  
575 richness in areas subjected to intermediate levels of disturbance (Sommer et al., 1993;  
576 Ninčević-Gladan et al., 2015). So in line with this view, high diversity does not necessarily  
577 correlate with “good” environmental conditions. Conversely, the presence of blooms could  
578 be perceived as “negative” by societies but can be often driven by natural conditions. As  
579 long as the pressure–state relationships are inadequately understood, ecologically  
580 meaningful boundaries and thus targets to assess GES cannot be defined for PH3.  
581 Unfortunately, we were unable to examine the behaviour of the indicator under different  
582 stressor scenarios. Whilst PH3 will need further development to support formal state  
583 assessment, the indicator can still be very informative on the health of the environment and

584 act as a “surveillance” indicator rather than an operational one. Although, “surveillance”  
585 indicators do not directly track state in relation to GES, they do provide complementary  
586 information (highlighting a « specific cause for concern ») that presents a broader and more  
587 holistic picture of state, and inform and support science, policy, and management (Shephard  
588 et al., 2015; Varkitzi et al., 2018; Bedford et al., 2018). In this respect, PH3, in its current state  
589 of development, will act as a warning signal by highlighting unprecedented or directional  
590 state shifts in the plankton communities of the marine pelagic habitat.

591

592 Detecting trends in the structure of phytoplankton communities is achievable but requires  
593 the collection of suitable data (Ajani et al., 2014). Long-term monitoring networks of  
594 sufficient spatial and temporal resolution are needed to distinguish the anthropogenic and  
595 natural processes that affect the phytoplankton abundance and composition, and to be able  
596 to detect significant changes in the community structure in a robust manner. Several  
597 transnational projects and conventions have already highlighted the need for appropriate  
598 monitoring programs to feed biodiversity indicators and associated parameters. The  
599 PERSEUS project, for example, pointed out the lack of quantitative data on pressures and a  
600 lack of spatial coverage, in particular offshore data on nutrients, phytoplankton and  
601 dissolved oxygen (Crise et al., 2015). For more complete regional assessments, in particular,  
602 better acquisition of region-wide plankton data and coherent monitoring programmes will  
603 still be required (Caroppo et al., 2013; OSPAR, 2017d; Varkitzi et al., 2018). In terms of  
604 sampling frequency, a minimum of bimonthly sampling is advised for estimating  
605 phytoplankton biodiversity (Uusitalo et al., 2013; OSPAR, 2017d). With regards to the  
606 analysis of the phytoplankton community data, light microscopy is the most commonly used  
607 laboratory technique for the determination of the abundance and species identification

608 (OSPAR, 2016). Whilst this method is time-consuming and requires a high degree of  
609 expertise (Havskum et al., 2004), detailed taxonomic data, containing information on the  
610 presence/absence and abundance of individual plankton species, are required to underpin  
611 the development of sensitive species and community-level indicators (Beaugrand et al., 2005;  
612 McQuatters-Gollop et al., 2017). In this respect, well-educated microscopists are necessary for  
613 obtaining reliable phytoplankton monitoring results (Lehtinen et al., 2012). Unfortunately,  
614 adequate funding to support plankton taxonomy in line with its value to science and  
615 decision making remains a key challenge to ensuring the availability of plankton data for  
616 marine policy and conservation (McQuatters-Gollop et al., 2017). Innovative analysis  
617 techniques exist (OSPAR, 2016; Karlson et al., 2016; Chust et al., 2017; Aubert et al., 2017) but  
618 it is difficult to find a “one size fits all” method for counting and characterizing the  
619 composition of the phytoplankton communities in marine systems, due to their intrinsically  
620 high spatial and temporal variability (Garmendia et al., 2013), and diversity of sizes (Sieburth  
621 et al., 1978). In any case, microscopic data will still be required to support and validate new  
622 analytical methods and to test indicators derived from these new types of monitoring  
623 (McQuatters-Gollop et al., 2017).

624

625 Whilst some authors remain sceptical of the community composition approach (e.g.  
626 Ninčević- Gladan et al., 2015), others have demonstrated successful applications of  
627 composition based metrics for water quality assessment, mainly developed for use in the  
628 WFD (e.g. Tett et al., 2008; Devlin et al., 2009; Facca et al., 2014). In most cases, these  
629 assessments were carried out using multimetric indicators because the inclusion of  
630 additional metrics can render an index more sensitive and robust (e.g. Hering et al., 2006;  
631 Rombouts et al., 2013). When selecting indicators, the aggregation (combined use of several

632 indicators for an ecosystem-based approach) should consider different elements of  
633 community response to environmental change, e.g. taxonomic and functional diversity,  
634 biomass, species composition and the presence of opportunistic or non-indigenous species  
635 (Lehtinen et al., 2012; Zettler et al., 2017). In case of the common OSPAR indicators, this type  
636 of aggregation could be achieved by combining each Pelagic Habitat (PH) indicator where  
637 the plankton community is considered at different resolutions, PH1 at the life-form level of  
638 the community, PH2 for the total biomass/abundance of the community and PH3 at the  
639 species level. Hence, by combining the information from these three indicators, a more  
640 holistic assessment of plankton dynamics can be obtained than from each indicator  
641 individually.

642

643 With the current OSPAR common indicators, the determination of the ecological quality of  
644 the pelagic habitat is based on the biological quality elements only, the plankton. According  
645 to Article 3 of the MSFD, however, “Good Environmental Status” (GES) for pelagic habitats  
646 is defined by “the structure, functions, and processes of the constituent marine ecosystems,  
647 together with the associated physiographic, geographic, geological and climatic factors,  
648 allow those ecosystems to function fully and to maintain their resilience to human-induced  
649 environmental change.” Even with a clear definition of GES, the variability in prevailing  
650 conditions of the marine environment makes recognising if we have reached GES  
651 challenging, especially for pelagic habitats. Therefore, a more integrated approach that also  
652 accounts for the non-biological components of the sea water will need to be developed  
653 (Ferreira et al., 2011; Rombouts et al., 2013). Recently, Dickey-Collas and colleagues (2017)  
654 discussed the challenges related to the concept of “good” environmental status of pelagic  
655 habitats and propose directions for reflection and research to effectively monitor progress

656 towards, or movement from, GES. In summary, the authors propose three conditions that  
657 should be met for pelagic habitats to be in GES: (i) all species present under current  
658 environmental conditions have access to the pelagic habitats essential to close their life  
659 cycles; (ii) biogeochemical regulation is maintained at normal levels; (iii) critical physical  
660 dynamics and movements of biota and water masses at multiple scales are not obstructed.

661

662 For now, the current determination of GES for pelagic habitats takes a pragmatic approach  
663 and largely relies on existing information, data and methodologies. Especially for pelagic  
664 habitats, monitoring all species groups in all pelagic habitat types in all localities is simply  
665 not feasible. At best, it is possible to monitor a selection of species groups, preferably species  
666 sensitive to environmental change over relatively short time-scales and where data can be  
667 collected to ensure regular updates (Van Strien et al., 2012 and references therein). Any  
668 outstanding issues can be addressed during subsequent MSFD cycles through, for example,  
669 the development of new methodologies (Danovaro et al., 2016), the gathering of additional  
670 data through monitoring programmes and further development of indicators (EC, 2011;  
671 Padegimas et al., 2017). In line with the ongoing work within OSPAR and other Regional  
672 Seas conventions, the further implementation of the MSFD will continue to be agreed with  
673 the stakeholders at transnational level and to be based on solid scientific knowledge (Varkitzi  
674 et al., 2018). The pilot study for the development of PH3 presented here is based on the  
675 outcome of the Intermediate Assessment 2017 and this type of preliminary assessment is the  
676 starting point of a long-term iterative process.

677

678

679

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694

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