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# Changes in marine phytoplankton diversity: assessment under the Marine Strategy Framework Directive

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**Abstract**

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

and October ($8x10^6$ cells $L^{-1}$). In this way, PH3 informs on different aspects of phytoplankton diversity from a community to a genus level. At the current stage of development, however, PH3 acts as a "surveillance" rather than an operational indicator since the relationship to GES is not directly tracked. In the future, by additional testing of PH3 and extending the geographical scope, the robustness of the assessment could be further determined across the OSPAR Maritime Area.

## Introduction

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

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

Coastal ecosystems face increasing human disturbances such as pollution and/or eutrophication (i.e. excessive nutrients or organic enrichments) that can drive marked changes in the plankton community dynamics and thus in its structural attributes, such as diversity, dominance or size structure. Phytoplankton, for example, show rapid responses to altered nutrient levels through changes in biomass and composition (Reynolds, 2006). Whereas the use of phytoplankton biomass for water quality assessment has a long history (Pasztaleniec, 2016), the evaluation of community composition has gained a more recent interest through the implementation of the Water Framework Directive (WFD) (Devlin et al., 2009; Uusitalo et al., 2013). In the WFD, three metrics, namely 'phytoplankton abundance', 'phytoplankton biomass' and 'phytoplankton taxonomic composition', are part of the biological quality elements (BQEs), i.e. organism groups which integrate the effects of various stressors such as nutrient enrichment, acidification, and, to some extent, hypoxia or habitat degradation (Lyche-Solheim et al., 2013). In contrast to measurements for chlorophyll *a* as a proxy for biomass, the assessment of the taxonomic composition of the phytoplankton assemblage could provide information about the whole community, including the importance of the different size-groups such as the pico- and nano-phytoplankton (Domingues et al., 2008).

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

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

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

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

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

This paper summarises the development of the OSPAR common indicator “Changes in plankton diversity” (PH3) for phytoplankton communities. The aim of PH3 is to characterise the phytoplankton community structure and to detect potential temporal shifts, preferably in relation to the environment. Frequently used diversity indices, mainly developed in the context of the Water Framework Directive, were preselected. Microphytoplankton counts obtained from three coastal time-series in the Western Channel and the north of the Bay of 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).
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/). Data on salinity (psu), temperature (°C), inorganic nutrients (ammonia,
216 nitrate, nitrites, silicate, phosphates; in μmol $L^{-1}$) and oxygen (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

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

## 2. Data analysis

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

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

#### 2.1.1. Ecological relevance

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

2.1.1.1. Indices based on richness (number of taxa)

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

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

Whilst species richness (*S*) is the simplest and most straightforward index to calculate, this estimate is strongly influenced by the sampling process (Peet, 1974; Rodriguez-Samos et al., 2014). To investigate the effect of sampling effort on our estimates of richness, the cumulative 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 $$\delta = 100\ (n_1 + n_2)\ /\ \mathrm{N} \qquad (2)$$

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 (Lyashevska 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

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

### 2.2. Measuring beta diversity

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

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

$$IVI = RDi + RF_i \quad (3)$$

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

$$RD_i = (n_i / N)*100 \quad (4)$$

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

371 $RF_i = (f_i/F)*100$ (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 (*Gj*) 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 *Gj* 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 $x10^6$ cells $L^{-1}$ in July and to a lesser extent to the genera *Skeletonema* spp. (1.5
460 $x10^6$ cells $L^{-1}$) in April and *Leptocylindrus* spp. in Mai (5.4 $x10^5$ cells $L^{-1}$) and September (6.13
461 $x10^5$ 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 (<$8x10^5$ 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 1.15x$10^7$ cells $L^{-1}$(Fig. 5b). Before and after the bloom, *Leptocylindrus* spp. (13%) was also
466 abundant (>8x$10^5$ cells $L^{-1}$). 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 (2.2x$10^6$ cells $L^{-1}$) and October (8x$10^6$ cells $L^{-1}$)
469 was responsible (Fig. 5c). Earlier in the year, smaller blooms were recorded in April for the
470 genus *Skeletonema* spp. (1.17 x$10^6$ cells $L^{-1}$) and in June for the Chaetocerotaceae (1.8x$10^6$ cells
471 $L^{-1}$). 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

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

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

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

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

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

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

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

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

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

With the current OSPAR common indicators, the determination of the ecological quality of the pelagic habitat is based on the biological quality elements only, the plankton. According to Article 3 of the MSFD, however, "Good Environmental Status" (GES) for pelagic habitats is defined by "the structure, functions, and processes of the constituent marine ecosystems, together with the associated physiographic, geographic, geological and climatic factors, allow those ecosystems to function fully and to maintain their resilience to human-induced environmental change." Even with a clear definition of GES, the variability in prevailing conditions of the marine environment makes recognising if we have reached GES challenging, especially for pelagic habitats. Therefore, a more integrated approach that also accounts for the non-biological components of the sea water will need to be developed (Ferreira et al., 2011; Rombouts et al., 2013). Recently, Dickey-Collas and colleagues (2017) discussed the challenges related to the concept of "good" environmental status of pelagic 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.
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679

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