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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 (2.2×10^6 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 indices for assessing GES of pelagic habitats under the MFSD. More specifically, we tested these diversity indices for their ecological relevance, mathematical consistency and link to marine hydrological factors.

Materials and methods

1. Phytoplankton and environmental datasets

Microscopic counts of phytoplankton data from the Western Channel and the north of the Bay of Biscay, France, were collated from two sources, namely RESOMAR-Pelagos (Pelagic database of the Réseau National des Stations et Observatoires Marins; http://resomar.cnrs.fr/Base-de-donnee-Pelagos) and REPHY (Réseau d'Observation et de Surveillance du Phytoplancton et des Phycotoxines;http://envlit.ifremer.fr/surveillance/phytoplancton_phycotoxines/presentation). The REPHY is implemented and managed by the French Research Institute for the Exploitation of the Sea (IFREMER). The database of RESOMAR-Pelagos hosts plankton data collected from most of the French coastal marine stations and observatories. From the RESOMAR-Pelagos database, we filtered for stations where samples were collected and analysed using consistent methodology, were sampled at a minimum monthly frequency, which contained minimal gaps in the sampling, and which simultaneously sampled nutrients and hydrological factors. This selection resulted in the station of SOMLIT-Astan (2007-2013, fig. 1), a coastal long-term monitoring station situated 4.6 km from the coast that is characterized by permanently mixed waters with limited continental influence. Twice a month, seawater samples are collected at 1 m depth using a 5 liters Niskin bottle for phytoplankton analysis. Samples are fixed with acid Lugol's iodine solution and then stored according to the methods described in Sournia (1978). Cell counts are made under an inverted light microscope at 200-400x magnification. Further details on phytoplankton quantification and identification protocols for SOMLIT-Astan can be found in Guilloux et al. (2013). Environmental data from the site are collected by the Station Biologique de Roscoff and hosted by the SOMLIT (Service d'Observation en Milieu LITtoral, INSU-CNRS) database; they were retrieved from their online platform (http://somlit.epoc.u-bordeaux1.fr/fr/). Data on salinity (psu), temperature (°C), inorganic nutrients (ammonia, nitrate, nitrites, silicate, phosphates; in μmol L^{-1}) and oxygen (ml L^{-1}) were used in the analysis.

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

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

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

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

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

Classical measures such as Shannon and Simpson's are based on species proportions and fail to measure changes in abundance if all species in a community are declining at the same rate (Buckland et al., 2011). To overcome this issue, the geometric mean index *G j*, for example, quantifies the average trend in relative abundance across species in the community (Buckland et al., 2011). Finally, evenness indices express the equitability of species abundance in the sample or the community (Washington, 1984). Here, we applied the Pielou's index (*J′*; Pielou, 1975).

2.1.2. Mathematical consistency

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

2.1.3. Link with environmental conditions

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

A standardized Principal Components Analysis (PCA; Jolliffe, 1986) was applied to the potential environmental correlates of phytoplankton diversity to determine: (1) the environmental variables that explained the largest variation in the data set, (2) the relationships among these potential environmental predictors, and (3) how the scores of the principal components were related to the phytoplankton diversity 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.

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

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:

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

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.

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

where f_i is the number of occurrence of the genus *i* and *F* is the total number of occurrence of all the genera.

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

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

2. Results

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

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

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

The Principal Components Analysis (PCA) investigated the relationships among the mean and seasonal variations in physico-chemical factors (Fig. 2), and the relationships of the PC with phytoplankton diversity indices (Table 2). Similar correlations were found for the different test sites, suggesting that the analyses explain the general behaviour of the index and that the responses are not only a function of the prevailing local environmental conditions. In SOMLIT-Astan, for example, the first Principal Component (PC1) explained 43% of the variation in the data where temperature, nitrate, phosphate and silicate contributed mostly (Fig. 2a). The PC2 was explained by salinity, oxygen and nitrite and accounted for 26% in the variation. For the seasonal variations in the environmental factors (Fig. 2b), the PC1 explained 28% and the PC2 explained 26%. However, in terms of the correlations with the PC and diversity indices, the seasonal variations in environmental factors are more strongly related to diversity than annual mean conditions (Table 2). For the richness group, D was the metric best explained by the seasonal variations in environmental factors for SOMLIT-Astan ($r^2 = 0.76$; $p<0.001$).

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

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

To investigate the seasonal and annual variations in the three aspects of diversity simultaneously, contour plots of genus richness (expressed here as D), dominance (expressed here as δ) and evenness (J') per sampling site are shown (Fig. 3). Since similar trends in biodiversity change were found for those indices that are strongly interrelated, only the contour plots of the three previously selected indices (indicated in bold in Table 3) are presented here. Here, both richness and dominance were highly variable between years and variations were site-specific. In contrast, the evenness was comparatively less variable and showed trends that were more similar than the ones encountered for dominance. For the longer time-series of Le Croisic and Ouest Loscolo, there was an increase in the number and duration of high dominance events along the period. For Le Croisic, for example, there seemed to be a trend where the start of the dominance period occurred earlier in the year from 2001 onwards. For Ouest Loscolo, the dominance period was nearly extended across all seasons with longer peak periods (from 2007) compared to earlier years in the time-series where the dominance periods were confined to spring and autumn times. This seasonal expansion of high dominance correlated with increased periods of low richness and evenness.

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

Whilst the contour plots for α diversity indices informed on the state of the community, the β index was able to detect significant temporal changes at the community (LCBD) and the genus level (IVI) on an annual basis. For Le Croisic, a year of relatively low richness and high dominance (2007) was followed by a year of high richness, with peaks in June-July and September (2008) (Fig. 3, Fig. S2b). The events in 2007 were marked by a relatively elevated value of the LCBD (0.26) indicating a significant shift in the phytoplankton community structure (Fig.4). Upon visual inspection of the IVI for the same year (Fig. 5a), the peak in dominance was due to the blooming of the species *Lepidodinium chlorophorum* (47%) with an abundance of 3.9 x10^6 cells L^{-1} in July and to a lesser extent to the genera *Skeletonema* spp. (1.5 x10^6 cells L^{-1}) in April and *Leptocylindrus* spp. in Mai (5.4 x10^5 cells L^{-1}) and September (6.13 x10^5 cells L^{-1}). The previous year at the same site was characterised by a community dominated by *Chaetoceros* spp. (32%) and *Gymnodinium* spp. (18%) with lower abundances (<8x10^5 cells L^{-1}). In 2014, a value of the LCBD (0.25) similar to that of 2007 was found, that also coincided with a bloom of *Lepidodinium chlorophorum* (77%), with an abundance of

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

Discussion

Ecological indicators based on key functional groups, such as phytoplankton, can provide sensitive and quantifiable indications of ecological changes and environmental perturbations in marine surface waters (Paerl et al., 2003; Rombouts et al, 2013). The common OSPAR Pelagic Habitat indicator "Changes in plankton diversity" was developed as a surveillance indicator to describe the phytoplankton community structure and to identify temporal changes or "events" within the assessment period. Since biodiversity is multi-dimensional, no single measure can meet all needs for assessing change (Buckland et al., 2017). It is, therefore, important to use PH3 as a composite indicator where the alpha diversity, i.e. the diversity within a site or sample, and the beta diversity that focuses on the rate of change, or turnover, in species composition are being considered. For this purpose, four indices were 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, *δ*), 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 (δ) 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 act as a "surveillance" indicator rather than an operational one. Although, "surveillance" indicators do not directly track state in relation to GES, they do provide complementary information (highlighting a « specific cause for concern ») that presents a broader and more holistic picture of state, and inform and support science, policy, and management (Shephard et al., 2015; Varkitzi et al., 2018; Bedford et al., 2018). In this respect, PH3, in its current state of development, will act as a warning signal by highlighting unprecedented or directional state shifts in the plankton communities of the marine pelagic habitat.

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

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

Whilst some authors remain sceptical of the community composition approach (e.g. Ninčević- Gladan et al., 2015), others have demonstrated successful applications of composition based metrics for water quality assessment, mainly developed for use in the WFD (e.g. Tett et al., 2008; Devlin et al., 2009; Facca et al., 2014). In most cases, these assessments were carried out using multimetric indicators because the inclusion of additional metrics can render an index more sensitive and robust (e.g. Hering et al., 2006; 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 towards, or movement from, GES. In summary, the authors propose three conditions that should be met for pelagic habitats to be in GES: (i) all species present under current environmental conditions have access to the pelagic habitats essential to close their life cycles; (ii) biogeochemical regulation is maintained at normal levels; (iii) critical physical dynamics and movements of biota and water masses at multiple scales are not obstructed.

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

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