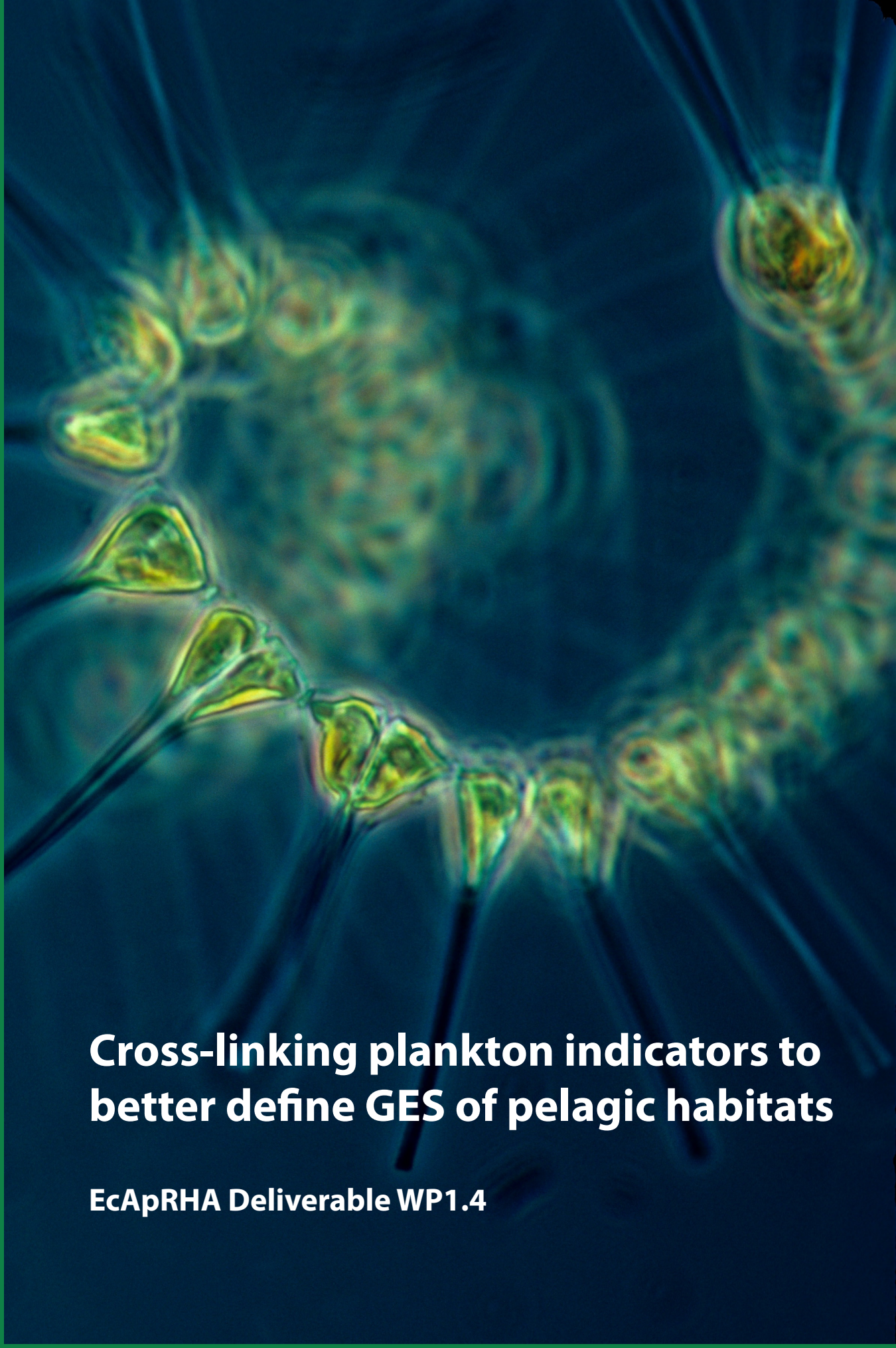

Cross-linking plankton indicators to better define GES of pelagic habitats

EcApRHA Deliverable WP1.4

Co-financed by the European Union

EcApRHA an Ecosystem Approach to Regional Habitat Assessments

2017

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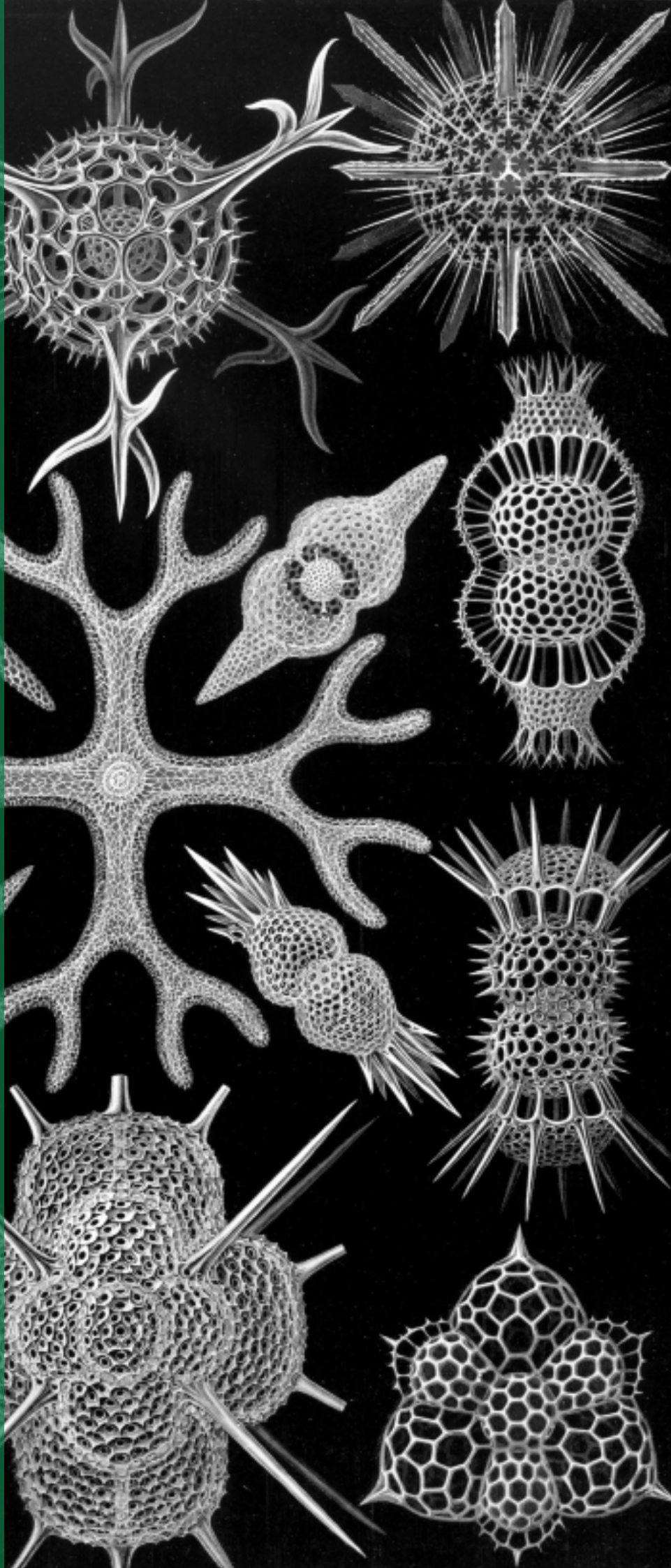

EcApRHA

The EcApRHA project (Applying an Ecosystem Approach to (sub) Regional Habitat Assessment) aims to address gaps in the development of biodiversity indicators for the OSPAR Regions. In particular, the project aims to overcome challenges in the development of indicators relating to the MSFD (Marine Strategy Framework Directive 56/2008/EU), such as Descriptor D1 (Biodiversity), D4 (Food webs) and D6 (Seafloor integrity), and to deliver an action plan to OSPAR that will enable monitoring and assessment at the (sub) regional scale, to contribute to OSPAR Intermediate Assessment 2017.

Indicators related to the benthic and pelagic habitats, as well as food webs, are investigated within the project at different levels (from data to indicator; from indicator to habitat assessment; from habitat to ecosystem assessment).

Acknowledgment

This report was produced as a result of the EcApRHA (Addressing gaps in biodiversity indicator development for the OSPAR Region from data to ecosystem assessment: Applying an ecosystem approach to (sub) regional habitat assessments) project. The project was co-financed by the European Union (EU). Grant No. 11.0661/2015/712630/SUB/ENVC.2 OSPAR

Executive summary

The multimetric approach is a methodological tool which can be used to study a range of systems, including GES assessment of marine waters. Three indicators (PH1, PH2 and PH3) are currently being developed in the frame of the OSPAR convention for the pelagic habitat component. The three PH indicators provide information on different and complementary aspects of the plankton community that, only when considered altogether, provide a holistic vision of the ecosystem which is central to GES assessment. The present document aims at combining their information for the first time, following a multimetric approach. For this purpose, it was decided that the Plymouth Marine Laboratory L4 station would be the focus of this deliverable for the period 2000-2014.

Different results were obtained from PH1, PH2 and PH3 regarding dates characterized by atypical plankton community structures, stressing their complementarity. However, similar results were also found for some sampling dates, suggesting that the complementary information conveyed by the three PH indicators shows potential for generating a higher-level indicator.

This work has also evidenced a number of gaps and issues in the integration of the three PH indicators that we address with guidelines. In particular, efforts should be devoted to overcome technical difficulties in the integration the PH3 indicators, especially regarding differences in temporal resolution. Future development of the indicators could involve complementary techniques to classical methods to overcome taxonomic constraints. In the frame of this project, the access to data in certain format was identified also found problematic. Creating a central database of pre-formatted data managed by a group of experts could also benefit the regional calibration of the indicators for areas where appropriate data are available. Establishing a clear and easily accessible report which details all the monitoring guidelines concerning the metrics used for the OSPAR PH indicators could also be beneficial for homogenising the monitoring and inter-comparability of data among contracting parties in the goal of regional marine management.

Acronyms

Chl *a*: Chlorophyll *a*
GES: Good Environmental Status
IVI: Importance Value Index
LCBD: Local Contribution to Beta Diversity
MSFD: Marine Strategy Framework Directive
PML: Plymouth Marine Laboratory
PCA: Principal Component Analysis

1 Introduction/Background

Policy mechanisms aiming at managing seas in an ecosystemic way require tools to monitor and to inform on the health of marine systems. Indicators of Good Environmental Status (GES) are thus developed to serve the implementation of the Marine Strategy Framework Directive (MSFD, 2000/60/EC).

Approaches based on the use of multimetric indices have been developing fast during the last decade (Nõges et al. 2009) and their theoretical basis is fairly well detailed in the scientific literature, supporting researchers in the design of such indices (e.g. Hering et al. 2006, Schoolmaster Jr. et al. 2012). Multimetric indices synthesize data from multiple levels of biological organization with the goal of deriving a single index that indicates the ecological status of a particular type of habitat (Schoolmaster Jr. et al. 2012). By combining metrics of different categories such as biomass, diversity or trophic structure, multimetric indices can indeed inform about the multitude of impacts of anthropogenic disturbances at different biotic levels in the ecosystem to provide a holistic picture of the studied system. Compared to single index metrics, this approach is powerful as it reduces uncertainty in the assessment while increasing the robustness of the assessment (Dale & Beyeler 2001, Herring et al. 2006, Borja & Dauer 2008). In recent years, multimetric indices were proven particularly helpful in the implementation of the Water Framework Directive and were developed to assess a variety of aquatic ecosystems (e.g. lakes: Gabriels et al. 2010, rivers: Gabriels et al. 2010, Ocampo-Duque et al. 2007, seas: Fano et al. 2003, Pachés et al. 2012). As a result, the multimetric index approach is fundamental to the development of GES indicators for the MSFD.

For the pelagic habitat component, the indicators developed in the frame of the OSPAR convention consider different levels of organization of plankton communities. Several multimetric indices constructed in recent years and described in the scientific literature include measurements of plankton communities that helped the development of the Pelagic Habitat indicators. These tools were particularly developed for phytoplankton of different aquatic systems with typical multimetric indices based on phytoplankton monitoring data integrating at least a measure of phytoplankton biomass based on chlorophyll a (Chl a) concentration analysis and of taxonomic richness based on light microscopic counts. Other complementary measures can include metrics on cell size-class (e.g. Lugoli et al. 2012, Laplace-Treyture & Feret 2016) or species dominance in the community (e.g. Facca et al. 2014). In comparison, very few multimetric indices have been developed for zooplankton communities, with the notable exception of the Zooplankton Assemblage Indicator developed for the assessment of US national lakes (Peck & Blocksom 2015) which considers in its metric selection process indices of richness/biomass/density, diversity/dominance, trophic guild, and taxonomic composition. Metrics for zooplankton and for phytoplankton can also be combined (i.e. integrated) in order to broaden the assessment of the ecological status of marine and freshwater ecosystems (e.g. Kane et al. 2009). From these examples, three Pelagic Habitat indicators are being developed in the frame of the OSPAR convention to capture complementary aspects of plankton community dynamics.

The OSPAR indicators proposed so far consider “Abundance/composition metrics” (PH2 – Biomass and abundance), “Functional metrics” (PH1 – Plankton lifeforms) and “Richness / diversity metrics” (PH3 – biodiversity indices). For the moment, these indicators are state indicators, aiming at describing the community structure and changes in this structure. Metrics on “Sensitivity / tolerance” are missing because the current scientific ability to identify precise anthropogenic pressures for pelagic habitats is limited (Hunsicker et al. 2016). Our approach, however, accounts for environmental/natural variables that could be influenced by human activities (e.g. nutrients, temperature, turbidity, turbulence, etc.). These environmental constrains are fundamental factors shaping plankton diversity and should always

be included from the very beginning of the development of the indicators. To date, the three indicators have not been considered together. The integration of these indicators has the potential to give a more holistic answer to how plankton communities response to environmental changes and/or anthropogenic stressors, which would constitute a more holistic indicator than the consideration of the single indicators separately.

The principal aims of this action are to combine information of the three PH indicators for the first time and to relate them with environmental factors.

It is worth stressing that the dynamics of plankton are highly variable both in space and time in addition to being strongly dependent on the physico-chemical properties in the environment. As a consequence, interpreting changes in plankton communities reflected by variations of PH1, PH2 and PH3 indicators, as in the present work, cannot be performed without considering these environmental factors. For this project, we focused on one station where data for the three indicators can be accessed and used in combination with data on the environmental factors that structure plankton communities. We selected datasets for which experts, with strong knowledge of the zone of consideration, could be easily contacted if needed for interpreting the results. Consequently, Plymouth Marine Laboratory's L4 station, based in the western English Channel, was chosen for the development of our methodological approach.

In addition to the primary aims of the action, we provide a working plan and tools for interpreting and better understanding how plankton communities as a whole respond to environmental changes.

This secondary aspect is justified by the limited time generally devoted to designing adequate assessment tools in most biodiversity assessment projects and was motivated by its potential need for future assessments and tests to be, for instance, conducted on other data sets and regions.

The French National Museum of Natural History (MNHN) is the leading institution for this action.

2 PH1-PH2-PH3 integration: the multimetric index approach

In this section, we propose an initial methodological approach for further developments and testing of the integration of the three indicators for any kind of plankton data set fitting the OSPAR indicator metric requirements. The integration of the three indicators, PH1, PH2 and PH3, is expected to provide a wider, holistic, picture of plankton diversity and dynamics by considering different levels of organisation of the community all together. Ultimately, the assessment of Good Environmental Status of pelagic habitats should consider both environmental/natural factors and human-induced pressures in order to define assessment thresholds for the three indicators.

We do not aim to synthesize current knowledge on multimetric indices here because recent reviews can already be found in the literature (e.g. Hering et al. 2006, Schoolmaster Jr. et al. 2012). Among these, the deliverable report D4.1-4 from the WISER project (Lehtinen et al. 2012) is of particular interest for the plankton component. This document reviews all multimetric indicators that were developed for assessing and managing phytoplankton communities in coastal and transitional waters. However, the exact same methodology could not be applied for the OSPAR pelagic indicators because no multimetric index developed so far considers community composition, diversity indices and functionality aspects of plankton simultaneously, in contrast to the integration of the PH indicators. The integration of the OSPAR indicators is, hence, challenging. Nevertheless, we built on this information to develop the present multimetric index approach.

Practical and well-defined steps are essential for building efficient management and assessment tools. In order to develop a multimetric index, Hering et al. (2006) notably set up a standardized procedure, which, we propose, can be adapted in our case as follow:

1. Selection of the most suitable form of multimetric index

2. Generation of a multimetric index

3. Setting class boundaries

4. Interpretation of result

This procedure should be particularly useful for identifying clear gaps and issues in the building of this approach for the pelagic habitat compartment. This methodological framework is discussed in the general conclusions of the present deliverable.

In order to define a suitable form of multimetric index, a bibliographic revview should always be thoroughly performed, as done individually for the development of the PH indicators, to identify recently developed multimetric indices that might also combine the different levels of organization of biological communities. As noted in the previous section, current examples for phytoplankton communities notably include Facca et al. 2014, Lugoli et al. 2012 or Laplace-Treyture & Feret 2016. However, these do not fit the aims of the PH development and integration that consider the structure of both zooplankton and phytoplankton communities for three different levels of organisation. From the literature review, if such index exists, it should be considered for plankton communities. Otherwise, a new form of multimetric index should be developed.

Between the first and second steps, the authors also suggest conducting metric selection by notably excluding irrelevant or numerically unsuitable metrics, which was already considered during the development of the PH indicators. The same authors also identified metric types relevant to the development of such a multimeric index. These correspond to (a) Abundance/composition metrics (e.g. Padisák et al. 2006, Ptacnik et al. 2009, Carmendia et al. 2010), (b) richness / diversity metrics (e.g. Sherrard et al. 2006, Weckström et al. 2007, Tsirtsis and Spatharis 2009), (c) sensitivity / tolerance metrics (e.g.Lugoli et al. 2012), and (d) functional metrics (e.g. Weckström et al. 2007, Henriksen et al. 2011). Although metrics on sensitivity/tolerance are not available for pelagic habitats (as mentioned in the previous section, current scientific knowledge does not allow disentangling anthropogenic pressures for pelagic habitats), the proposed OSPAR indicator metrics are in adequacy with these relevant metric types, making their integration quite promising already.

The association of these metrics in combining different aspects of the organisational levels of plankton communities can be challenging as it corresponds to the association of different data units. This is notably the case of the PH indicators as these have been developed individually so far, by applying specific statistical tools (state-space approach for PH1, time-series anomalies calculation for PH2) which do not consider directly the inter-comparison of the PH indicators. In this situation, multivariate statistical techniques can be used to tease out the mechanisms that shape communities. These methods also allow analysing in an efficient way the data and providing visualizations of potential interactions among variables, which can be approached at different levels of organization. Multivariate statistics have successfully been applied to study the relationships between multiple biological variables of interest with environmental variables or anthropogenic pressures (e.g. Facca et al. 2014). Different methods can be used, depending on the characteristics of the metrics/data (e.g. continuous or discrete values). Table 1 (from Kleyer et al. 2012) details how to select an appropriate multivariate analysis depending on the considered questions and types of data.

After selecting relevant metrics for each indicator, PH1, PH2 and PH3, the generation of a multimetric index requires consideration of the different metrics at once. This point is one of the main challenges of the procedure. A simple procedure is to combine information from the three indicators simultaneously to relate environmental changes or pressures to the changes in the plankton community highlighted by the indicators.

Setting threshold values for the resulting multimetric index is necessary to assess if GES is reached or not. In the development of OSPAR indicators, threshold values are defined by identifying a reference period and by the use of statistical tool to set limits based on the data of the reference period. This task is challenging for pelagic habitats as the drivers/pressures shaping the plankton communities are poorly understood. So far, reference conditions have not been clearly set up for the PH indicators. PH1 considers a reference period (called “analysis period” later on in the document to avoid confusion) for computing the Plankton Index (PI) but this methodological step does not address GES assessment. The analysis for generating PH indicators is currently conducted on complete time-series. Further work is required for the definition of reference periods for the PH indicators. This issue is also raised in the deliverable report WP1 1.1. This gap needs to be addressed through scientific discussion within the pelagic communities and in the knowledge of environmental and pressures data. These limits will be different according to the area of consideration. Experts from each area need to be consulted for the definition of reference periods.

Ecological questions	Unit of analysis	Dependent variable (yr)	Phylogeny	Criteria			Methods
				Within species trait variability	Species frequency v. occurrence	Single v. multiple traits	
1- How do average trait expressions of communities respond to environmental gradients?							
1a How do average trait expressions of communities change along environmental gradients?	Average species or individuals	Community trait composition	Not relevant	Preferable	Both possible	Both possible	CWM-RDA, RLQ, double CCA
1b Do average trait expressions of *a priori* groups of species (e.g. dominants, invasives) respond differently than the rest of the community?	Average species or individuals	Community trait composition	Not relevant	Possible	Abundance needed for dominance	Both possible	CWM-RDA, RLQ, double CCA
1c To what extent is community functional response driven by species replacement or phenotypic variation?	Average species or individuals	Community trait composition	Not relevant	Inter-treatment variability	Both possible	Both possible	
1d How do patterns of trait correlation at the community levels change with environmental conditions?	Average species or individuals	Community trait composition	Not relevant	Preferable	Both possible	Pairs of traits	Violle et al. (2007)
2- How do trait expressios of species respond to environmental gradients?							
2a. Which traits predict species response to environmental gradients?	Species	Species position	Possible	Can be used as predictor	Both possible	Both possible	Cluster regression, RDA-sRegTree, RDA-mRegTree, OMI-GAM, RLQ, double CCA
2b. Which traits predict species niche breadth?	Species	Species range	Possible	Average trait values per species	Both possible	Both possible	OMI-GAM, RLQ, double CCQ
2c. How do groups of species with similar trait expressions respond to environmental gradients?	Species		Possible	Possible (ecotypes)	Both possible	Both possible	Cluster regression, RDA-sRegTree, RDA-mRegTree, OMI-GAM, RLQ, double CCA
2d. Is the response to the environment of *a priori* groups of species (e.g. dominant v. non-dominant or invasive v. non-invasive) related to the same traits?	Species	Species properties (invasiveness, dominance)	Not applicable	Possible	Both possible	Both possible	Cluster regression, RDA-sRegTree, RDA-mRegTree, OMI-GAM, RLQ, double CCA

CCA: canonical correspondence analysis, RDA: redundancy analysis, RLQ: a double interia analysis of two arrays (R and Q) with a link expressed by a contingency table (L)

Table 1: Selection of an appropriate multivariate analysis based on question and type of data (from Kleyer et al. 2012)

When the reference period is defined, qualitative categories can be built. Categories are necessary in the multi-metric approach described by Hering et al. (2006) which are made in relation to boundaries (and are really useful for helping management decisions). Categories can be assessed theoretically, as done for the PH2 state indicators (i.e. based on the statistical distribution of the index), or with literature or expert-based information, as presented by the example of the phytoplankton production indicator (Figure 1).

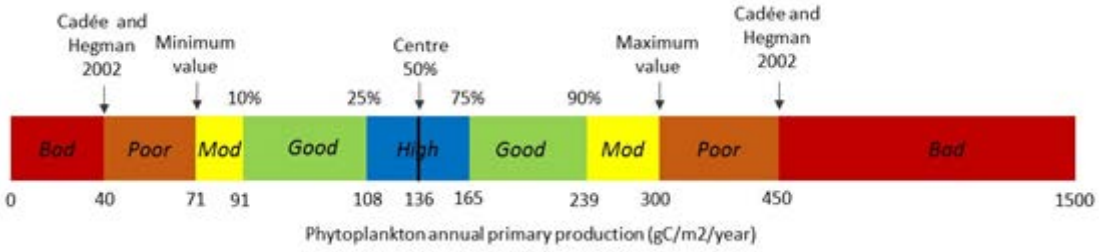

Figure 1: Boundaries and categories used for the FW2 indicator (primary production) based on literature and theoretically boundaries

The definition of reference periods and of relevant categories should be addressed in future developments of the PH multimetric index

Finally, the interpretation of the results should be done in the knowledge of the local/specific area plankton dynamics but also taking into account the physico-chemical characteristics of the zone of interest for which the multimetric index has been calculated. Interpretation of the results should be done for each area with the participation of local experts.

Summary of the Pelagic Multimetric index building approach

The different steps of the Pelagic Multimetric index building-approach are summarised by Figure 2. This diagram should be used to identify the clear gaps and issues (see Section 6) related to the building of this multimetric index in the future.

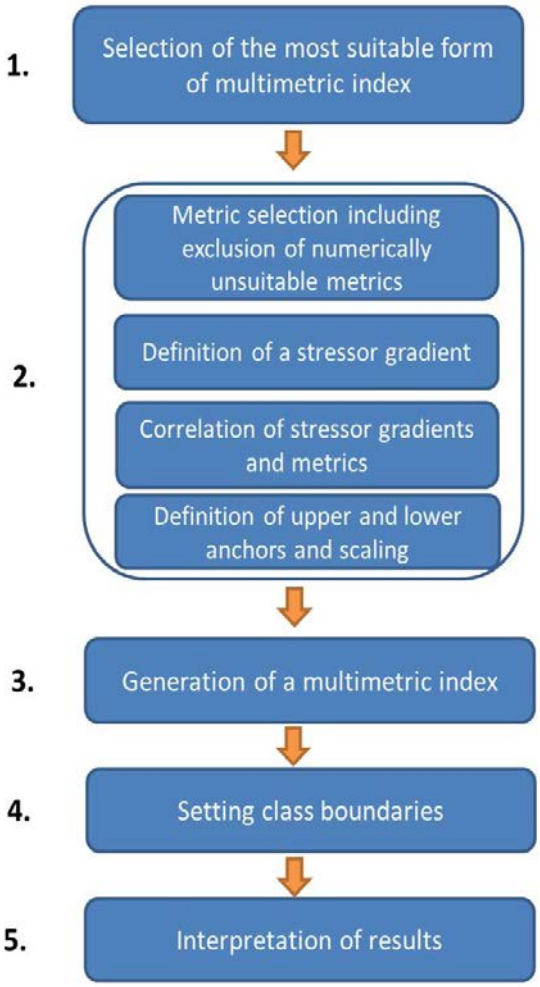

Figure 2: Diagram showing the procedure steps for the building of the Pelagic Multimetric Index approach

3 Methodology

Two of the Indicators, PH1 and PH2, have already been calculated for the L4 station (OSPAR 2017 assessment) but PH3 had not been assessed yet. Some of these results are available through the OSPAR 2017 assessment. Since some refinements have been made due to updates on the provided L4 data and on the selected time-period, we provide methodology details for the calculation of the PH1 and PH2 indicators, as well as the updated results. For the diversity indices, PH3, index selection had to be conducted for the zooplankton compartment but was already performed for phytoplankton as part of the OSPAR Intermediate Assessment 2017 (OSPAR, in preparation). Environmental data are necessary for the interpretation of the three indicators. The data provided for L4 are presented with general information on the study area.

3.1. Study area: the L4 station

The Western English Channel is an area for which long time-series, suitable for the testing of the indicators, exist. The L4 station is a long-term monitoring station located in the English Western Channel, located approximately 16 km south-west of Plymouth, UK in a water depth of approximately 54m (Figure 1). With weekly sampling augmented with a data buoy, L4 is the most intensively sampled station in a cluster of sites known as the Western Channel Observatory (WCO) (www.westernchannelobservatory.org.uk). The long term history of these sites is described in

Southward et al. (2005) and Smyth et al. (2015), while a modern updates of both the sampling methods and the plankton variability is summarized in Widdicombe et al. (2010) and Atkinson et al. (2015).

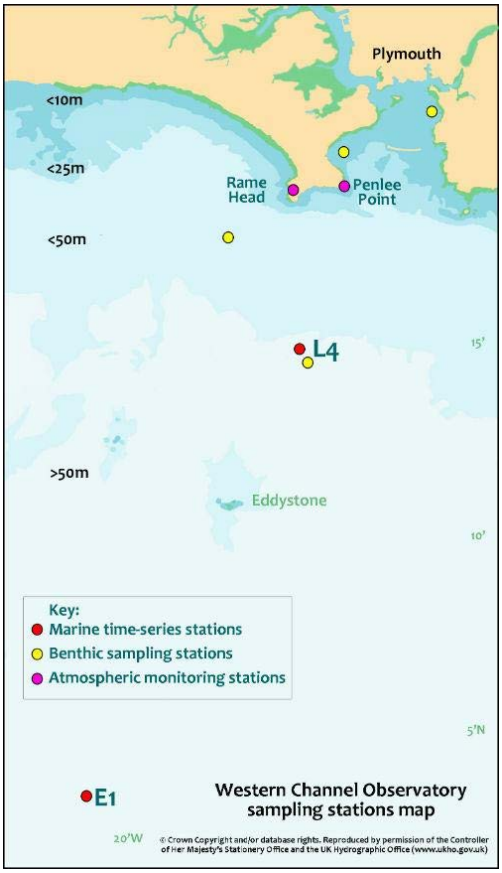

Figure 3: Localisation of L4 station in the Western English Channel: red dots signify pelagic time series and the data buoys

This station has benefited from long-term scientific monitoring for a wide range of abiotic and biotic metrics although in a heterogeneous way (different sampling periods, different institutes realizing monitoring, etc...). In the frame of the collaboration with the Plymouth Marine Laboratory (PML), the environmental data provided are summarised in Table 2.

Data	Metric	Detail	Sampling device	Analysis	Time series
Biological data	Phytoplankton	Taxonomic counts			Oct. 2012 - Dec. 2014
		Total Chl *a*	Niksin bottle	HPLC	March 1999 - Dec. 2014
	Zooplankton	Taxonomic counts	WP2 net 200µm		March 1988 - Dec. 2015
Environmental data	Sea surface temperature				March 1988 - Dec. 2012
	Salinity				April 1996 - Dec. 2014
	Nutrients	Nitrite			Jan. 2000 - Dec. 2015
		Nitrate			
		Ammonia			
		Silicate			
		Phosphate			

Table 2: Biological and environmental data for the L4 station provided in the frame of the EcApRHA project

In order to investigate the relationships between the indicators and the environmental variables, all variables have to be generally considered for a common time period. As shown in this table, the environmental data are heterogeneous and were not available for a same time period. In order to make the most efficient use of these data, i.e. to use as many environmental variables as possible, data have to be considered only from January 2000 since this is the time when most of the provided data are available. Values for salinity were not available for the years 2014 and 2015. In our analysis, instead of using salinity measures taken at 10m deep for these years, we use averages of salinity at 0-50m depth, which were provided.

3.2. PH1 indicator analysis for L4

PH1 "Changes in plankton communities" features a "Plankton Index" of lifeform pairs which has been developed to track changes in the state of the plankton in marine waters over time. The main features of the method are: (i) the grouping of planktonic species into functional types or lifeforms; (ii) the display of changes in the abundance of each of these lifeforms using a state-space approach; (iii) calculating a Plankton Index (PI) to quantify possible changes in the state of the plankton relative to baseline or starting conditions; and (iv) relating trends in the PI to trends in human pressures and climate change indices. When examined in ecologically-relevant pairs (Table 2), lifeforms can provide an indication of changes in: the transfer of energy from primary to secondary producers (changes in phytoplankton and zooplankton); the pathway of energy flow and top predators (changes in gelatinous zooplankton and fish larvae); benthic/pelagic coupling (changes in holoplankton (fully planktonic) and meroplankton (only part of the life-cycle is planktonic, the remainder is benthic) (Table 3; see Gowen et al., 2011). Monthly lifeforms were calculated for L4 and annual PI values were calculated from 2000 to 2014. Departing from the OSPAR assessment process, which only used a subset of these as a reference period, years from 2000 until 2014 were used as reference period to provide a meaningful comparison with the annual anomalies calculated in PH2. Detailed methodologies for this indicator, and the datasets used, are provided in the PH1/FW5 OSPAR assessment sheet (OSPAR, in preparation).

Lifeforms	**Additional criteria**	**Confidence**	**Explanation**
Diatoms v. dinoflagellates		High	Dominance by dinoflagellates may be an indicator of eutrophication or of change in water column stability and may result in less desirable food webs
Gelatinous zooplankton v. fish Larvae/eggs	Ctenophores and cnidaria	High	Indicator of energy flow and possible trophic pathways
Small copepods v. large copepods	Adults <1.9mm (not nauplii or eggs)	High	Size based indicator of food web structure and energy flows
	Adults >2mm		

Carnivorous zooplankton v. non-carnivorous zooplankton		Low	Indicator of energy flow and balance between primary consumers and secondary consumers
Crustaceans v. gelatinous zooplankton		High	Indicator of energy flow and possible trophic pathways
Large microphytoplankton v. small microphytoplankton	>20 m cells, not colonies.	High	Size-based indicator of the efficiency of energy flow to higher trophic levels
	<20 m cells, not colonies.		
Microphytoplankton v. non-carnivorous zooplankton	Biomass (example Chl, PCI)	High	Indicator of energy flow and balance between primary producers and primary consumers
	Abundance		
Diatoms v. autotrophic and mixotrophic dinoflagellates		Low	Shift in primary producers may indicate eutrophication
Pelagic diatoms v. tychopelagic diatoms		High	Indicator of benthic disturbance and frequency of resuspension events
Nuisance and/or toxin-producing diatoms v. diatoms Or Nuisance and/or toxin-producing dinos v. dinos		Low	Shift in algal community towards nuisance and/or toxic species which have the potential to impact other higher trophic level indicators
Holoplankton v. meroplankton		High	Indicator of strength of benthic-pelagic coupling and reproductive output of benthic versus pelagic faunas
Ciliates v. microflagellates	Including tintinnids	Low	Shift from primarily autotrophic to a more heterotrophic system
	All species < 20 µm		

Table 3: Lifeform pairs consist of two ecologically-relevant lifeforms. The 'Additional criteria' column contains supplementary information regarding particular lifeforms

3.3. PH2 indicator analysis for L4

PH2 “Changes in plankton biomass and abundance” is based on chlorophyll a (Chl a) or PCI index (CPR) as a proxy and on abundances of zooplankton (using copepods) to represent the balance between production/import and mortality/export of phytoplankton and zooplankton. The methodology involves time-series analysis for identifying significant changes in the data set of interest. More specifically, anomalies in plankton biomass and abundance are detected and are then ranked according to their distribution to establish a level of change (small, important or extreme changes). The details for the analysis of this indicator are provided in the assessment of PH2 as a contribution to the OSPAR Intermediate Assessment 2017 and CEMAP guidelines (OSPAR, in preparation). Data for total phytoplankton biomass and total copepod abundance are used as monthly means.

3.4. PH3 indicator

3.4.1. Aims and methodological concept

PH3 ”Changes in plankton diversity” corresponds to a multimetric index estimating the biological and ecological quality of a pelagic ecosystem by considering the structure of the plankton community. For zooplankton and phytoplankton, biodiversity indices are combined to focus at three complementary aspects of plankton community structure, namely heterogeneity, diversity, and contributions of each taxa to community diversity. Local contributions to beta diversity (LCBD, Legendre & De Caceres 2013) use variance in taxa distribution among sampling units to inform about the heterogeneity in the plankton community. More practically, this metric enables the identification of atypical community structures which can be considered for index calibration in future assessments, or, instead, correspond to degraded areas in need of restoration. Knowledge on the diversity and community composition of these areas provides a complementary picture of the community composition to assess if the community is degraded or not. Because phytoplankton and zooplankton respond to different environmental drivers (e.g. Chl a being a measure of phytoplankton biomass might not be so relevant in the analysis for phytoplankton as an environmental parameter but can drive population dynamics in zooplankton), index selection should be conducted for both groups of organisms. Dominance and diversity indices (Menhinick index and Hulburt index) have already been selected for phytoplankton in the assessment of PH3 as a contribution to the OSPAR Intermediate Assessment 2017 (OSPAR, in preparation). Yet, these indices had to be identified for zooplankton. A suitable selection is described in the following section.

3.4.2. PH3 analysis for zooplankton at L4

The present section of the methodological approach for the PH3 indicator is given for zooplankton, but is similar to the work carried out for phytoplankton, and aims to provide a methodological framework, based on the L4 station example, for how to select diversity and dominance/evenness indices.

3.4.2.1 The zooplankton data

The zooplankton community at L4 has been sampled since March 1988 using two replicate hauls of a WP2 net of 200 µm mesh size, towed vertically from bottom to surface. The taxonomic resolution has not been performed to the species level for all taxa, with many taxa determined at the order level (i.e. total Siphonophore), suborder level (i.e. Tintinnida) or genus level (i.e. Clione spp. or Appendicularia spp.) and has not been focused on the determination of rare taxa. Diversity indices or generic indices

require the consideration of similar taxonomic unit within the data set. As such, it was not possible to use all the zooplankton taxa at L4 for the PH3 assessment. However, copepods are often the focus of zooplankton monitoring and are usually much better identified than any other taxonomic group. For the L4 data, taxa in the subclass of Copepoda are all determined at least to the species or genus level. We make the assumption that the taxa determined at the genus level mostly correspond to one main species. For instance, Oithona spp. is mostly represented by Oithona similis in this area. Accordingly, we considered these genera as species in our analysis but we acknowledge that this assumption could lead to a bias in our interpretation of the results. The considered copepod taxa are presented in the Annexes (A1). No zooplankton data were available for August 2000 due to unavailability of a ship for sampling. As such, this month was not considered in the analysis. In order to use the data acquired with a homogenous frequency, and in link with the indicator work at the OSPAR level, monthly means of taxa abundances were used for the analysis of the different indices.

3.4.2.2 Statistical analysis

The computing of LCBD values follows Legendre & De Caceres (2013) on a Hellinger-transformed abundance matrix. Permutation tests allowed the identification of significant LCBDs (significance level alpha=0.05).

The most common indices of diversity are presented in the Annexes (A2). From their formula, it is clear that mathematical convergences might occur among them. Bandeira et al. (2013) actually investigated the mathematical convergences among the most common diversity indices that we presented in this table (Simpson, Gleason-Margalef, Menhinick, Brillouin, Shannon, Patten, Piélou and Hurlbert). A first classification of these indices can be realized based solely on a mathematical demonstration. Bandeira et al. (2013) made three groups of indices, reducing an initial number of 8 indices to 3.

Group 1= { Brillouin, Shannon, Simpson's reciprocal (Gini), Hurlbert},

Group 2= { Piélou, Patten},

Group 3= { Gleason-Margalef's, Menhinick's}

The mathematical convergences within the indices of group 1, and among the ones of group 2, have been clearly established. Gleason-Margalef's and Menhinick's indices have been grouped together but have to be considered independently and provide complementary information. Bandeira et al. (2013) have validated the demonstration of these convergences, and thus classification, with plankton data. They use data of phytoplankton and zooplankton communities from two neighbouring bays in the north-west Mediterranean, which are differently affected by anthropogenic pressures. The convergence of the indices was shown through strong statistical correlation within the two first groups and has been validated through plankton data over three consecutive years. We used this classification, validated on phytoplankton data, as a basis for the first selection of the indices which will be used for the PH3 assessment for zooplankton.

No simple specific richness index was used because these indices are highly correlated to sampling effort and to the level of taxonomic expertise, which has the potential to heavily bias the results. Within the Group 1 of Bandeira et al. (2013), the Brillouin index was chosen based on the information provided in Annexe A2, notably since it is less sensitive to sampling effort than the Shannon index. Within Group 2, the Patten index was preferred since the Pielou index is more sensitive to the taxonomical level of determination and requires that the whole community is as much as possible known (which is not the case for L4). Both the Menhinick and Gleason-Margalef's are considered. The data were normalized with the log(x+1) transformation for indices not considering normalization in their formulas.

A principal component analysis (PCA) was conducted on the provided environmental factors (centred and reduced) and was followed by correlations between each principal component and the pre-selected indices to identify those most sensitive to environmental variation to study in relation to the zooplankton community at L4. Following this, Pearson correlations among indices were performed to find the less redundant and, hence, the most complementary dominance and richness indices. For significant LCBD values, we report the corresponding dates and richness and dominance index values.

This work was completed by the computing of an Importance Value Index (IVI) in case of high dominance. For more clarity in the result section, we only show figures for the three dates characterized by the highest LCBDs (LCBD values for these dates were also found significant from the permutation test). IVIs allowed us to further investigate the presence of potentially undesirable species in the community, which could stress the need for restoration. IVIs provide information on species contribution to community structure and are calculated as follow:

IVI = relative density + relative frequency

…where relative density corresponds to the ratio of the number of individuals of the considered species over the total abundance and the relative frequency is the number of occurrence of the considered species over the number of species in the sample (Curtis and McIntosh 1950, Mukherjee et al. 2010).

As a result, the use of LCBDs, selected diversity indices and IVIs altogether provides a global picture of the whole community by synthesizing information at the community heterogeneity at the regional level (here, time series level, LCBD), community composition level (diversity indices) and species level (IVI). This approach thus provides the ground for defining reference conditions and degraded communities.

The procedure used for computing PH3 (including the selection of composition indices) is summarized in Figure 4.

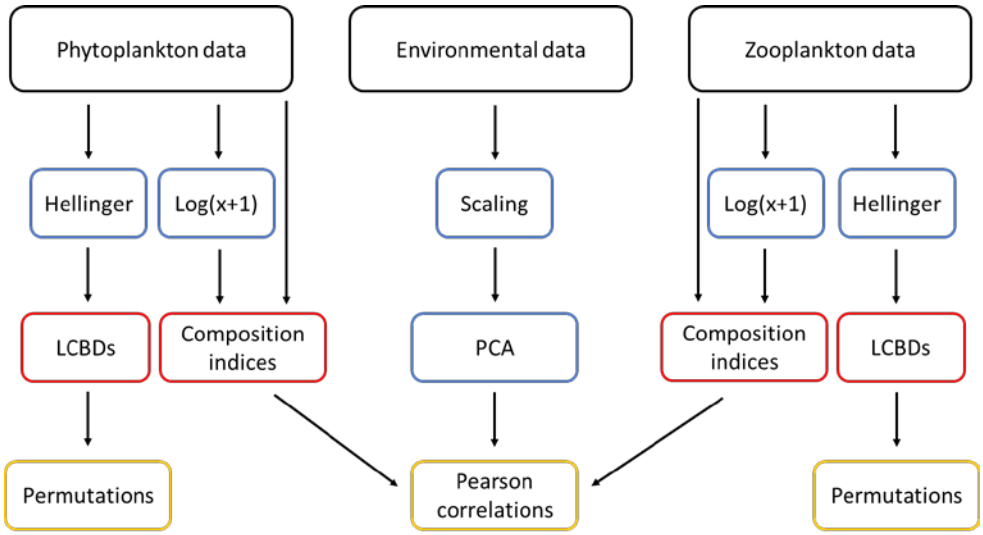

Figure 4: Summary of the procedure for computing PH3 (including composition index selection). Black boxes indicate data types, blue boxes indicate data transformation, yellow boxes indicate statistical tests and red boxes indicate PH3 indices.

3.4.3. PH3 analysis for phytoplankton at L4

For the phytoplankton component of PH3, case-studies have been previously performed on long time-series of phytoplankton community data (genus level) from data sampled at five French stations. The multivariate analyses performed on these data sets, which are essentially comparable to the L4 data, constitute the background for the selection of the indices used to analyse the phytoplankton community at the L4 station. Other criteria, including mathematical properties or ease of interpretation, were also considered in this process. As such, species richness of phytoplankton was not retained because it was shown to be highly correlated to the sampling effort and to the level of taxonomic expertise, and can easily be biased. Menhinick's richness index (D) was found to be the most sensitive index to changes in environmental conditions. Hulburt's dominance index (delta) was also selected. Furthermore, as stressed by Facca et al. (2014), this index is expressed as a percentage and can easily be interpreted. Therefore, we decided to use these two indices for the PH3 analysis of the phytoplankton community at L4.

In order to use data acquired at a homogenous frequency, and in link with the indicator work at the OSPAR level, monthly means of taxa abundances from microscopic counts were used for the analysis of the indices. To calculate the indices, abundances at the genus level were considered. As for zooplankton data, a PCA followed by a correlation between the principal components and the indices were conducted to check that the pre-selected indices were also adequate for representing variations of the phytoplankton community at L4 as a response to environmental conditions (ammonia, nitrate and nitrite values were summed to obtain dissolved inorganic nitrogen concentrations and Chl a was not considered). For these analyses, environmental data were normalized with log-transformation. LCBDs were also computed for phytoplankton data and used together with Hulburt and Menhinick indices to characterize phytoplankton communities at atypical dates. As for zooplankton, these results were completed with information provided by IVIs for result interpretation.

No phytoplankton data were available from L4 for December 2011. As such, this month was not considered in the analysis. Species abundances (number of cells per ml, see Widdicombe et al. 2010 for details on data collection) were normalized (log(x+1) transformation) to reduce heterogeneity in the data set before computing the Hulburt index. For the same reason, LCBD computing required a Hellinger transformation (i.e. each value in a data matrix is divided by the square root of its marginal sum of squares) of the abundance matrix. The analysis was repeated with and without potentially heterotrophic genera (which potentially do not belong to phytoplankton per se, see Annexe A3), but similar results were found. We only present here the results for the analysis that excluded potentially heterotrophic genera. As for zooplankton, we performed permutation tests to assess the significance of the computed LCBDs (significance level: alpha=0.05) and completed the results with IVIs (for clarity IVIs are shown only for the three dates characterized by the highest LCBDs; the corresponding LCBDs were also found significant from the permutations).

4 Results

4.1. PH1 for L4

In order to make a meaningful comparison with PH2, the whole analysis period used for PH1 corresponds to the whole time-series analyzed in this report, i.e. 2000 to 2014; in other words, 2000-2014 acts as the period of analysis (referred to as "reference period" for ease, even though it is not a

traditional reference period). This is because PH2 determines anomalies based on the whole time-series, and does not use a reference condition/period. The plankton index (PI) was calculated for all of the lifeforms shown in Table 3 at L4 using an analysis period of 2000 to 2014; below three examples are given for comparison. Figure 5 shows the large phytoplankton and small phytoplankton annual PI values, because the analysis period is so large there are no statistically significant years of change. The strongest change occurred in 2004, 2006, 2007, and 2011 (lowest PI values), these can be explained by lower abundance of small phytoplankton relative to large phytoplankton during 2004, 2006 and 2007. 2011 is however an anomalous year due to the similar timings in peak abundance and larger abundance of small phytoplankton.

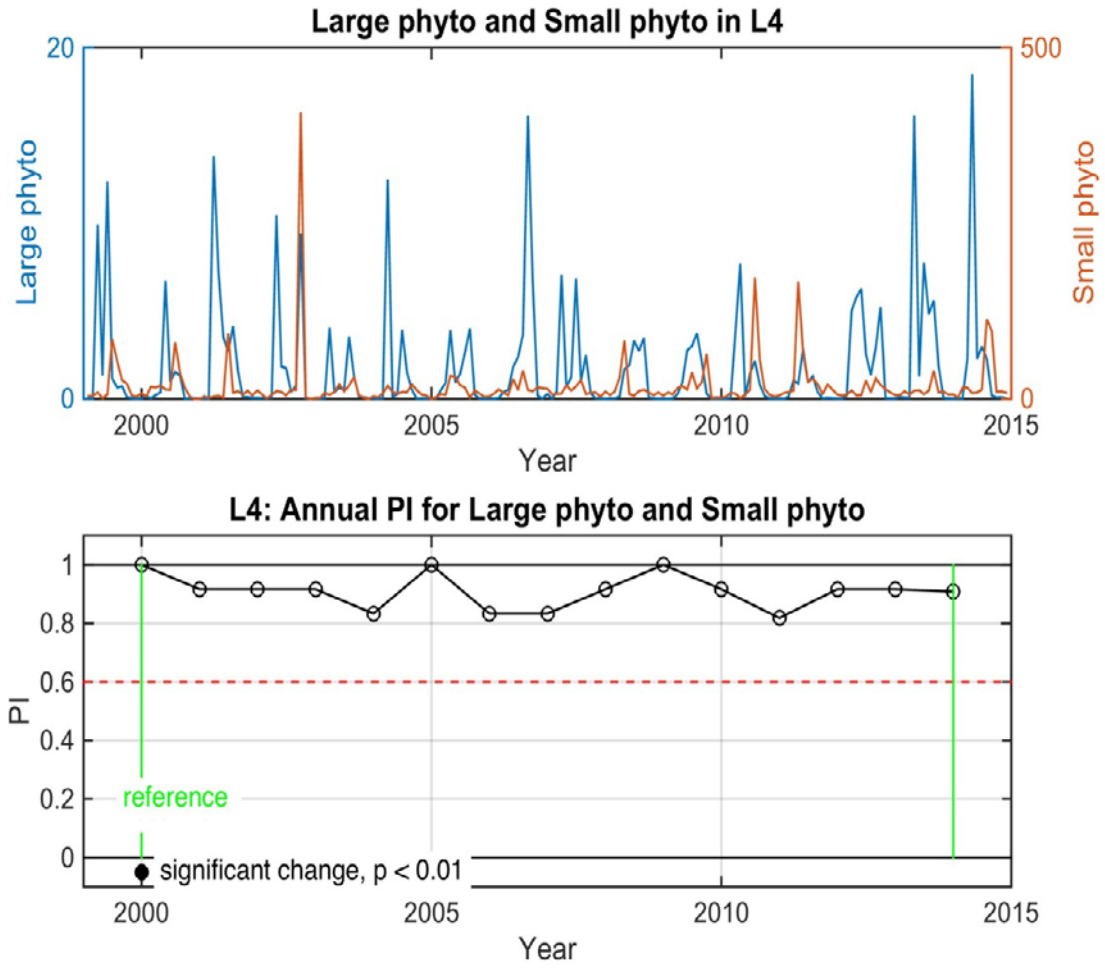

Figure 5: Annual PI time-series plotted with the monthly plankton data at L4 for the lifeform pair; large phytoplankton against small phytoplankton. Top subplot = Monthly time-series of large phytoplankton (blue) and small phytoplankton (orange) abundances at L4. Bottom subplot = Annual time-series of PI values that are calculated by comparison to the analysis envelope for the period 2000 to 2014. Annual PI not significantly different from the starting condition ($p>0.01$) = open circle. Annual PI significantly different from the starting condition ($p<0.01$) = closed circle (black).

Figure 6 is an example of a zooplankton lifeform pair; small copepods and large copepods. The lowest PI value occurs in 2002, where there are relatively higher abundances of large zooplankton compared to the whole time-series. Again, there are no significant changes in the annual PI due to the analysis period encompassing the whole time-series period.

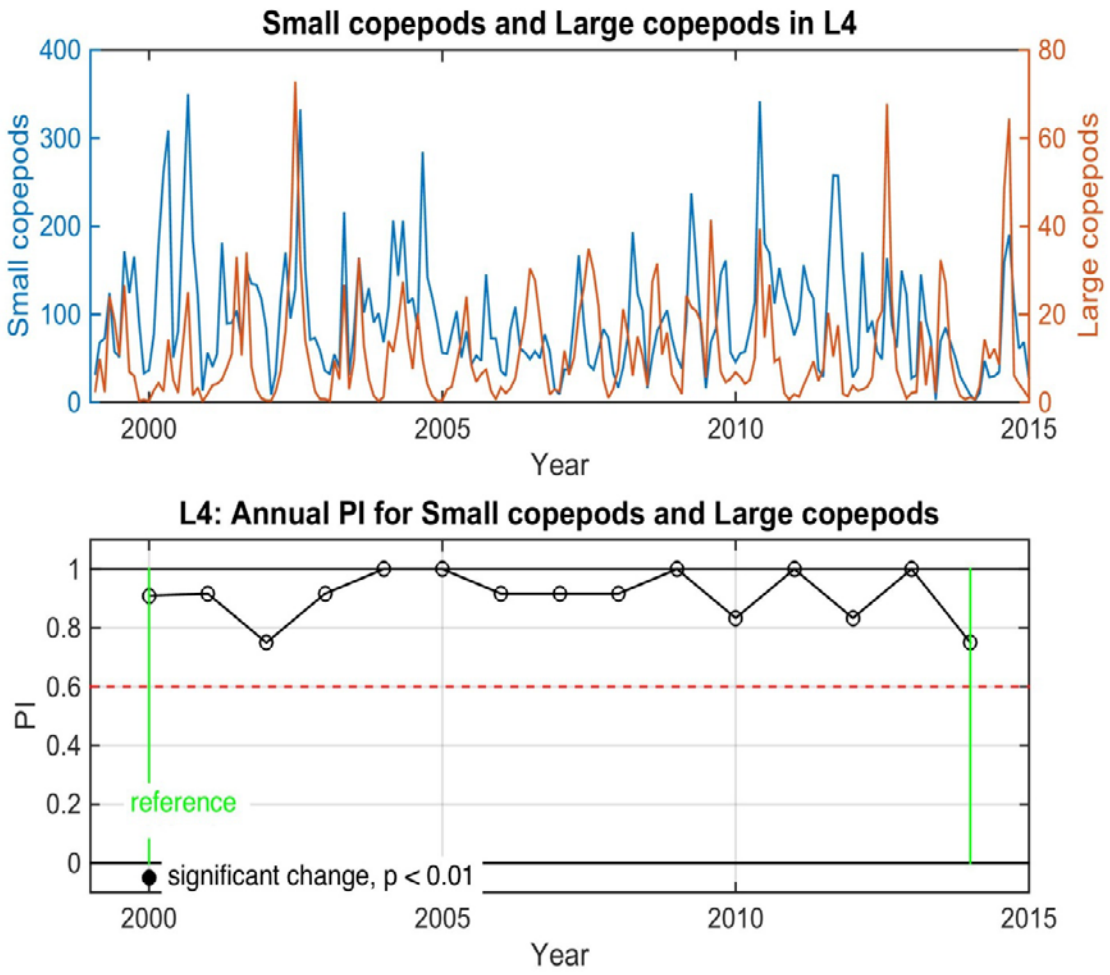

Figure 6: Annual PI time-series plotted with the monthly plankton data at L4 for the lifeform pair; small copepods against large copepods. Top subplot = Monthly time-series of small copepods (blue) and large copepods (orange) abundances at L4. Bottom subplot = Annual time-series of PI values that are calculated by comparison to the analysis envelope for the period 2000 to 2014. Annual PI not significantly different from the starting condition ($p>0.01$) = open circle. Annual PI significantly different from the starting condition ($p<0.01$) = closed circle (black).

Figure 7 shows the annual plankton index for the lifeform pair holoplankton and meroplankton, an additional significance band has been added for ($p<0.05$) to aid comparisons with PH2. The lowest annual PI value occurs in 2004, and gives a significant result where $p<0.05$; this was due to an anomalous low abundance of meroplankton relative to holoplankton.

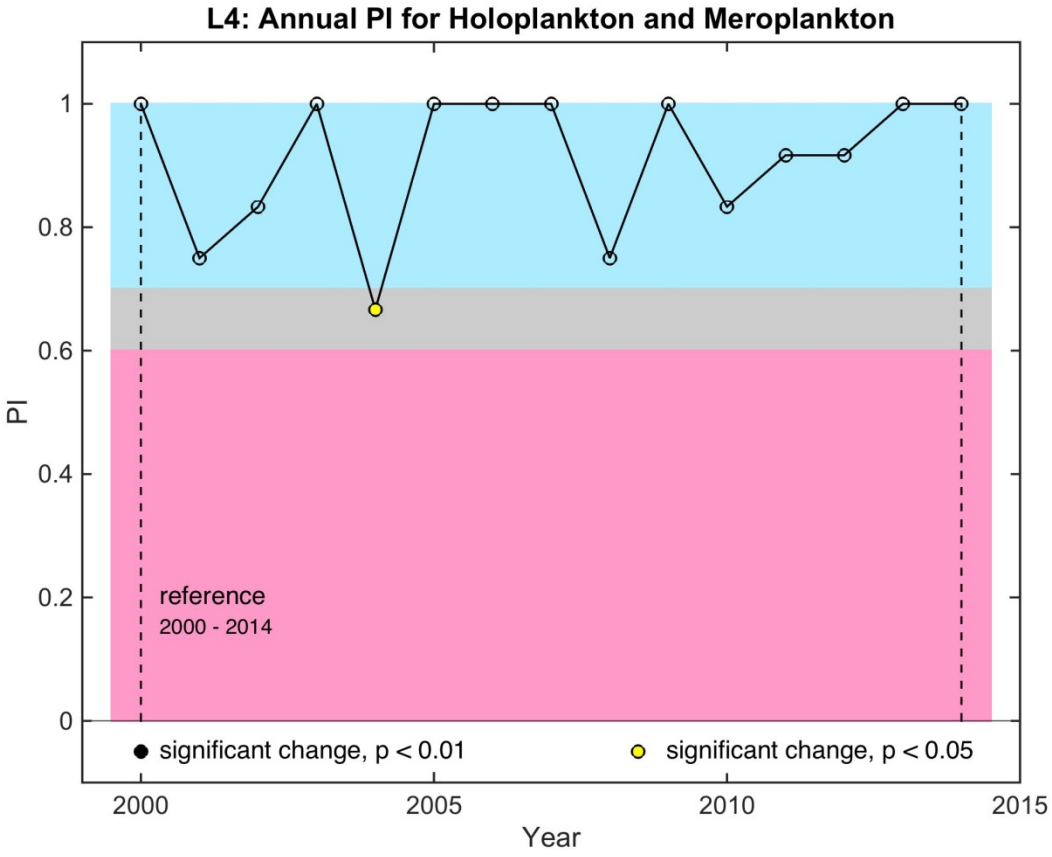

Figure 7: Change in the annual Plankton Index for the lifeform pair Holoplankton and Meroplankton recorded at station L4 from 2000 to 2014, using the analysis conditions 2000 to 2014. Light blue area is the threshold for no significant change from the starting conditions, grey is a significant change for $p < 0.05$, and pink is a significant change for $p < 0.01$. Changes in the annual Plankton Index do not necessarily indicate a deterioration of environmental conditions; they do, however, indicate change from the reference conditions (= analysis period).

4.2. PH2 for L4

We calculated anomalies for both phytoplankton (Figure 8) and zooplankton (Figure 9), for the period 2000-2014 at the annual and at the monthly scales. The annual anomalies are averages of the monthly anomalies. It was decided to classify the anomalies based on percentiles, a common statistical method to present results, in order to communicate the results to a broad audience. The 5, 25, 50, 75 and 95 percentiles have been used to categorize the anomalies within a whole time-series (see Figure 8b). Three categories are used:

- “small change” which corresponds to the anomalies within the 25 and 75 percentiles,
- “important change” which corresponds to the anomalies within the 5 and 25 percentiles and within the 75 and 95 percentiles,
- “extreme change” which corresponds to the anomalies within the 0 and 5 percentiles and within the 95 and 100 percentiles.

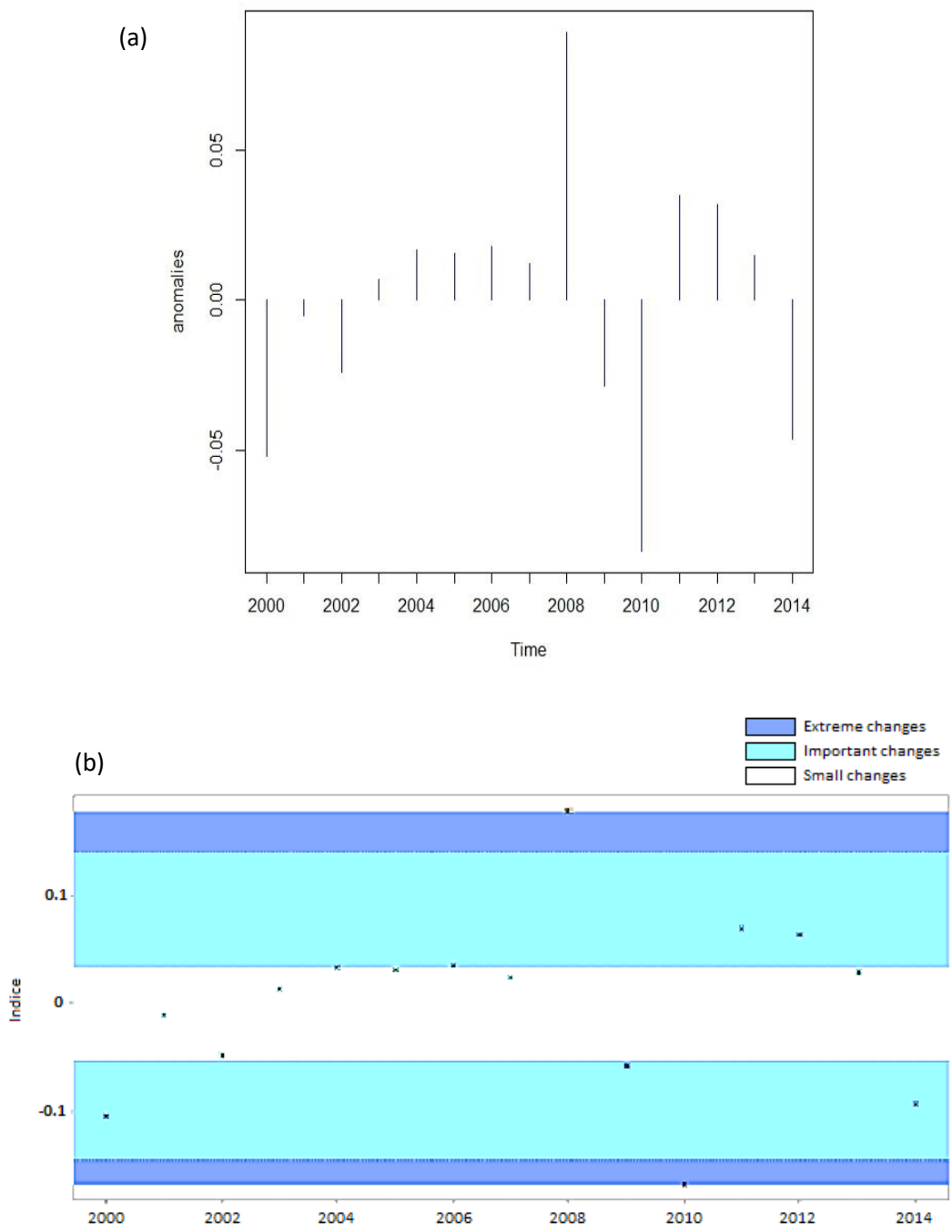

Figure 8: Graph (a) of the annual anomalies for phytoplankton (with total Chl *a* used as proxy of total phytoplankton biomass) for the L4 station for the period 2000-2014. The same anomalies are presented but classified into the 3 categories defined so far for the PH2 indicator (b)

Two years, 2008 and 2010, show strong anomalies in the phytoplankton total biomass, which appear in the extreme change categories. Most of the years present anomalies within the 25 and 75 percentiles, thus categorised as small change. However, these values are mostly at the limit between small change and important changes, particularly between 2004 and 2006.

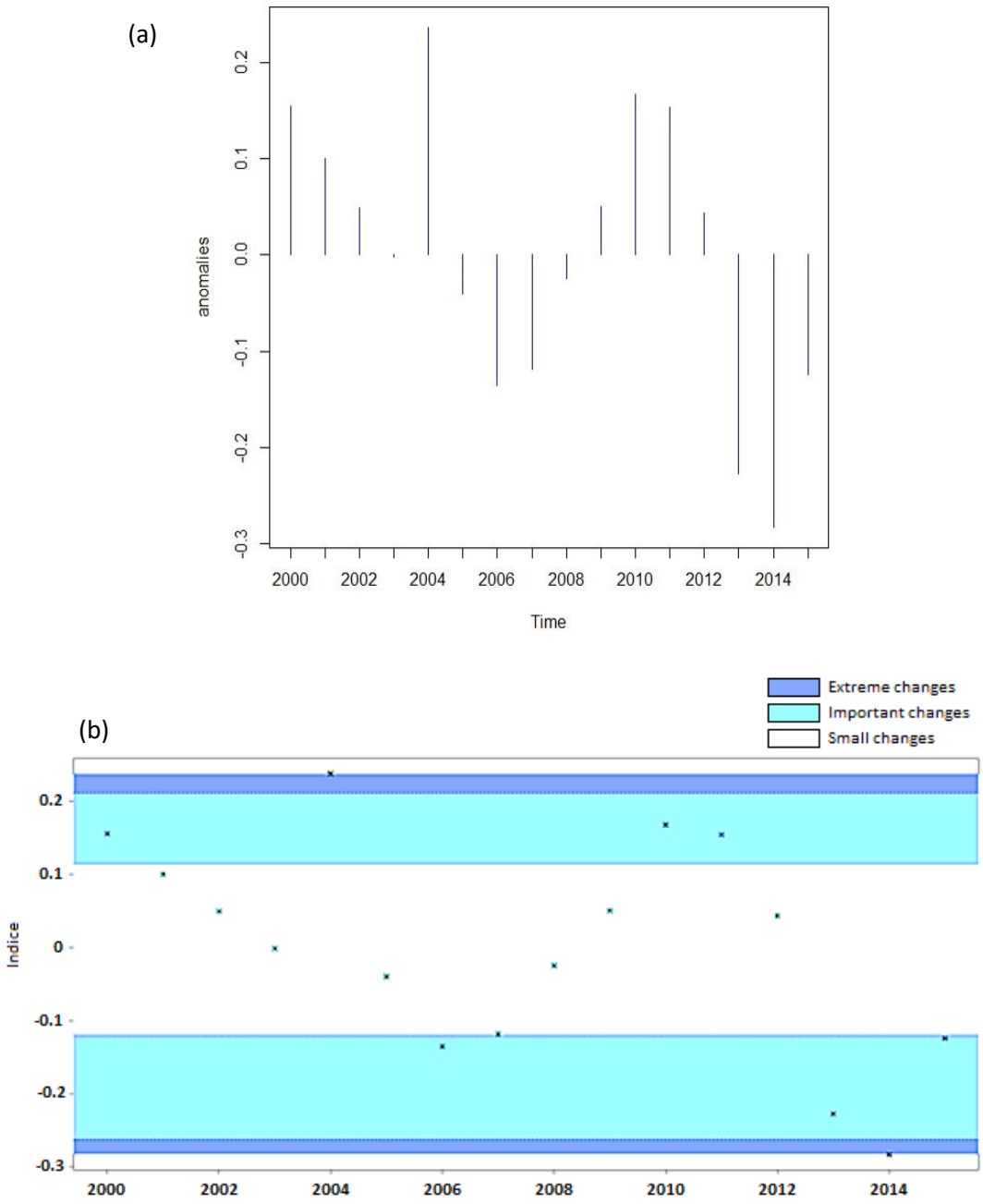

Figure 9: Graph (a) of the annual anomalies for zooplankton (with total copepod abundance used as a proxy of total zooplankton) for the L4 station for the period 2000-2014. The same anomalies are presented but classified into the 3 categories defined so far for the PH2 indicator (b)

The years 2004 and 2014 show particularly strong anomalies in the total copepod abundance, which appear in the extreme change categories. As a comparison, 2004 was also a year for which the PH1 indicator, for the lifeform holoplankton/meroplankton found a significant ($p<0.05$) annual PI value. Although a few years (2000, 2006, 2010, 2011, 2013 and 2015) were classified under the important change category, most of the years present anomalies within the 25 and 75 percentiles (i.e. small change).

A significant monotonic trend was found for the period 2000-2015 (using non-parametric Spearman test, p-value=0.019) at the significance level alpha=0.05.

The monthly anomalies (Figures 10 and 11) can complement our findings as they provide greater detail for each year.

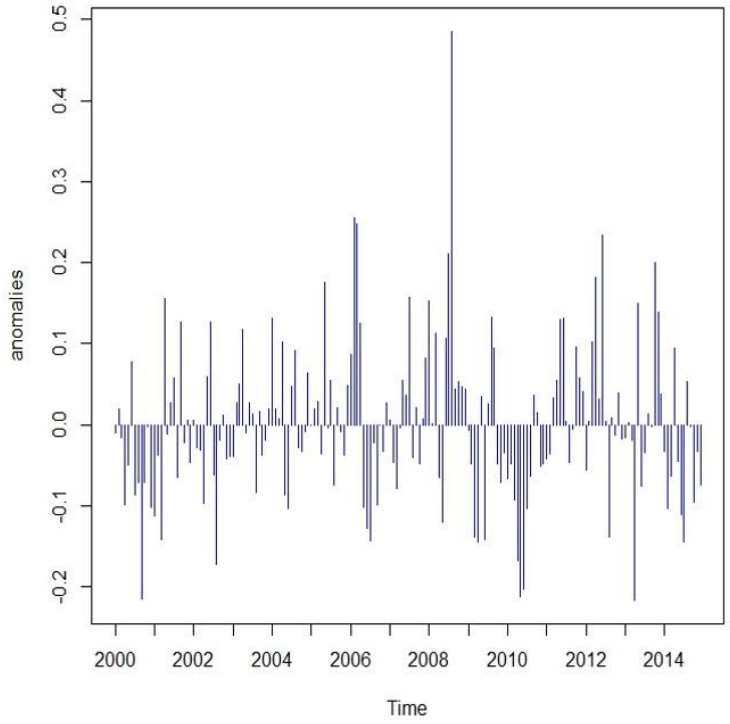

Figure 10: Graph of the monthly anomalies for phytoplankton for the L4 station for the period 2000-2015

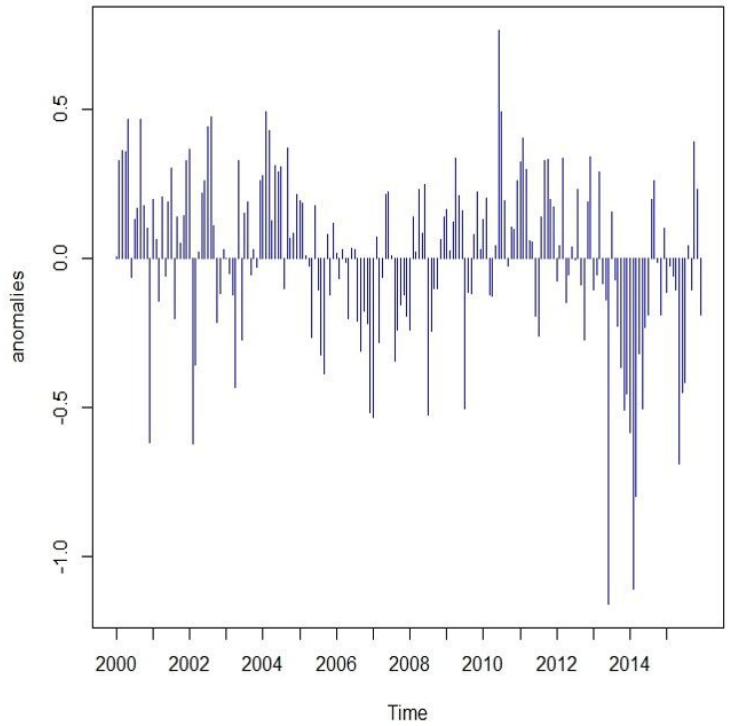

Figure 11: Graph of the monthly anomalies for zooplankton for the L4 station for the period 2000-2015

4.3. PH3 for L4

4.3.1. Indicator results for phytoplankton

Temporal fluctuations of phytoplankton richness (Menhinick index) and dominance (Hulburt index, following log(x+1) transformation) indices are presented in Figure 12. Their sensitivity to environmental conditions is shown in Figure 13.

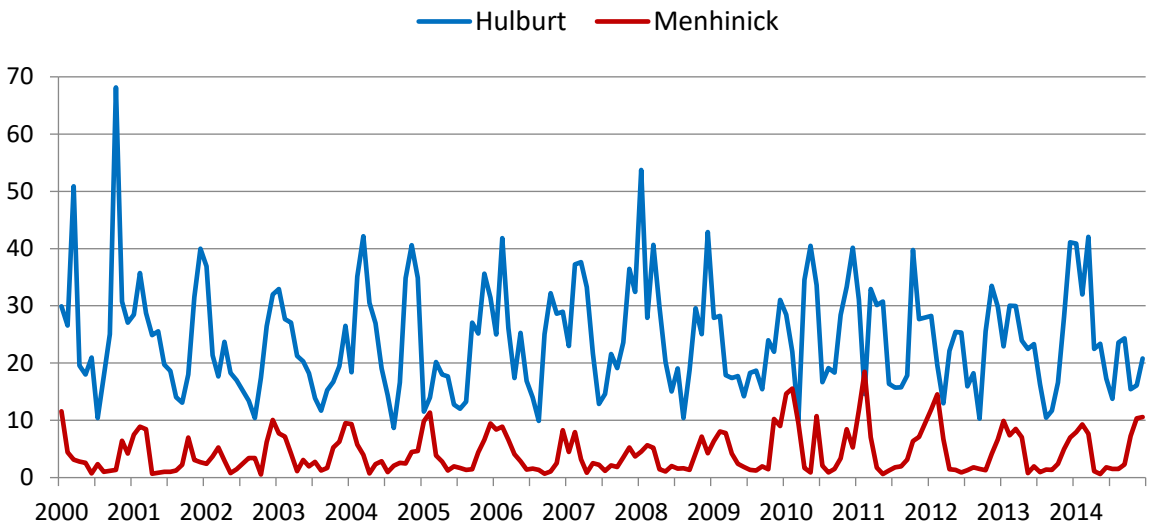

Figure 12: Temporal dynamics of richness (Menhinick index, in red) and dominance (Hulburt) indices for phytoplankton at L4

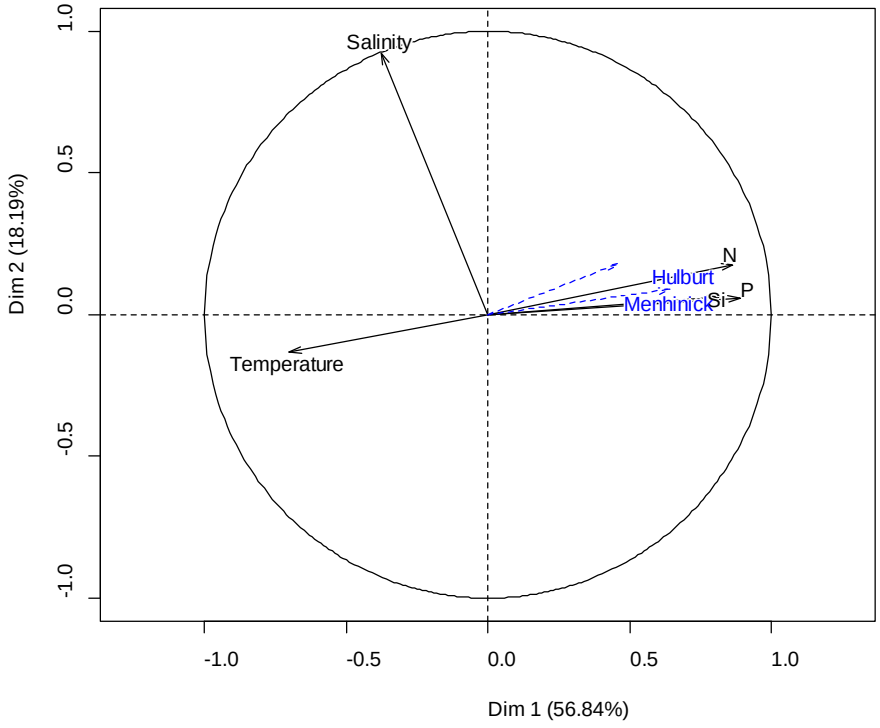

Figure 13: Results from PCA conducted on L4 environmental factors, with composition indices for phytoplankton as supplementary variables.

In Figure 13, the smaller the angles between environmental variables (represented as black arrows) and the principal components (represented as dashed black lines), the more the variables contribute to the construction of the principal components. The first component (horizontal axis) explains 56.84% of the environmental variability, and the second (vertical axis) explains 18.19% of the environmental variability. The blue arrows indicate how the indices, added as supplementary variables, are correlated with the principal components representing environmental conditions at L4. We found significant correlations between the first axis and the Menhinick ($r=0.64$, $p<0.001$) and the Hulburt ($r=0.46$, $p<0.001$) indices. The second component was correlated to the Hulburt index ($r=0.18$, $p<0.001$). Yet, following the Guttman-Kaiser criterion (also called the "broken stick" rule), only the first axis was significantly explaining environmental variance (although both axes are required to visually present the results). This confirms that both indices computed for phytoplankton reflect well the environmental conditions at L4.

LCBDs were computed for the considered time series on abundances of phytoplankton genera and are presented in Figure 14.

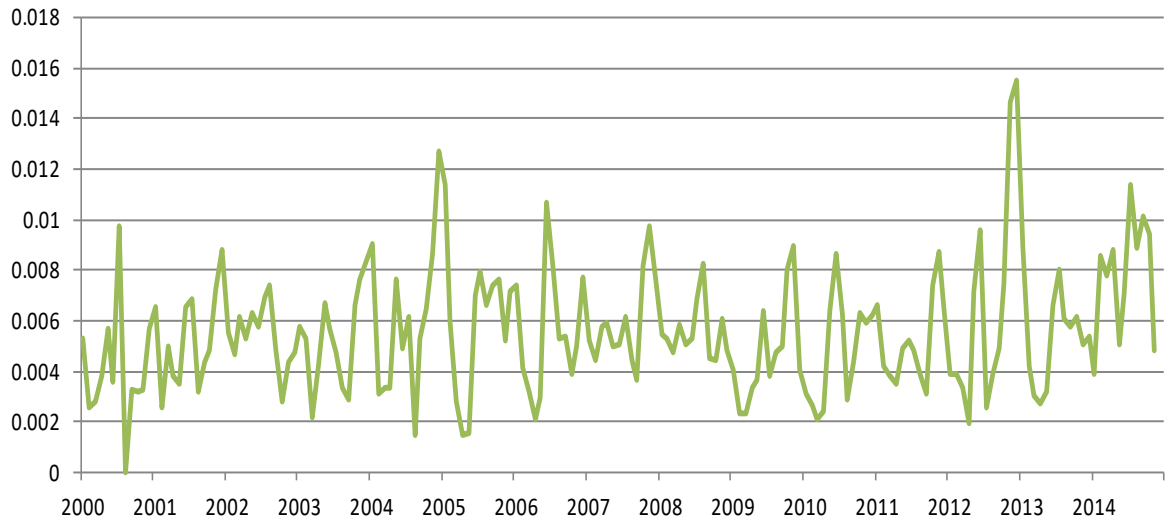

Figure 14: Local contributions to beta diversity (LCBD) for the phytoplankton community at L4

Results from permutations on LCBDs are presented in Table 4, with the corresponding values of the Menhinick and Hulburt indices.

Date	Menhinick	Hulburt	LCBD	p-values
8/2000	1.2454	15,9868	0.0097	0.009
9/2000	1.3840	19,9908	0.0089	0.031
12/2000	4.4590	25,5611	0.0103	0.005
1/2001	7.6640	23,6604	0.0091	0.020
3/2001	7.6544	24,8924	0.0087	0.001
4/2001	0.7985	23,4206	0.0114	0.001
5/2001	1.0070	23,0909	0.0110	0.047
2/2002	4.0604	20,0203	0.0089	0.008
4/2002	3.4116	19,8486	0.0081	0.001
5/2002	0.8640	16,8136	0.0099	0.008
10/2002	0.6253	16,7274	0.0136	0.008
4/2003	1.2855	19,2142	0.0100	0.023
12/2003	9.5976	18,9784	0.0084	0.004
4/2004	0.8337	26,8674	0.0102	0.002
8/2005	2.1846	12,2754	0.0093	0.007
9/2005	2.2149	22,5196	0.0105	0.009
9/2006	1.0618	21,795	0.0107	0.034
10/2006	1.7690	24,4713	0.0101	0.015
4/2007	1.3006	25,3633	0.0098	0.001
7/2008	**2.5864**	**15,8973**	**0.0088**	**0.001**
5/2009	3.6052	14,542	0.0085	0.040
11/2009	12.5888	19,1267	0.0096	0.018
4/2010	**2.2965**	**29,675**	**0.0110**	**0.030**
5/2010	**1.3769**	**28,8241**	**0.0115**	**0.013**
2/2012	17.8471	15,7223	0.0081	0.013
4/2012	2.0610	19,4497	0.0092	0.026
5/2012	1.9802	21,9489	0.0091	0.002

Table 4: Index values of phytoplankton communities and corresponding LCBD values significantly different from the others obtained via permutation tests (see Legendre & Gauthier 2014, significance level: alpha=0.05). Red rows indicate the three dates with the highest LCBD values. Sampling dates for years with abnormal community traits identified from PH1 or PH2 are highlighted with a bold font.

The analysis of LCBDs for the phytoplankton community at L4 station reveals that periods of high LCBD values, highlighted in blue in Table 4, are generally characterized by low richness (i.e. low values of Menhinick index) and high dominance (i.e. high values of Hulburt index). To better capture community structure for the dates characterized by the highest LCBDs, we calculated IVIs and represented them in Figure 15.

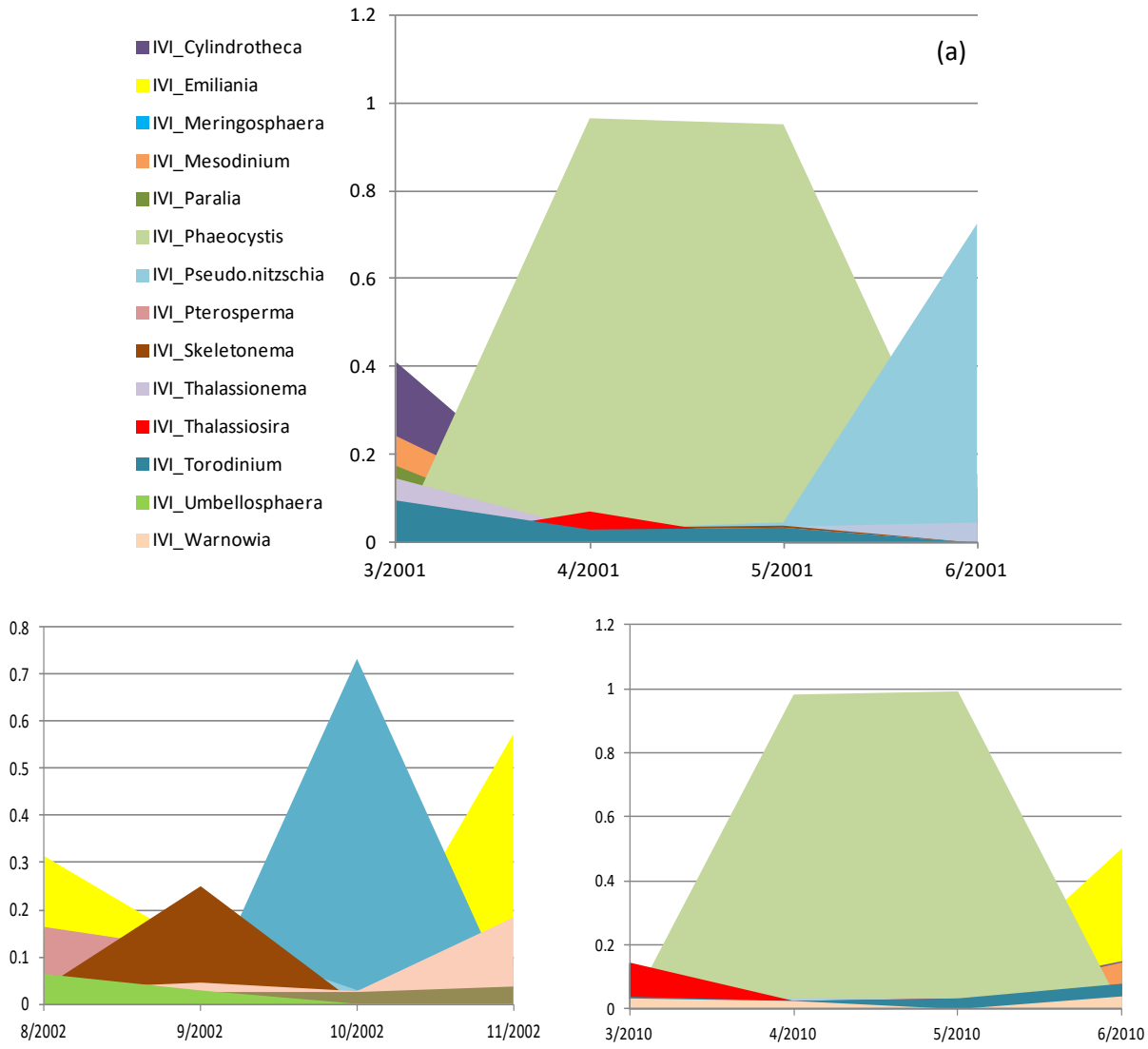

Figure 15: Important index values (IVI) for phytoplankton communities at L4 for periods characterized by high LCBDs, i.e. May 2001 (a), October 2002 (b), April and May 2010 (c)

IVIs calculated in case of high LCBDs, high dominance and low richness indicate that October 2002 is marked by an unusual bloom of a siliceous Chromista of the genus Meringosphaera while May 2001, April and May 2010 correspond to harmful algal blooms of the Haptophyte genus Phaeocystis. **This last result is also supported by anomalies in the phytoplankton biomass detected by PH2 for the year 2010.**

4.3.2. Indicator results for zooplankton

We estimated the sensitivity of each pre-selected index for zooplankton with environmental conditions at L4. To this end, we performed- a PCA on the provided environmental variables (centred and reduced) and calculated correlations between principal components and each index. The results are presented in Figure 16.

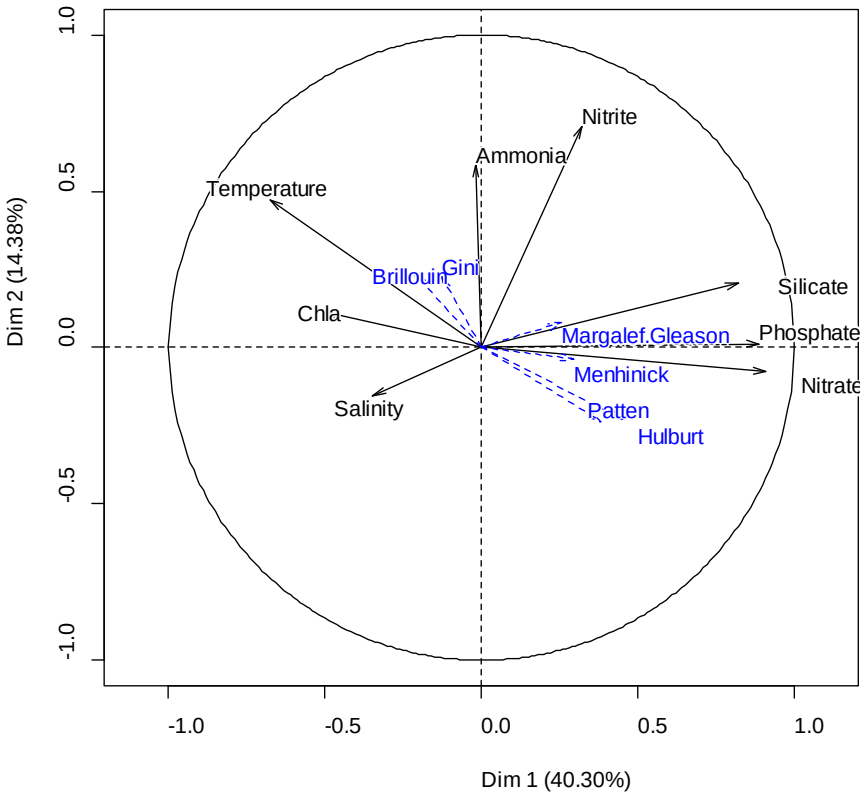

Figure 16: Results from PCA conducted on L4 environmental factors, with composition indices for zooplankton as supplementary variables

Using the Guttman-Kaiser criterion, only the first and second components were found to significantly explain environmental variance, explaining respectively 40.30% and 14.38% of the variance. Following the PCA, significant correlations were found between the first axis with Patten (r=0.45, p<0.001), Hulburt (0.38, p<0.001), Menhinick (r=0.29, p<0.001), Margalef-Gleason (r=0.26, p<0.001) and Brillouin indices (r=-0.21, p<0.001). The second component was correlated to the Brillouin (r=0.24, p<0.001), Gini (r=0.23, p<0.001), Patten (-0.23) and Hulburt (r=-0.24, p<0.001) indices.

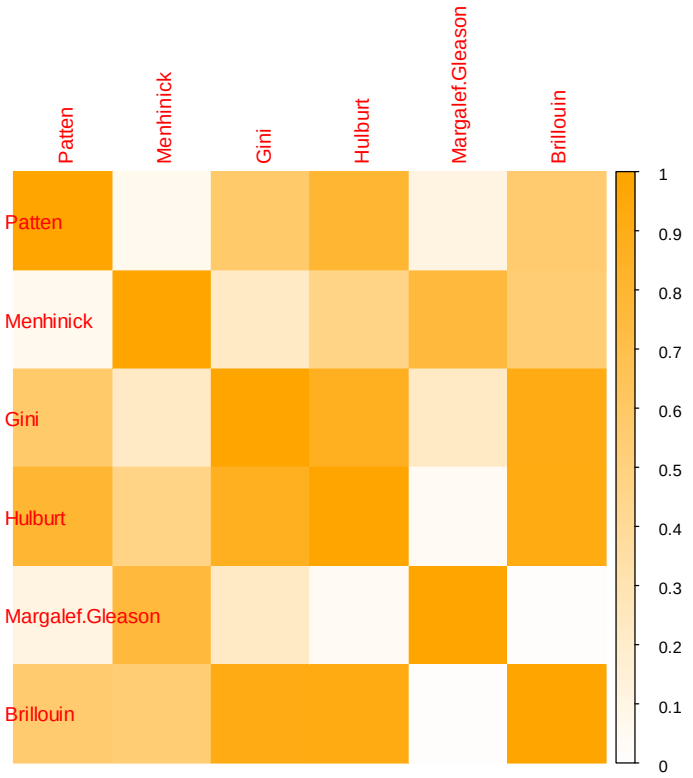

Figure 17: Correlogram indicating correlations among absolute values of the pre-selected indices for zooplankton at L4

Pearson correlations between indices were calculated to assess redundancy among these metrics. Results are presented in Figure 17, where colour intensity indicates absolute values of the coefficients obtained from correlations between the pre-selected indices (dark orange indicating high correlation and white indicate no correlation). It can be concluded that the indices of Patten and Margalef-Gleason tend to have lower correlation coefficients with the rest of the indices, indicating low redundancy in the information they convey.

In light of the results presented in Figures 16 and 17, Patten's index was retained to assess dominance in the zooplankton community at L4. Menhinick and Margalef-Gleason indices are good candidates to reflect species richness and scored the two highest correlations for diversity indices with environmental variables (Fig. 14). Moreover, they both scored low correlations with the Patten index (based on absolute regression coefficient value), indicating possible complementarities with this index. We decided to retain Menhinick's richness index because it scored both the lowest correlation with the Patten index (r=0.026 against r=-0.14 for Margalef-Gleason index) and the highest correlation with environmental variables (r=0.23 against r=0.16 for Margalef-Gleason index). As a result, Patten and Menhinick indices were retained to assess zooplankton community composition at L4. The temporal dynamics of these two indices are presented in Figure 18.

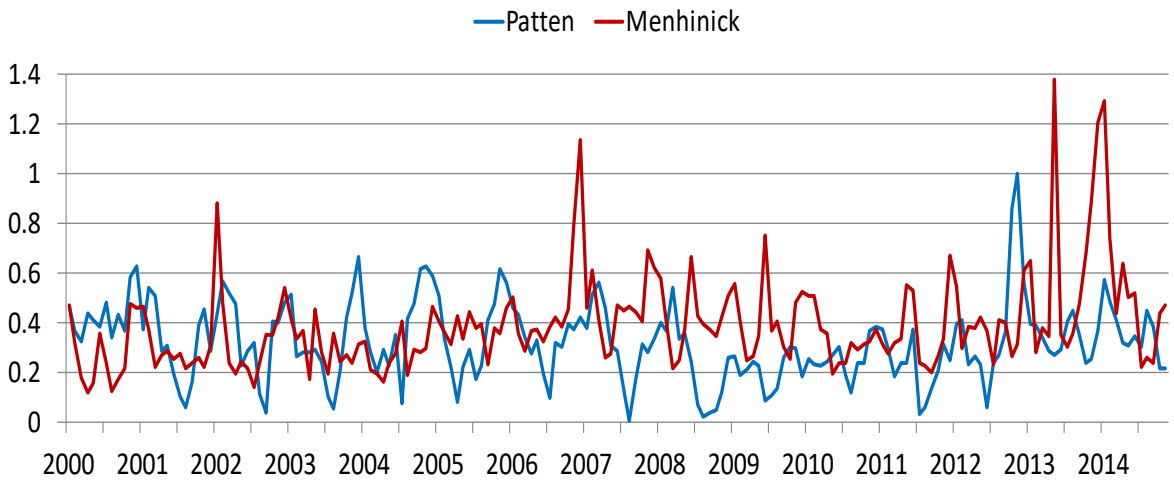

Figure 18: Temporal dynamics of richness (Menhinick index, in red) and dominance (Patten index, in blue) indices for zooplankton at L4

LCBDs were computed for the considered time series on copepod abundances and are presented in Figure 19. Low LCBDs correspond to typical community composition, while high LCBDs indicate atypical communities. Dates with the lowest and the highest LCBDs are presented in Table 5, with the corresponding values of the Menhinick and Patten indices.

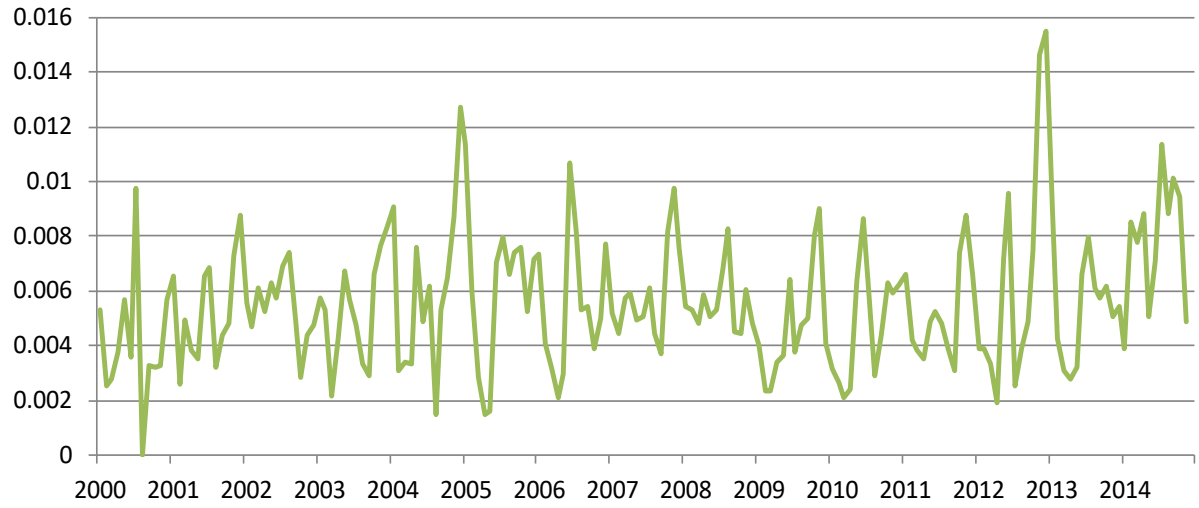

Figure 19: Local contributions to beta diversity (LCBD) for the zooplankton community at the L4 station

In Table 5, we present dates characterized by significant LCBDs and their corresponding Menhinick and Patten index values.

Date	Menhinick index	Patten index	LCBD	p-values
7/2000	**0.24100841**	**0.483484**	**0.00973605**	**0.041**
12/2004	**0.29751737**	**0.627982**	**0.01269268**	**0.006**
1/2005	0.46392543	0.589699	0.01133315	0.014
6/2006	**0.37413167**	**0.329836**	**0.01065836**	**0.028**
11/2007	0.40569697	0.314948	0.00974494	0.042
6/2012	0.4248631	0.233366	0.00957868	0.048
11/2012	0.26610861	0.862072	0.01461197	0.002
12/2012	0.31524601	1	0.01547921	0.001
7/2014	0.52262291	0.348789	0.011373	0.016
9/2014	0.262217042	0.450419	0.01014691	0.023
10/2014	0.23928366	0.386603	0.00943641	0.038

Table 5: Richness and dominance index values of zooplankton communities and corresponding LCBD values significantly different from the others obtained via permutation tests (see Legendre & Gauthier 2014, significance level: p=0.05). The three dates with the highest LCBD values correspond to the red rows. Sampling dates for years with abnormal community traits identified from PH1 or PH2 are highlighted with a bold font.

The highest significant LCBD values reflect atypical community structure. The corresponding richness and dominance index values inform us whether these correspond to communities of high conservation values or, instead, if they indicate degraded communities. The three highest LCBDs correspond to December 2004 and to November and December 2012 and are characterized by the highest values of Patten's dominance index. During these periods, Margelef-Gleason index values are locally low but do not account for the lowest values in the time-series. In order to identify the species responsible for high dominance for these dates, we calculated IVIs. The results are presented in Figure 20.

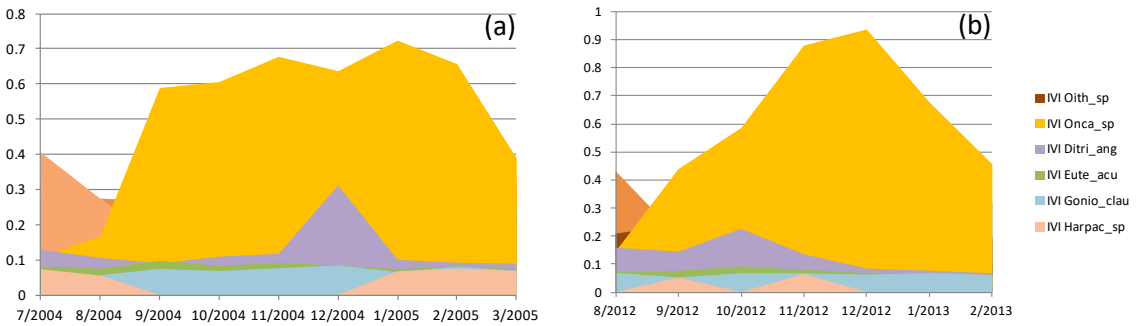

Figure 20: Important value indices (IVI) the zooplankton community at the L4 station during periods characterized by high LCBDs, i.e. December 2004 (a), November and December 2012 (b) From the IVIs, it appears that the three periods characterized by high LCBDs correspond to periods marked by the dominance of copepods of the genus *Oncaea*, which can dominate the zooplankton community during the winter when food resources are scarce (Eloire et al. 2010, Tanimura et al.

1997). December 2004 is also marked by the high abundance of *Ditrichocorycaeus anglicus*. Both *Oncaea* spp. and *D. anglicus* are thermophilic and opportunistic species known to gain advantage in the community when temperatures increase (Valdès et al. 2007). This result is remarkable because PH1 (lifeform holoplankton/meroplankton) and PH2 both found 2004 to be characterized by an atypical zooplankton community structure.

5 Integrating results from PH1, PH2 and PH3

PH1 "Changes in plankton functional communities", PH2 "Changes in plankton biomass and abundance" and PH3 "Changes in plankton diversity" provide complementary information on aspects of plankton community structure related to the ecological status of pelagic habitats. As previously reported, each index pointed out different changes in phytoplankton and zooplankton communities, stressing the limited redundancy and high complementarity among them. Importantly, the three indicators have also spotted similar periods of changes in the plankton community. Accordingly, our ability to generalize how changes in one indicator can translate to changes in another is currently limited. Yet, PH1, PH2 and PH3 all found anomalies in the zooplankton community structure in 2004. Whilst the large analysis period of PH1 limited its sensitivity for the L4 data, PH2 and PH3 both found atypical structure of the phytoplankton community in 2010.

A difficulty in combining the results of the different PH indicators lies in the resolution of their outcome (see Section 6). Indeed, the PI for PH1 provides results for each year (although it is based on monthly measurements) while PH3 returns monthly values. PH2 can be interpreted for both years and months. We addressed these differences by examining the results on the lifeform time-series provided by PH1 but no clear connection could be established. This should be considered in future assessments and tests. Because the computing of the PH1 reference envelope was based on along the whole temporal period, significant PI values were only detected for the zooplankton component. Consequently, we can compare results of PH2 and PH3 for phytoplankton on a monthly basis. More explicitly, 2010 was marked by an extreme change in phytoplankton biomass (seen from PH2). Low richness and high dominance by the genus *Phaeocystis* (detected by PH3 and completed with IVIs) in May are associated with strong negative anomalies (detected by PH2). We further investigated if atypical environmental conditions, especially ratio of nutrients which are known to condition the growth of phytoplankton (referring to possible limitation conditions), could have favoured bloom formation of one or few single species. To this end, we calculated monthly averages of N:P, Si:N and Si:P ratios from the provided data by omitting missing values. We compare in Table 6 them to the corresponding months of bloom formation in 2010 (see also Figure 15).

Date	N:P	Si:N	Si:P
Mean (±SD) April	9.04 (±23.20)	1.08 (±0.49)	7.63 (±24.31)
Mean (±SD) May	4.57 (±9.07)	3.10 (±8.46)	7.51 (±13.34)
April 2010	17.33	0.25	4.32
May 2010	4.29	1.098	4.71

Table 6: Nutrient ratios for spring (green rows) and autumn (yellow rows) blooms detected by the PH3 indices at L4 and the corresponding monthly averages.

From Table 6, it can be seen that an elevated N:P ratio coincides with the start of the *Phaeocystis* bloom. These conditions are known to favour eutrophication, as well as HAB *Phaeocystis* bloom formation (Gypens et al. 2007, Lefebvre et al. 2001, Lancelot et al. 1987). Further analyses should be conducted in order to confirm these observations and test human impact on this system.

PH1 found a significant PI for the pair holoplankton/meroplankton in 2004, indicating an atypical lower abundance of meroplankton relative to holoplankton during this period. This result suggests weak benthic-pelagic coupling and reproductive output of benthic versus pelagic faunas. This period coincides with a decrease in copepod abundance. PH3 also revealed the high abundance of the thermophilic and opportunistic copepod *D. anglicus* in December 2004. These organisms can be favoured in the community when water temperature is warmer. We compare observed monthly water temperatures for the period corresponding to the increase in abundance of *D. anglicus* (see Figure 20) with monthly averages for the period 2000-2014 in Table 7.

Date	Temperature (°C)
Mean (±SD) August	16.44 (±0.30)
Mean (±SD) September	15.89 (±0.35)
Mean (±SD) October	15.00 (±0.19)
Mean (±SD) November	13.81 (±0.28)
Mean (±SD) December	11.44 (±0.36)
August 2004	14.66
September 2004	17.08
October 2004	16.07
November 2004	13.10
December 2004	13.12

Table 7: Surface water temperature for months detected by the PH3 indices at L4 and the corresponding monthly averages. Unusually high temperatures are indicated in bold red font.

The measures reported in Table 7 suggest that unusually high water temperatures in September 2004 correspond to periods of marked increases in the abundances of thermophilic copepods detected by PH3. These changes might be associated to changes in copepod biomass and in benthic-pelagic coupling detected to PH2 and PH1 could reflect a consequence of current environmental changes on pelagic habitats. Further analyses should be conducted in order to strengthen this possible link to anthropogenic pressures.

6 Conclusions and perspectives

The multimetric approach is a methodological tool which can be used to study a range of systems, including GES assessment of marine waters. Three indicators are currently being developed in the frame of the OSPAR convention for the pelagic habitat component: PH1 “Changes in plankton functional communities”, PH2 “Changes in plankton biomass and abundance” and PH3 “Changes in plankton diversity”. As mentioned in the previous sections of this document, the three PH indicators

provide information on different and complementary aspects of the plankton community that, only when considered altogether, provide a holistic vision of the ecosystem which is central to GES assessment. The main aim of this deliverable is to combine information of the three indicators for the first time. For this purpose, long-term and high quality data are needed. It was thus decided that the Plymouth Marine Laboratory L4 station would be the focus of this deliverable for the period 2000-2014. Moreover, PH1 and PH2 have already been calculated for the L4 station (OSPAR 2017 assessment, in preparation) but PH3 needed to be computed for the phytoplankton compartment and to be developed for zooplankton.

Different results were obtained from PH1, PH2 and PH3 regarding dates characterized by atypical plankton community structures, stressing their complementarity. However, the three PH indicators detected anomalies in the zooplankton community structure in 2004, with significant PI value for the pair holoplankton/meroplankton (PH1), marked decrease in copepod abundance (PH2). PH3 found the species composition of December 2004 to be atypical in the time series and characterized by low diversity and high dominance. The use of IVIs detailed the high abundance of the thermophilic and opportunistic copepod *D. anglicus* for this month, which started increasing in proportion to other species in the community when temperatures were higher than usual. Furthermore, PH2 and PH3 both found abnormal community structure for phytoplankton in 2004. A change that might be linked to disturbed nutrient ratios during the spring.

This work has also evidenced a number of gaps and issues in the integration of the three PH indicators. Among them, the temporal resolution of the results produced by each indicator limits our ability to compare their results. As mentioned in the previous section, PH1 produces PI values for years while PH3 produces monthly values. Although it could be considered to compute PH3 for years, this can be seen as problematic in light of plankton dynamics, which are characterized by seasonal successions (justifying *per se* the use of monthly data instead of yearly). The details provided by the lifeform time series were used to address this issue. However, no clear connections with the results provided from the other PH indicators could be established. Findings from PH1 might have been limited by the use of a long period for determining seasonal patterns of lifeform ratios. Efforts should be devoted to overcome these technical difficulties, likely inherent to the integration of separately developed indicators.

In the frame of this project, one of the main issues encountered concerns the access to data (also highlighted in EcApRHA deliverable reports WP1 1.1 and 1.2, OSPAR in preparation). The time-frame available for performing the planned analysis and preparing this deliverable was notably constrained by the time when the data were provided. Accordingly, future investigations should be conducted in order to strengthen the links between the three PH indicators, their common interpretation (i.e. their integration into a general multi-metric index) and potential links to the environmental data. Connected to this issue, lies the recurring problem of data formatting. Indeed, data are typically provided in a range of formats and of data sets (even from the same partner institute), which further limits the time that can actually be devoted to the processing/analysis of the data and to result interpretation. Adopting a general format for the data could be a solution and was notably discussed for national monitoring programs (e.g. annual RESOMAR conference 2016 in France). The formatting of the data should ideally be realized by the institutes in charge of this data collection as the time available for biodiversity assessment project is typically short and involves researchers working on short-term contracts. Creating a central database of pre-formatted data could also benefit the regional calibration of the indicators for areas where appropriate data are available.

In the future, complementary techniques to classical microscopy (and to colorymetric analyses for phytoplankton) such as metagenomics could be considered to provide metrics for the calculation of PH1, PH2 and PH3 indicators (cf. EcApRHA WP1 report 1.2). In this respect, the work by Wang et al. (2012) constitutes a notable example were a wide range of data, including biological data with genetic units, is considered to assess how microbial communities respond to natural and anthropogenic environmental variables. Statistical tools are currently available for such a future development of the PH indicators.

Finally, we end this work by providing larger recommendations at the OSPAR level, which are summarised in the following bullet points:

- Address and resolve the issues of data access from public institutions to enable access for use within OSPAR assessments in future

- Clear need for the creation of a database of inter-comparable marine biological data and environmental/pressure data at the OSPAR level, and for which the data clearly match defined monitoring guidelines (ensuring data quality)

- Need for a long-term group of experts (potentially at the OSPAR level) responsible for the creation and running of a European database and ensuring the quality of collected monitoring data. This should be considered for the long-term, clearly not at the end of the directive process

- Need for the establishment of a clear and easily accessible report including all the monitoring guidelines concerning the metrics used for the OSPAR PH indicators. This is required in order to provide clear recommendations for homogenising the monitoring and inter-comparability of data among Contracting Parties in the goal of regional marine management

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Annexes

A1. Taxa/species of copepod summed for calculating total copepod abundance at the L4 station

AphiaID	**Taxa/Species name**
104633	*Metridia lucens*
149755	Total Acartia clausi
104474	*Candacia armata*
104494	*Centropages chierchiae*
104496	*Centropages hamatus*
104499	Total Centropages typicus
104501	*Isias clavipes*
104722	*Anomalocera patersoni*
104686	*Parapontella brevicornis*
104736	*Labidocera wollastoni*
104878	Total Temora longicornis
104879	*Temora stylifera*
104462	*Calanoides carinatus*
104466	Total Calanus helgolandicus
104193	*Calocalanus spp.*
104161	Total Clausocalanus spp. (Calculated)
104510	*Total Ctenocalanus vanus (Calculated)*
104685	*Total Paracalanus parvus (Calculated)*
104515	*Total Pseudocalanus elongatus (Calculated)*
104173	*Subeucalanus spp.*
104164	*Microcalanus spp.*
104521	*Diaixis hibernica*

104563	*Paraeuchaeta hebes*
104229	*Scolecithricella spp.*
106485	*Oithona spp.*
128690	*Oncaea spp.*
128805	*Ditrichocorycaeus anglicus*
115341	*Microsetella sp*
116162	*Euterpina acutifrons*
346509	*Goniopsyllus clausi*
1102	Harpacticoid unidentified
1104	Siphonostomatoida

A2. The most common biodiversity indices with their formula, main characteristics description, advantages and limitations

Index	Formula	Type and use
Species richness	$R = S$ S: the total amount of species in the sample/community.	Diversity index - simple, basic and widely used (Magurran 2003) - often considered with its standard deviation (as an assessment of variation in species between samples/communities) - requires a good knowledge of the taxonomy - highly sensitive to sampling effort (Kemton 1979).
Shannon-Weaver's entropy (H') *Also called*: - Shannon index - Shannon–Weaver (or Shannon–Wiener) index	$H' = -\sum_{i=1}^{S}[\frac{n_i}{N} \times \ln(\frac{n_i}{N})]$ n_i: abundance of species i S: total amount of species N: total abundance of all species (Shannon & Weaver 1949)	Diversity index - very common and well known - quantifies the uncertainty (entropy or degree of surprise) associated with predicting to which species a randomly chosen individual belongs to depending on community structure (Shannon 1948) - increases as both the richness and the evenness of the community increase - commonly used together with Simpson's index - sensitive to sampling effort (Hubalek, 2000) - not sensitive to diversity changes in time (Boyle et al. 1990) - difficult interpretation when comparing communities (Kerloff 2010)
Margalef-Gleason index Other names: Margalef index	$D_{Margalef} = (S-1)/\ln(N)$ S: total number of species in the sample/community N: the total abundance of all species	Diversity index - a simple measure of biodiversity, easy to compute - high values indicate high diversity

	(Margalef 1951, 1958)	- highly sensitive to sampling effort and to the structure of the community, including weak variations in the amount of species in the sample/community (Magurran 2004, Gamito 2010) - can be use in conjunction with indices sensitive to evenness or changes in dominant species, such as the dominance Berger-Parker index.
Gleason index	$\mathrm{D}_{\mathrm{Gleason}} = \mathrm{S}/\ln(\mathrm{N})$ S: total number of species in the sample/community N: total abundance of all species (Ludwig & Reynolds 1988)	Diversity index - a simple measure of biodiversity, easy to compute - high values indicate high diversity - sensitive to richness aspects of biodviersity and to change in sample size, gear, and handling procedures (Kumar & Hyde 2004).
Menhinick index	$D_{\mathrm{Menhinick}} = S/\sqrt{N}$ S: total number of species in the sample/community N: total abundance of all species (Menhinick 1964)	Diversity index - a simple measure of biodiversity, easy to compute - high values indicate high diversity -extremely sensitive to sampling effort - efficient for evaluating eutrophication (Karydis & Tsirtsis 1996).
Brillouin index	$HB = \frac{\ln N! - \sum_{i=1}^{S} \ln n_i!}{N}$ Also found expressed as: $HB = \frac{1}{N} \times \ln\left(\frac{N!}{\prod_{i=1}^{S} n_i!}\right)$ n_i: abundance of species i	Diversity index. - not sensitive to the abundances of common species - adapted for samples not randomly collected or for small communities but leads to Shannon-Weaver's index for large N and n_i values (Margalef 1958, Piélou 1975, Magurran, 1988, Legendre & Legendre, 1998). - can only be used only when the complete community is known (hence, rarely usable in practice) and sampling is done without replacement

	S: total amount of species N: total abundance of all species (Brillouin, 1956)	- it cannot be used when abundances are expressed as biomass or productivity (Legendre & Legendre 1998). - difficult, if not impossible, to compute in case of large amounts of individuals - difficult interpretation : depending on sample size, it can yield higher values for less evenly distributed communities (see Peet 1974) - no variance for this index - no statistical tests are needed to demonstrate significant differences (Magurran 2003).
McIntosh index	$$U = \frac{N - \sqrt{\sum_{i=1}^{S} n_i^2}}{N - \sqrt{N}}$$ n_i: abundance of species i S: total amount of species N: total abundance of all species (McIntosh 1967)	Diversity index - shows how individuals are distributed in a homogenous way in the sample. - highly influenced by the sample size. - rarely used
Berger-Parker (d)	$$d = n_{max}/N$$ n_{max}: abundance of the most abundant species in the sample/community N: total abundance of the sample/community (Berger et al. 1970)	Dominance index - gives the fraction of total of individuals contributed by the dominant species (Caruso et al. 2007) - easy to compute - allows comparison between sites for similar sampling efforts - should not be considered for species-poor communities (i.e. under 15 species) - the reciprocal of the index (1/d) is often used

Simpson index *Also called*: - Herfindahl–Hirschman index	$$\lambda = \sum_{i=1}^{S} (\frac{n_i}{N})^2$$ n_i: abundance of species i S: total amount of species N: total abundance of all species (Simpson 1949) If the dataset is small, and sampling without replacement is assumed, the probability of obtaining the same type with both random draws is: $$l = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N-1)}$$ (Piélou 1969) When derived from concentration, it is advised to use a modified version of the index, the Gini-Simpson index: $$\widehat{D} = 1 - \lambda \quad \text{or} \quad \hat{l} = 1 - l$$ (Gini 1912, Simpson 1949)	Dominance index - expresses the probability that any two individuals drawn at random from an infinitely large community belong to the same species - the inverse of the Simpson index (1/ λ) or simply 1- λ are widely used too (Hill 1973). - sensitive to important variations in abundant species, does not give much weight to rare species (Gimaret-Carpentier et al. 1998) - if the dataset is very large, sampling without replacement gives approximately the same result, but in small datasets the difference can be substantial. In this situation, a modified version should be used: l (or 1- l) - use the Gini-Simpson index, $\widehat{D}$, to derive the index from concentrations (Simpson 1949)
Patten index	$\mathrm{R} = (\mathrm{H'_{max}}\text{-}\mathrm{H'})/(\mathrm{H'_{max}}\text{-}\mathrm{H'_{min}})$	Dominance index

	H': value of the Shannon-Weaver index for the considered sample/community H'_{max}: highest value of the index among the different samples/communities H'_{min}: lowest (Patten 1962)	- assesses predominance through evaluating redundancy in the community, represents the way of which the individuals are distributed among species and gets a measurement of the predominance of one or some species (Bandeira et al. 2013) - often used together with the Shannon-Weaver index so that R estimates the rate of abundance of the species while being independent of the base of the logarithms (Whilm 1967).
Brillouin's evenness	$E = HB/HB_{max}$ Also found expressed as : $E = H'/\ln S$ HB: value of the Brillouin index for the considered sample/community HB_{max}: highest value of the index among the different samples/communities S: total amount of species (Piélou 1975)	Evenness index - describes the portion of rare species in a sample/community - difficult to compute (because it requires computing Brillouin indices)
Hulburt	$\delta = 100 \text{ x } (n_1+n_2)/N$ N: total abundance of all species in the sample/community n_1: abundance of the most abundant species in the community n_2: abundance of the second most abundant species in the community	Dominance index - percentage reflecting the fraction of individuals belonging to the two most abundant species in the community (Hulburt 1963). - common use of 100-Hulburt as the portion of individuals belonging to rarer species in the community.

Hurlbert (PIE)	$PIE = \left(\frac{N}{N-1}\right) \times (1 - \sum_{i=1}^{S} (ni/N)^2)$ Where n_i is the abundance of species I, S is the total amount of species and N is the total abundance of all species (Hulbert 1971).	Evenness index - represents the probability of intraspecific encounter - an alternative to Shannon more sensitive to losses and changes in relative abundance of species (Boyle et al. 1990). - insensitive to temporal changes in the community (Boyle et al. 1990). - easily interpreted as a probability - unbiased by sample size, although the variance increases at small N - important analog in population genetics (equivalent to the calculation of heterozygosity, H) - can be used as a measure of interspecific competition.
Piélou index	$J' = H' / H'_{max}$ H': Sannon index value corresponding to the considered sample/community H'_{max}: maximum value of the Shannon index in all the considered samples/communities (Piélou 1966, 1975).	Evenness index - reflects of the repartition of individuals within the species, independently of the specific richness or the degree of incertitude existing for a type of individual taken randomly in the population (Pielou, 1966) - allows to see if a community can be highly dominated by some species (particularly by opportunistic species which are often in high abundances in communities) - ranges between 0 (only one species dominates the community) and 1 (equi-repartition of species) - sensitive to the sampling effort as the total number of species in the community (S) is hard to measure.
Jaccard index Also called: *Jaccard* similarity coefficient	$J = S_c/(S_a+S_b+S_c)$ S_a: number of species present in sample A S_b: number of species present in sample B	Similarity index - most ancient and simple similarity index to compare different samples or communities

	S_c: the number of species present in both samples (Jaccard 1901, 1912)	- very straightforward as it corresponds to the fraction of species shared between samples - pairwise measures can notably be used to examine how the index values vary with distance or environmental differences between sites - rarely used to study marine systems (but see Danilov & Ekerlund 1999) although condisered to be one of the best measurements to detect the appearance of new species during successions (see Bandeira et al. 2013).
Saprobic index	$$SI = \frac{\sum_{i=1}^{S}(K_i n_i)}{\sum_{i=1}^{S} n_i}$$ n_i: abundance of species i S: total amount of species N: total abundance of all species K_i: numerical value corresponding to the preferred saprobic zone of the species i (oligosaprobic : K_i =1; β-mesosaprobic: K_i = 2; α-mesosaprobic: K_i = 3; polysaprobic: K_i = 4) (Pantle & Buck 1955, Liebmann 1962)	Biotic index - developed to assess the impact of water pollution on species assemblages, corresponds to the weighted mean of all individual indices and indicates the saprobic zone as follow: SI = 1.0 - < 1.5 : oligosaprobic SI = 1.5 - < 2.5 : β-mesosaprobic SI = 2.5 - < 3.5 : α-mesosaprobic S = 3.5 - 4.0 : polysaprobic - based on the saprobic system corresponding to four zones of gradual self-purification (from pollutants): the polysaprobic zone, the α-mesosaprobic zone, the β-mesosaprobic zone, and the oligosaprobic zone. These zones are characterised by indicator species, certain chemical conditions and the general nature of the bottom of the water body and of the water itself. - should not be used in conditions of turbulent currents (Chandler 1970). - insensitive to rare species. - requires the organisms normally occurring in each of the river classification zones for a particular region to be known so that they can be assigned to a preferred saprobic

		zone during the calculation of the index. This information can only be obtained by detailed studies of the water systems, including precise identification of the individual species (Chapman 1992).

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A3. List of potentially heterotrophic or mixotrophic genera in the phytoplankton data set.

Similar results for PH3 were found for analyses conducted on phytoplankton data sets including or excluding the following genera:

AphiaID	**Genus name**
109473	*Amphidinium*
109517	*Amphidoma*
415082	*Ascampbelliella*
292897	*Askenasia*
292899	*Balanion*
109474	*Cochlodinium*
341301	*Didinium*
109462	*Dinophysis*
109515	*Diplopsalis*
183562	*Epiplocylis*
183543	*Eutintinnus*
172431	*Favella*
NaN	*Gymnodinium* (colourless only)
109476	*Gyrodinium*
172434	*Helicostomella*
109477	*Katodinium*
109499	*Kofoidinium*
101190	*Laboea*
101179	*Leegaardiella*
101180	*Lohmanniella*
109490	*Nematodinium*
109500	*Noctiluca*
109528	*Oxytoxum*
196836	*Parafavella*
172321	*Peritromus*
109466	*Phalacroma*
109485	*Polykrikos*
109487	*Pronoctiluca*
292924	*Proplectella*
425488	*Prorodontida*
109553	*Protoperidinium*
109555	*Pyrophacus*
292925	*Rhabdoaskenasia*
183566	*Salpingella*
101185	*Strobilidium*
101198	*Strombidinopsis*
101195	*Strombidium*
247913	*Tiarina*

732976	*Tintinnid*
163780	*Tintinnopsis*
101196	*Tontonia*
143943	*Uronema*
163573	*Vorticella*

ISBN: 978-1-911458-24-1

Publication Number: EcApRHA1.4/2017

This report was produced as a result of the EcApRHA (Addressing gaps in biodiversity indicator development for the OSPAR Region from data to ecosystem assessment: Applying an ecosystem approach to (sub) regional habitat assessments) project. The project was co-financed by the European Union (EU). Grant No. 11.0661/2015/712630/SUB/ENVC.2 OSPAR