Spatial patterns of phytoplankton composition and upper-ocean biogeochemistry do not follow carbonate chemistry gradients in north-west European Shelf seas

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Abstract

A key difficulty in ocean acidification (OA) research is to predict its impact after physiological, phenotypic and genotypic adaptation has had time to take place. Observational datasets can be a useful tool in addressing this issue. During a cruise in June-July 2011, measurements of upper-ocean biogeochemical variables, climatically active gases and plankton community composition were collected from northwestern European seas. We used various multivariate statistical techniques to assess the relative influences of carbonate chemistry and other environmental factors on these response variables. We found that the spatial patterns in plankton communities were driven more by nutrient availability and physical variables than by carbonate chemistry. The best subset of variables able to account for phytoplankton community structure was the euphotic zone depth, silicic acid availability,

mixed layer average irradiance and nitrate concentration (59% of variance explained). The spatial variations in phytoplankton and coccolithophores species composition were both found to be more strongly associated with nutrients and physical variables than carbonate chemistry, with the latter only explaining 14% and 9% of the variance, respectively. The plankton community composition and contribution of calcifying organisms was not observed to change under lower calcite saturation state (Ω) conditions, although no regions of undersaturation (Ω < 1) were encountered during the cruise. Carbonate chemistry played a more prominent, but still secondary, role in determining dinoflagellate and diatom assemblage composition (20% and 13% of total variance explained, respectively). Nutrient and physical variables also explained more of the spatial variations of most climatically-active gases and selected biogeochemical response variables, although some also appeared to be influenced by carbonate chemistry. This observational study has demonstrated that ocean acidification research needs to be set in context with other environmental forcing variables to fully appreciate the primary, or indeed secondary, role that increasing $f\text{CO}_2$ has on biological communities and associated biogeochemical rates.

Keywords

Ocean Acidification, Carbonate Chemistry, Nutrients, Shelf Seas, Climatically Active Gases, Biogeochemical Variables, Diatoms

1. Introduction

The ongoing increase in seawater CO_2 and reduction in seawater pH associated with the uptake of fossil fuel-derived CO_2 from the atmosphere (often termed ocean acidification [OA]) will have many direct and indirect effects on the chemistry and biology of the ocean

(Shi et al., 2010). Coastal economies, including those along the coast of northwest Europe, are often dependent on calcium carbonate-producing organisms, such as shellfish (e.g., cockles, mussels, and oysters). It has been observed that in some areas elsewhere in the world these fisheries are collapsing due to changing carbonate chemistry composition (Dickson, 2010; Callaway et al., 2012). For at least nine years, the US and Mexico West Coast shellfish industry has observed high larval mortality in hatcheries and poor larval recruitment success for some species in the wild, especially during periods of strong deep-water upwelling (Cooley and Doney, 2009; Dickson, 2010).

Previous work on OA has also suggested that impacts are not limited to seafloor organisms, but can also affect organisms and biogeochemical processes in open water away from the coast. There is no clear agreement, heavily debated on either side, whether planktonic calcifying organisms are negatively or positively impacted. Coccolithophores are an example of calcifying organisms that have shown relatively mixed responses from OA (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Langer et al., 2009; Bach et al., 2015). Other marine phytoplankton (e.g., diatoms) have also been shown to change their elemental composition (C/N/P) in response to changing CO_2 concentration, but again these changes are species-specific and sometimes opposite responses are obtained in different studies (Engel et al., 2005; Schulz et al., 2008). It has been proposed that increasing levels of fCO_2 in the surface ocean will lead to accumulation of dissolved organic carbon (DOC) and transparent exopolymer particles (TEP), with important implications for the functioning of the marine carbon cycle (MacGilchrist et al. (2014) and references therein).

Primary production also has the potential to be affected by OA. For example, during a summer 2014 cruise on the northwest European Shelf (the same cruise as is the subject of this paper), considerable variability in carbon-fixation dynamics (such as growth rates and the contribution from small cells) in manipulation experiments were observed (Richier et al.,

2014). It was also found that the net production of particulate organic carbon (POC) was strongly correlated with net changes in CO_2 level.

During the same bioassay experiments as in Richier et al. (2014), an association was found between higher nitrous oxide (N_2O) concentrations and lower temperatures, which was attributed to increased gas solubility (Clark et al., 2014). Furthermore, a consistent increase in dimethyl sulfide (DMS) and a decrease in the precursor dimethyl sulfoniopropionate (DMSP) in response to high CO_2 was also observed (Hopkins and Archer, 2014). However, other studies previously observed a decrease in DMS under high CO_2 concentrations (Hopkins et al., 2010) or no effect of elevated CO_2 on concentration of DMSP (Lee et al., 2009).

To maximize our understanding of the effects of environmental stressors on complex oceanic ecosystems and biogeochemical processes, multivariate statistical approaches are required. Multivariate techniques are ideally suited to studies where multiple response variables are measured in the same observational or experimental study; they have been used extensively for terrestrial ecological studies and fisheries studies, e.g. Beaugrand and Reid (2012). These analyses are used to test complex hypotheses about regional differences and relationships between variables (Havenhand et al., 2010). Used in this way, observational data can inform us of ocean change impacts and complement more targeted and more finely-detailed experimental research (Collins et al., 2014; Sunday et al., 2014). Some earlier field studies have also analyzed linkages between carbonate chemistry and species composition in an OA context using multivariate statistics (e.g. Charalampopoulou et al. (2011); Charalampopoulou et al. (2016)). Frigstad et al. (2013) reported the need for comprehensive studies on all available environmental data (with lots of variables) to determine causal relationships.

The main goal of this paper is to investigate quantitatively the links between natural spatial variations in environmental factors and: i) plankton biodiversity and community

structure, ii) climatically-active gases and, iii) biogeochemical variables, across the highly

dynamic environment of the northwest European Shelf waters. Trying to identify which

combination of environmental variables drives biogeochemical variables is difficult due to the

complexity of responses, interactions, and spatial variability. Here, we used a multivariate

statistical approach to address this problem and identify statistically the strongest

combinations of environmental variables associated with the response variables. In doing so,

we were able to determine which response variables were most influenced by changes in sea

surface carbonate chemistry gradients. As such, this study represents a large and

comprehensive addition to the literature on OA impacts on the surface ocean, as deduced from

observational data.

2. Methods

Samples were collected between June– July 2011 during the RRS *Discovery* research

cruise (D366) in northwest European Shelf waters (Fig. 1), as part of the UK ocean

acidification (UKOA) research programme. The data reported in this study came from

multiple measurements collected within the mixed layer. Several previous papers describing

results of experimental and observational work on this cruise have been published in a 2014

special issue (Tyrrell and Achterberg, 2014). Here we present an overarching statistical

analysis of the observational data. Across the cruise more than 20 environmental variables

(physical, nutrients and carbonate chemistry), >120 plankton species abundance

measurements and 22 other response variables (biogeochemical variables and climatically-

active gases) were collected at 64 stations (some of them were visited twice) (Fig. 1). All data

included in the paper are available from the British Oceanographic Data Centre (Ribas-Ribas

et al., 2014a).

2.1. Environmental data

Temperature and salinity were taken directly from the calibrated CTD data. Analyses of nitrate and nitrite (NO_x), phosphate (PO_4), and silicic acid ($Si(OH)_4$) were undertaken using a segmented flow auto-analyser (Skalar San+) following methods described by Kirkwood (1989). Excess nitrate relative to phosphate (N*) was calculated as NO_3 - (PO_4 x 16) and excess silicic acid relative to nitrate (Si*) was calculated as $Si(OH)_4$ - NO_x (Poulton et al., 2014). Analysis of high quality seawater carbonate chemistry measurements are detailed in Ribas-Ribas et al. (2014b).

Mixed layer depths (MLD) were calculated as the shallowest depth at which the density difference ($\Delta\sigma_t$) with the surface waters was more than $\Delta\sigma_t = 0.05$ kg m^{-3}. This threshold has been previously used on the eastern European continental shelf (Hickman et al., 2012). Daily incident irradiance (Ed^{0-}), in terms of photosynthetically active radiation (PAR), was integrated from dawn to dusk from the PAR sensor on the RSS *Discovery*. The euphotic zone depth (Zeup) was defined as the depth at which irradiance was 1% of near-surface values, with an optical depth of 4.6 (Kirk, 1994). K_d was calculated for pre-dawn casts using the same method as Charalampopoulou et al. (2011); regression analysis between turbidity (measured using a turbidity sensor on the CTD frame) and K_d ($r^2 = 0.493$; $p < 0.001$) values calculated for daytime stations were used to predict K_d for pre-dawn casts. The mixed layer average irradiance (Ed^{ML}) was calculated following Kirk (1994) using a combination of Ed^{0-}, K_d, and MLD as in Equation 1:

$$Ed^{ML} \text{ (mol PAR m}^{-2}\text{ d}^{-1}) = Ed^{0-} (1 - \exp^{-Kz}) / K_z \qquad\qquad \text{(Eq. 1)}$$

where $K_z = K_d \cdot$ MLD. The variable Ed^{ML} describes the mean light experienced by a particle being mixed from the surface to the base of the mixed layer. A two day average was used as

this accounts for incidental light and recent light history. This is especially important for morning measurements, where light data from the sampling day is most representative of the time after sampling.

In all, 19 environmental variables were measured at the same stations where the phytoplankton variables were measured (Table 1). Prior to statistical analysis, multicollinearity of the environmental variables within each environmental group (nutrients, carbonate chemistry, and hydrographical variables) and overall (all groups combined) were determined using Pearson's product moment correlations. For pairs of collinear variables ($\rho > 0.95$; e.g. dissolved inorganic carbon/total alkalinity (DIC/A_T)), we checked the analysis with both variables to examine if they both produced the same statistical outcome and then, if they did, one of the variables was removed from the subsequent analysis.

If the explanatory variables had a skewed distribution they were transformed appropriately (Quinn and Keough, 2002). All variables were normalized (subtracting the mean and dividing by the standard deviation for each variable) prior to being constructed into a resemblance matrix using Euclidean distance (Equation 2):

$$D = \sqrt{\Sigma_i(y_{i1} - y_{i2})^2} \qquad \text{(Eq. 2)}$$

where y_{i1} and y_{i2} result from the pre-treatment transformation and subsequent normalisation.

2.2. Phytoplankton data

Methods for coccolithophore counts are detailed in Poulton et al. (2014). After filtering and mounting as permanent slides, coccolithophore cell counts and species identification were carried out under cross-polarised light using a Leitz Ortholux microscope (X1000, oil immersion).

Microplankton community samples collected from the CTD casts were preserved in 2% acidic Lugol's iodine solution and stored in cool, low light conditions. The Utermöhl method for quantitative phytoplankton analysis (Edler and Elbrächter, 2010) was used for taxonomic identification and quantitative analysis of microplankton greater in cell size than 10 μm.

Spatial variation in the plankton community was assessed individually for each phytoplankton group (diatoms, dinoflagellates, and coccolithophores), as well as for all groups combined (with and, in some cases, without unidentified naked dinoflagellates). Abundance data for all groups were aggregated to the genus level, and screened to keep taxa that appeared in ≥1% of the samples; in each of the plankton groups, taxa were log transformed (log[X+1]) to down-weight the contributions of the dominant species prior to being constructed into a similarity matrix using the Bray-Curtis similarity coefficient (Equation 3):

$$S_{jk} = 100 \left\{ 1 - \frac{\sum_{i=1}^{p} y_{ij} - y_{ik}}{\sum_{i=1}^{p} (y_{ij} + y_{ik})} \right\} \tag{Eq. 3}$$

where y_{ij} represents the entry in the ith row and jth column of the data matrix for the ith species in the jth sample and y_{ik} is the count for the ith species in the kth sample.

To assess if the calcifying plankton group contributed less to the total phytoplankton community in regions of low pH/calcite saturation state ($\Omega_{calcite}$), the total phytoplankton abundance data was sub-divided into calcifying and non-calcifying species. Diversity indices (total number of individuals [N], total number of species [S], and species richness [H', the Shannon-Wiener Index]) of (i) calcifying and (ii) non-calcifying groups were determined, as well as the (iii) ratio of diversity indices between calcifying:non-calcifying groups. The Shannon-Wiener Index was determined by (Equation 4):

$$H' = \sum (P_i . \log(P_i)) \tag{Eq. 4}$$

where P_i is the proportion of the total count arising from the ith species.

2.3. Response variable data

Methods used for the analysis of DMS, DMSP, N_2O, methane (CH_4), POC, particulate organic nitrogen (PON), particulate organic phosphorus (POP), TEP, chlorophyll-a (Chl-a) biogenic silica (BSi), and dissolved oxygen (DO), can be found in Richier et al. (2014) and references therein (see Table 3 in Richier et al. (2014)). Herein, C:N, C:P, N:P are the ratios of POC:PON, POC:POP, and PON:POP, respectively.

2.4. Statistical analysis

Phytoplankton community assemblages sampled across the northwestern European Shelf were analysed using multivariate approaches with PRIMER-E v6 (6.1.13) and PERMANOVA + (1.0.3). Table 2 summarizes the statistical analyses used in this study.

2.4.1. Principal components analysis

A principal component analysis (PCA) was used to visually represent the variation across all the environmental forcing variables (physical, carbonate chemistry, and nutrients) and to assess if natural groupings found within the data were similar to those found in the phytoplankton community assemblage.

2.4.2. Cluster analysis

Natural groupings in the environmental data were differentiated using cluster analysis of the group average and statistically compared using similarity profile ('SIMPROF') analysis. Similarity percentages ('SIMPER') analysis were then used to determine which

variables are characteristic of each environmental cluster, variables which cumulatively contribute least to 50% of Euclidean distance (and are therefore most 'similar') between stations grouped in a cluster; as well as discriminating variables which contribute the most toward 50% of total Euclidean distance between pairs of clusters.

2.4.3. Principal co-ordination analysis

To visualise the natural groupings of the phytoplankton community, a principle coordination analysis (PCO) was employed. PCO is an unconstrained plot where samples are projected onto a best fitting plane, by which the axes aim to capture as much variability from the (Bray Curtis or Euclidian distance) resemblance matrix as possible.

2.4.4. Analysis of similarities

Analysis of similarities (ANOSIM) was carried out to determine whether the phytoplankton assemblages varied between *a priori* specified groups of stations that are characterized by differing environmental characteristics, determined independent of the phytoplankton analysis.

The global value of R varies between 1 and -1; values greater than 0 indicate greater variation between groups than within group, where 1 would indicate all stations within a group are more similar to each other than to any station from another group. Global R and associated significance values were used initially to determine whether significant differences were present ($p < 0.05$); pairwise comparisons were investigated further for combinations with lower p values (≤ 0.1) and higher R statistics (≥ 0.4) (Clark et al., 2014). Subsequent SIMPER analysis was then used to examine the discriminating species (those contributing to 50% of dissimilarities (Bray-Curtis) between clusters) in each pairwise comparison, which were then compared to the corresponding environmental variables.

2.4.5. Mantel Test

To assess if the spatial variations in plankton groups were related to carbonate chemistry gradients rather than other environmental factors, all three environmental forcing groups (nutrients, carbonate chemistry, and hydrographical variables) were separately, and in combination, correlated to the plankton groups using a non-parametric Mantel Test (RELATE analysis). The measure of agreement between the matrices was assessed with Spearman's rank correlation coefficient (rho) with permutations to test the null hypothesis of no agreement in the multivariate patterns between the two independently derived resemblance matrixes.

Significant correlations were analysed further using BEST analysis (BVstep, 999 permutations) to determine a subset of variables within the environmental group that best matched the biotic pattern in the phytoplankton group. BEST analysis performs a permutation-based multivariate analysis of biotic-abiotic relationships to link the patterns in the response variables to those in the environmental variables. The BEST analysis finds which subset of environmental variables produce the highest Spearman's rank correlation between a Bray-Curtis similarity matrix (responses) and Euclidean distance matrix (environmental). The permutation test indicates the probability of achieving this correlation by chance, therefore providing a significance test for the correlation.

To gauge if the community structure of the phytoplankton were related to carbonate chemistry gradients rather than other environmental factors, the analysis as described above was repeated but each CTD station was first standardised to the total number of individuals within each sample. A distance-based linear model (DISTLM) was used to model the relationship between the environmental forcing variable group (nutrients, carbonate chemistry, and hydrographical variables) and the phytoplankton abundance. Marginal tests were used to assess the (i) relative influence of each independent environmental variable, and (ii) each forcing variable group on the phytoplankton assemblage using R^2 selection criteria.

Sequential testing in a step-wise model was then used to assess the best subset of forcing variables (overall and within each environmental group) that can be used to model the variability within the phytoplankton community. The modified Akaike Information Criterion (AIC_C) selection criterion was used for the step-wise model due to its capability of handling a small number of samples relative to the number of predictor variables available. The distance based linear model was repeated for the three sub-groups within the phytoplankton: coccolithophores, diatoms, and dinoflagellates.

RELATE analysis was also used to correlate the multivariate response of the diversity indices across the three environmental variable groups. As the diversity indices were measured on different scales, they were normalised prior to being constructed into a resemblance matrix using Euclidean distance. Significant correlations were analysed further for individual diversity indices using BEST analysis to isolate a sub-set of carbonate variables that best matched the biotic data.

2.4.6. Multiple linear regression model (LM) and generalized linear model (GLM)

Correlations between the independent explanatory/response variables (Table 1) and the response variables (climatically-active gases and biogeochemical variables) were analysed across the northwestern European Shelf using either Pearson's correlation coefficient (ρ) or rho, dependant on the heterogeneity of the response variables. Significance ($p < 0.05$) of the correlations between the response variables and the independent explanatory variables was generated using permutations (9,999 permutations). The combination of forcing variables (carbonate chemistry, nutrient, and physical variables combined) that had the greatest influence on the response variables was assessed using step-wise multiple linear regressions. If the response variable maintained homogeneity of variance and a normal distribution, a multiple linear regression model (LM) was used. If the distribution of the response variable

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was skewed, a generalized linear model (GLM) was obtained. The significance of the coefficients within each of the models was analysed using either analysis of variance (for the LM) or the Wald Test (for the GLM), and significant differences from the null model were assessed using the F- statistic (for the LM) and the Chi-squared statistic (for the GLM). Prior to analysis, multicollinearity between the explanatory variables was assessed using Pearson's correlation. Variables with a correlation > 0.95 were removed. Redundant variables (those that maintained a high collinearity with another variable) were substituted into the regression analysis in a stepwise fashion to assess their overall influence on the significance. Model inference and goodness of fit was assessed with AICc, and either residual deviance of variance using R^2 (for LM) or log-likelihood ratios (for GLM). Model validation was assessed by comparing the residual variance/ deviance against the fitted values. Spatial autocorrelation was visually assessed through variograms and all residuals were compared to explanatory variables and assessed for patterns.

3. Results

3.1. Environmental forcing and response variables

PCA grouped most environmental variables into either physical (more related to PC2-Env) or carbonate chemistry and nutrients (PC1-Env). Response variables were mostly grouped into climatically active gases (PC1-Res) and biogeochemical variables (PC2-Res). The range of environmental variability encountered across the D366 cruise is detailed in Table 1 (see also Tyrrell and Achterberg (2014)). The PCA of all environmental variables combined captured 57.3% of the spatial variability across the first two principle components (Fig. 2A). The first principle component accounts for 33.8% of the variability within the data and correlated strongly (Pearson's correlation, $\rho > 0.8$ or $\rho < -0.8$) to the MLD, $Si(OH)_4$, fCO_2, and HCO_3^- and negatively with saturation states. The second principle component accounts

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for 23.5% of the variability within the data and correlated positively with salinity and A_T and negatively to temperature and $f\text{CO}_2$ (ρ <-0.77). The PCA of response variables combined captures 48.2% of the spatial variability across the first two principle components (Fig. 2B). The first principle component correlated strongly with POC and PON and the second principle component correlated positively with POP and negatively with the ratio of C:P.

Cluster and subsequent SIMPROF analysis of environmental variables highlighted 12 significantly different clusters at the 5% significance level (Fig. 3A). The clusters were then located geographically (Fig. 3B). This showed a large degree of heterogeneity in environmental variables; however, some of the environmental clusters were strongly geographically separate, allowing the identification of several important geographic regions: South of Cornwall (cluster 'l'), open Atlantic (cluster 'f' and 'g' (both together)), Skagerrak/Norwegian coast (cluster 'b'), or with coastal influence (cluster 'c' and 'e') which receive large freshwater inputs from the English, Belgian, Dutch, German, and Danish coasts.

3.1.1. Climatically-active gases

Analysis of correlations between the climatically-active gases and environmental forcing variables revealed that one or more carbonate chemistry variables significantly influenced DMS, DMSP, and N_2O concentrations, but had no influence on CH_4 (supplementary material, Fig. 1S). Multiple linear step-wise regression found temperature was the main forcing variable influencing the concentration of N_2O across the northwestern European Shelf (supplementary material, Table 1S). For this climatically-active gas, an increase in temperature results in a decline in its concentration. Methane concentration was most influenced by MLD and PO_4, with an increase in MLD associated with lower CH_4 concentration, whilst an increase in PO_4 increased CH_4 concentration. DMS and DMSP concentration were influenced by the carbonate chemistry gradients in concert with other environmental variables. Salinity, Zeup, and $f\text{CO}_2$ had the greatest influence on DMS

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concentration, with the increase in both salinity and $f\mathrm{CO}_2$ causing declines in DMS concentration, whilst Zeup increased concentration. Salinity, N*, NO_3, $\mathrm{Si(OH)}_4$, Zeup, and $f\mathrm{CO}_2$ had the greatest influence on DMSP concentration, explaining 80% of the deviance within the data. Increases in salinity, N*, and $\mathrm{Si(OH)}_4$ positively influenced the concentration of DMSP, whilst $f\mathrm{CO}_2$, NO_3, and Zeup had a negative association.

3.1.2. Biogeochemical variables

Correlations between environmental forcing variables and biogeochemical variables revealed that the carbonate chemistry gradients were only related to the elemental stoichiometry (C:N and C:P) (supplementary material, Fig. 2S). Multiple linear step-wise regressions found that the variables within the physical and nutrient forcing groups had a greater influence on the biogeochemical variables than did the carbonate chemistry variables (supplementary material, Table 1S). N* and $\mathrm{Ed}^{\mathrm{ML}}$ had the greatest influence on POC, with both variables increasing its concentration. Zeup, MLD, seafloor depth, salinity, and Si* had the greatest influence on PON, while MLD and HCO_3^- had the greatest influence on POP. Elemental stoichiometry of C:N was influenced by the combination of temperature, HCO_3^-, and $f\mathrm{CO}_2$. Increased temperature and HCO_3^- concentration increased the C:N elemental stoichiometry, whilst increases in $f\mathrm{CO}_2$ decreased the ratio. C:P stoichiometry was influenced by DIC and Si*, with both variables declining the ratio with their increased concentration, and the N:P ratio was most influenced by temperature, N*, and $\mathrm{Si(OH)}_4$.

POP, C:P, and C:N were significantly influenced by HCO_3^-, DIC, and $f\mathrm{CO}_2$. It was found that POC was strongly correlated with DIC. All biogeochemical variables except C:P were significantly influenced by hydrographic variables (mainly temperature, MLD, and salinity). Nutrients were significantly correlated to all biogeochemical variables except POP and C:N. In our study with natural variation, C:P and C:N are negatively correlated with carbonate chemistry whereas N:P showed no influence of any carbonate chemistry variable.

Independently, one or more of the carbonate chemistry variables influenced the concentrations of TEP, chlorophyll-*a*, and dissolved oxygen (DO) (supplementary material, Fig. 3S). Multiple linear step-wise regressions found the combination of seafloor depth, salinity, N* and DIC had the greatest effect on TEP concentration, with DIC having a negative association with TEP concentration (supplementary material, Table 1S). The combination of Ed^{ML}, temperature, salinity and fCO_2 had the greatest influence on the concentration of DO. An increase in fCO_2, temperature and salinity concentrations resulted in a decrease in concentration of DO, whilst Ed^{ML} had a positive influence on its concentration. Temperature, Zeup, NO_3, DIC, A_T and fCO_2 had the greatest influence on chlorophyll-*a*, all together accounting for 68% of the deviance within the data. For biogenic silica, Zeup, seafloor depth, temperature and $Si(OH)_4$ had the greatest influence, together explaining 50% of the deviance within the data.

Saturation states of aragonite ($\Omega_{aragonite}$) and calcite ($\Omega_{calcite}$) are statistically different between regions (Fig. 4A). For example, the Irish Sea (clusters 'a' and 'i' in Fig. 3) had the lowest values and was statistically different from all of the other regions. The Skagerrak also had low $\Omega_{calcite}$ and $\Omega_{aragonite}$ values and was statistically different from all the other regions. Generally, the Atlantic sector was similar to the Celtic Sea. In the Bay of Biscay we found the highest values, statistically separate from the entire study region. The English Channel and Norwegian coast were not statistically distinct from one another. However, the phytoplankton community structure was not influenced by regions of low pH/ $\Omega_{calcite}$. The multivariate response of the species diversity indices (S, N, and H') in calcifying groups, non-calcifying groups, and the ratio between calcifying: non-calcifying groups was not found to correlate to the carbonate chemistry gradients in the northwest European shelves (Fig. 4B).

3.2. Phytoplankton community

The phytoplankton community composition, either as a whole or segregated into separate taxonomic groups, did not correlate to the spatial patterns in carbonate chemistry gradients. However, the multivariate pattern of the phytoplankton community assemblage did correlate significantly to the variation in nutrient pools (rho = 0.237, p = 0.021), and BEST analysis found that PO_4, N*, and Si* had the greatest correlation to the phytoplankton community structure (rho = 0.268, p = 0.039). Only the diatom community composition was found to significantly correlate to the nutrient environmental forcing variables (rho = 0.304, p = 0.001), which BEST analysis matched to the concentrations of NO_3 and $Si(OH)_4$ (rho = 0.333, p = 0.001). Combining all environmental forcing variables together, H^+, $Si(OH)_4$, and NO_3, were found to best explain the patterns in the multivariate diatom abundance data (rho = 0.341, p = 0.005).

ANOSIM revealed no significant difference in the phytoplankton community composition (R = 0.13, p = 0.085) between natural environmental clusters (see section 3.1, Fig. 3A). However, following the removal of unidentified naked dinoflagellates, ANOSIM did reveal some significant differences in the phytoplankton community composition (R = 0.211, p = 0.014) between natural environmental clusters (Fig. 3A), driven by variation in a number of species including coccolithophores (*E. huxleyi*), dinoflagellates (*Gymnodinium* spp. and *Noctiluca* spp.), and diatoms (*Rhizosolenia* spp., *Dactyliosolen* spp., and *Guinardia* spp.). Interestingly, ANOSIM revealed no significant difference in the phytoplankton group totals (R = 0.109, p = 0.113) between natural environmental clusters.

There were 9 natural groups found in the community structure of the phytoplankton data (graph not shown). These 9 groups were predominantly split on the basis of unidentified naked dinoflagellate community abundance. However, this group skewed the data due to it representing a large taxonomic clade, while all other groups were individual species aggregated to genus. Therefore additional analyses were made with naked dinoflagellates

removed. Their removal resulted in 2 distinct groupings within the phytoplankton community (Fig. 5A), which were distinguished by the dominance of the dinoflagellate *Gymnodinium* spp. in Group A (contributing to 78% similarity between samples within this group) and the coccolithophore *E. huxleyi* in Group B (contributing to 61% similarity between samples within this group).

Coccolithophores formed 7 natural groupings within the community (Fig. 5B). The dominance of *E. huxleyi* caused the greatest differences between the groups, with *E. huxleyi* dominating 100% of the samples in group F and none of the samples in group A. *Gephyrocapsa muellerae* and *Syracosphaera marginaporata* were also discriminating species. *E. huxleyi* was an important discriminating species between many clusters, largely explained by high dominance in clusters 'b', 'j', and 'l' and comparatively lower dominance in cluster 'g' (Fig. 3A). There is slight evidence of an association between increased *E. huxleyi* dominance and some carbonate chemistry variables, with decreased A_T and CO_3^{2-} being discriminating variables in 40% and 20% of the pairwise comparisons, respectively. Physical parameter groupings were more strongly linked to *E. huxleyi* dominance, with shallower MLD and decreased salinity both being discriminating variables in 60% of comparisons each, associated with increased dominance. Increased *E. huxleyi* dominance was also linked to increased temperature and seafloor depth in a single comparison (20%) each. Increased *E. huxleyi* dominance also showed relatively clear links to nutrients, with low phosphate also being a discriminating variable in 60 % of pairwise comparisons, and lower nitrate in an additional comparison. Additionally, when analysing coccolithophores independently, *E. huxleyi* dominance was more closely linked to shallow MLD, high irradiance, and lower salinity (see also Poulton et al. (2014)).

Excluding the unidentified naked dinoflagellates, the dinoflagellate group formed 5 natural groupings within the community structure (Fig. 5C). Group A and B was dominated

by *Gymnodinium* spp. (contributing to 78% similarity between samples within this group) and *Noctiluca* spp. (contributing to 72% similarity between samples within this group), respectively. A combination of *Ceratium* spp., *Scrippsiella* spp., *Katodinium* spp., and *Gymnodinium* spp. dominated the community structure of dinoflagellate group E (contributing to 73% similarity between samples within this group), whilst group C and D only had one CTD sample within their cluster. ANOSIM revealed no significant difference in the dinoflagellate community composition (R = 0.012, p = 0.415) between natural environmental clusters determined in the cluster analysis (Fig. 3A). However, following removal of the unidentified naked dinoflagellates, there is evidence of a relationship between the dinoflagellate community composition and environmental variables: ANOSIM revealed some significant differences in the dinoflagellate (excluding unidentified naked dinoflagellates) community composition (R = 0.188, p = 0.02) between natural environmental clusters (Fig. 3A).

In both the ANOSIM and conceptual model analyses, dinoflagellate assemblage composition was most closely linked to carbonate chemistry and the physical variables. These explained 20 and 21%, respectively, of variability in the dinoflagellate assemblage composition, with DIC/HCO_3^- being the best overall subset of variables involved.

Diatoms formed 4 natural groupings within the community structure data (Fig. 5D). Group A had only one sample, which was segregated from the other significant clusters due to the dominance of *Corethron* spp. within this CTD sample. For instance, *Corethron* had high dominance (42 %) in cluster 'h' but was very low/absent in 'b', 'j', and 'l'. Groups B, C, and D were dominated by *Rhizosolenia* spp., *Guinardia* spp., and *Coscinodiscus* spp., respectively. When analysing diatoms independently, ANOSIM revealed significant differences in the diatom community composition (R = 0.226, p = 0.009) between natural environmental clusters (Section 3.1, Fig. 3A). We identify *Rhizosolenia* spp. as a key diatom

species, responsible for separating several different clusters either due to its high dominance (~65 %) in cluster 'b' or its low dominance (<5%) in 'h', 'c', and 'g' (Fig. 3A). The species that contribute to group separation were further investigated in section 4.2 to help identify which environmental parameters were behind these distributions.

We summarize how the variance in phytoplankton community composition in the northwest European Shelf in summer 2011 related to forcing factors in Fig. 6. All environmental forcing variables combined accounted for 59% of the variation within the phytoplankton community (Fig. 6A). The best overall subset of variables to predict the variation in the phytoplankton were $Si(OH)_4$ and N^*, which together account for 15% of the variation. The nutrient group explained the greatest proportion of variance within the phytoplankton abundance when the environmental variables were subdivided into groups. The combination of all environmental forcing variables accounted for 41, 62, and 58% of the variability within the separate plankton groups for coccolithophore, diatom, and dinoflagellate community structure, respectively (Fig. 6B-D). The best subset of variables to describe the variation in the diatom community assemblage were NO_3, $Si(OH)_4$, and CO_3^{2-}, which together accounted for 24% of the variation (Fig. 6D).

4. Discussion

We conducted a very large measurement campaign across a heterogeneous region within which environmental conditions varied considerably. We then undertook a rigorous statistical analysis of the data. Looking over the entirety of our results, it is apparent that while many potential response variables were apparently unrelated to carbonate chemistry, others were related to carbonate chemistry. For those response variables that correlated with carbonate chemistry, OA may well affect them. Where no correlations were found, it does not necessarily mean that those response variables will not be affected at all by OA. Other

possible causes of a lack of correlation include: i) OA impacts exist but are too small to have been detected by our approach, ii) OA will impact the variables, but not within the range of carbonate chemistry encountered during our cruise, iii) OA will impact these response variables, but not under the specific environmental conditions encountered during our cruise (for instance only at higher temperatures than are found around the British Isles), and iv) OA will impact these response variables, but only when a different set of biological species are present (for instance only when the phytoplankton assemblage is dominated by picophytoplankton, as did not on our cruise). The evidence we compiled and analysed does not support a strong impact of OA on most variables but conversely does not rule it out.

We did not find any saturation state values as low (0.8) as in the US Pacific Northwest coast (Feely et al., 2008), where the oyster hatcheries are reported to be failing (Cooley and Doney, 2009; Dickson, 2010). However, the carbonate chemistry of the surface ocean is rapidly changing with OA. When interpreting the results of our analyses, it is important to be aware of the range of carbonate chemistry variation we measured on our cruise. For instance, during our cruise we sampled surface waters that varied in fCO_2 between 264 and 411 µatm, in pH between 8.03 and 8.20, and in aragonite saturation state between 2.1 and 3.0 (Ribas-Ribas et al., 2014b). The northwest European Shelf seas in summer could become undersaturated in aragonite (<1) with a fCO_2 in equilibrium with an atmosphere of 1150 µatm (considering alkalinity constant and equal to an absorption of anthropogenic CO_2 of 180 µmol kg^{-1}). In winter, taking into account the lowest temperature reported by Bozec et al. (2006) of 5.1°C the under-saturation would be reached at 750 µatm, which is less than double of actual levels. In terms of the general progression of OA, it will take 84 ± 24 years for the fCO_2 of surface seawater to increase by 147 µatm (the range of variation on our cruise), according to the average rate of increase measured at the major time-series sites in the global ocean (Bates et al., 2014). Likewise, the ranges of variability we encountered in pH and $\Omega_{aragonite}$

correspond to 98 ± 25 and 106 ± 19 years respectively of OA impacts at current rates according to the time-series measurements (Bates et al., 2014). These considerations lead to some limitations in the likely applicability of our results, if they turn out not to apply outside the range of environmental variability from which they were derived. For instance, for the study region around the British Isles, if OA continues at current rates then by the year 2142 (125 years from now) the carbonate chemistry of the whole region will no longer overlap at all with the range of environmental variation that we experienced and the inferences from our analyses might then no longer apply.

We demonstrate that observational data can inform us on ocean change impacts and complements experimental research. It is vital to systematically link responses seen in laboratory studies to changes seen in natural populations so that we can detect and understand changes to marine phytoplankton in changing oceans (Collins et al., 2014). Experimental research has certain shortcomings: i) a general inability to address the possibility of adaptation to the imposed change (Sunday et al., 2014); ii) a tendency to simplify both the abiotic and biotic features of the natural environment (Collins et al., 2014), for example, many laboratory studies change only a single environmental factor (usually $f\mathrm{CO}_2$), whereas numerous simultaneous environmental changes occur naturally; iii) ignorance of the recent life history of phytoplankton population, which will affect the strength and effectiveness of natural selection (Collins et al., 2014). For example, species in environments historically exposed to unpredictable changes of $f\mathrm{CO}_2$ might have greater adaptability than those in a more predictable $f\mathrm{CO}_2$ regime (Sunday et al., 2014).

4.1. Climatically-active gases and biogeochemical variables

CH_4 and $\mathrm{N}_2\mathrm{O}$ were not found to be significantly correlated with carbonate chemistry, whereas DMS and DMSP were statistically influenced by $f\mathrm{CO}_2$. A consistent increase in DMS

and decrease in DMSP in response to high CO_2 was observed in mesocosm studies during the same cruise (Hopkins and Archer, 2014). However, other studies have observed a decrease in DMS under high CO_2 concentrations (Hopkins et al., 2010) or no effect on concentration of DMSP in elevated CO_2 (Lee et al., 2009). We observed in these variables a strong agreement between observational data and results of bioassay experiments conducted on the same cruise, but more studies are needed to fully elucidate the response of DMS/DMSP to increased CO_2. Hydrographic variables and nutrients had significant effects on all the climatically-active gases studied. Clark et al. (2014) found a loose association between N_2O and temperature at the stations where they measured N_2O production rates. In contrast, we found a strong statistical association; maybe because of the higher range of variability and greater number of stations (all CTD stations) included in our analysis. This association between higher N_2O concentration and lower temperature is most likely caused by increased gas solubility (Clark et al., 2014).

DIC and $f\text{CO}_2$ explained a significant amount of variation in TEP, chlorophyll-*a*, and dissolved oxygen (DO), but not in biogenic silica. Hydrography and nutrients are important variables in explaining the biogeochemical variables. Several studies have examined the relationship between TEP and $f\text{CO}_2$ in experimental manipulation studies (Engel, 2002; MacGilchrist et al., 2014) or model studies (Schneider et al., 2004); all of them concluded that rising CO_2 will enhance TEP concentration and therefore that TEP would be expected to positively correlate with DIC. Our findings contradict those of manipulation studies, with TEP negatively correlated with DIC (p-value < 0.001). How the experiments were acidified may have influenced their results, because A_T and not DIC or pH are responsible for the changes (Passow, 2012). However, multiple linear regression in natural conditions highlight DIC and not A_T as a main factor of change (supplementary material). Notably, Richier et al. (2014) also found that net production of POC was strongly correlated with net changes in DIC.

Dissolved oxygen appeared to be influenced by carbonate chemistry when in conjunction with other forcing variables; DO was seen to have lower values under elevated fCO$_2$. Dissolved oxygen and chlorophyll-*a* are the linking point of our two separate analyses of phytoplankton and response variables, reflecting the complexity, variability, and co-variation of the system. Thus, for example, our observations related DO with temperature, light, and fCO$_2$ but clearly temperature affects DO concentration via solubility. Equally, chlorophyll-*a* is also influenced by light availability, while the light environment will also be affected by chlorophyll-*a* in terms of its attenuation in the water column.

4.2. Phytoplankton community

In combining all parts of the analysis, it is apparent that the spatial variation in total phytoplankton community was most closely linked to the variation in physical and nutrient variables, rather than carbonate chemistry during summer 2011.

Throughout most of the region, *E. huxleyi* characterized the phytoplankton community, with only a few stations, including a number of those in the Celtic Sea, lacking this species (see also Poulton et al. (2014)). Both in relation to the phytoplankton community as a whole and within the coccolithophore community, the relative dominance of *E. huxleyi* was largely linked to physical variables; MLD, Ed^{ML}, salinity, and Zeup, as well as the nutrient variables; particularly PO$_4$ concentrations and the relative availability of nitrate to phosphate (N*). These patterns are in general agreement with much of the literature: coccolithophore blooms are often promoted by the onset of surface stratification, associated with high irradiance and temperatures (Tyrrell and Merico, 2004). Ed^{ML} and pH explained much of the variation in coccolithophore distribution and community composition in a study from the North Sea to the Norwegian Sea (Charalampopoulou et al., 2011). We hypothesise that this is because *E. huxleyi* is more tolerant of high light irradiances compared to other

species; however, there is scant laboratory data to fully explore this. Adaptations to high light irradiances are thought to be an important factor supporting coccolithophore dominance in highly stratified surface waters during summer (Nanninga and Tyrrell, 1996). Studies on *E. huxleyi* have indicated that light saturation occurs at much higher irradiances, and that there has been no evidence of photo-inhibition, even at very high irradiances which have inhibitory effects on other phytoplankton groups (Rost and Riebesell, 2004).

While this was clear when analysing coccolithophores independently, links between irradiance and *E. huxleyi* dominance within the total phytoplankton community were less clear. In one of the comparisons, increased dominance is seen in the Skagerrak (cluster 'b') associated with lower Ed^{ML} and phosphate, and higher temperature and seafloor depth. However, we note that the Skagerrak and Norwegian coast also had a shallow MLD and that incident irradiance here was particularly low. This observation could have been due to the persistence of *E. huxleyi* cells grown prior to measurements, when incident irradiance and consequently Ed^{ML} may have been greater.

Young et al. (2014) examined coccoliths from the same cruise on the northeast European Shelf and concluded that the main differences between coccolithophore assemblages were between oceanic and neritic communities, i.e. whether the environmental location was on the shelf or in deep water. This was not entirely reflected in our analysis of the coccolithophore community, with increased seafloor depth also linked to higher *E. huxleyi* dominance. However this observation was largely due to the very high dominance in the Skagerrak region, which featured an extremely shallow and warm surface mixed layer, yet deep seafloor depth. Other regions with notably high *E. huxleyi* dominance were typically shelf waters while a more mixed assemblage was found offshore (Poulton et al., 2014). Semi-enclosed shelf waters typically had greater riverine input leading to lower salinities, which would also strengthen the stratification of the water column, as seen in the Skagerrak.

Some of the dinoflagellates in the acidic Lugol's samples were likely to be heterotrophic, especially the small (<20 μm) unidentified ones. The photoautotrophic dinoflagellates require CO_2 for carbon fixation. However, the concentration of dissolved CO_2 in seawater is relatively low and hence autotrophs require it to be concentrated within the cell via carbon concentrating mechanisms (CCMs). These CCMs vary widely among taxa, with a range of mechanisms and efficiencies (Giordano et al., 2005). Consequently, increases in fCO$_2$ may be beneficial to some phytoplankton, though the extent of this will differ according to methods of carbon acquisition (Riebesell and Tortell, 2011). This could provide some explanation for the observed links between carbonate chemistry and the species composition of both diatom and dinoflagellate assemblages. Most phytoplankton have highly efficient CCMs and are typically not CO_2 limited at current fCO$_2$ (Doney et al., 2009), but those with less efficient CCMs, or that rely on passive diffusion of CO_2, are likely to benefit from increases in CO_2. Alternatively, elevated CO_2 concentration could reduce the energy required for CCMs, which in turn, could be allocated to other cellular processes (Riebesell and Tortell, 2011). Additionally, phytoplankton regulate cellular pH levels, and the ability to cope with increased concentration of H^+ has generally been shown to scale with size; large (>50 μm) celled phytoplankton experience greater natural variation in H^+ concentration and are consequently better adapted to this (Richier et al., 2014). Different sizes of phytoplankton could explain some species specific responses: small phytoplankton (<50 μm) are more greatly affected by proton concentration fluctuations due to bigger surface area to volume ratios than larger phytoplankton (Richier et al., 2014).

The typical temporal patterns in diatom community structure in the North Atlantic Ocean and northwest European Shelf seas have been well documented (Lange et al., 1992; Leterme et al., 2005); strong and clear links can be seen between nutrients and physical structure of the water column and diatom dominance in features such as the spring bloom. It could be

expected that similar links would be observed spatially. Dissolved nutrient concentrations, particularly silicic acid, and their relative proportions (Si*) were found to be important variables to explain variation in the total phytoplankton, both in the BEST analysis and in the conceptual model. This could be indicative of the influence of diatoms on the total community; these are the only group which have a requirement for silicic acid, and where present diatoms can be strong competitors for nutrients (Egge and Aksnes, 1992). Though no single diatoms species was a major feature (such as *E. huxleyi* or *Gymnodinium* spp.), as a whole diatoms were a modest proportion of the community and could potentially influence overall community dynamics.

Physical and nutrient variables also had a strong influence on the species structure of the diatom assemblage. Diatom size, nutrient requirement, and nutrient acquisition vary widely amongst different species. High dominance was largely associated with more acidic conditions and a shallower, warmer, and nutrient depleted mixed layer. *Rhizosolenia* spp. regularly occur during the later stages of diatom blooms and are able to persist well into summer stratified and nutrient depleted conditions (Villareal, 1987). Meanwhile some species had greater dominance in conditions more typically associated with diatom blooms, such as a deeper, cooler, and more nutrient rich mixed layer.

Interestingly, each of the analyses pointed to some link between diatoms and carbonate chemistry and the ANOSIM analysis indicated potential species-specific responses to carbonate chemistry conditions. *Rhizosolenia* spp. were seen to cope well with more acidic conditions, which reflects some previous studies (Sommer et al., 2015). In contrast, *Corethron* spp. were typically found in less acidic environments. This could reflect the past productivity of the environment: low pH will indicate bloom and high abundances in some areas whereas high pH will be indicative of post-bloom and low abundances in other areas.

5. Conclusions

We used observational data from a cruise to investigate controls on the spatial patterns of phytoplankton species and other response variables. When considering all of our results together, the spatial variations in total phytoplankton assemblage were found to be more strongly associated with variations in nutrient and/or physical variables than with carbonate chemistry gradients. The plankton community abundance, contribution of calcifying organisms, and composition did not change greatly between higher $\Omega_{calcite}$ and lower $\Omega_{calcite}$ conditions. However, carbonate chemistry, alongside physical and nutrient variables, is calculated to play a more prominent role in determining dinoflagellate and diatom assemblage compositions.

Climatically-active gases and biogeochemical variables showed some influence of carbonate chemistry variables; however nutrients and hydrography appear to be more important in explaining their spatial variations.

We report results for boreal summer when phytoplankton communities were dominated by dinoflagellates (Poulton et al., 2014), with the coccolithophore community dominated by *E. huxleyi*, whilst diatoms were in low abundances. Future studies should focus on seasonal variability when the phytoplankton community changes and temperature and other environmental variables will play an important role in producing variations in saturation states. Although we did not observe saturation states low enough that they could affect calcifying organism, caution should be taken as winter studies or regions affected by upwelling could experience much lower saturation states.

At this stage of OA research, it is essential to carry out micro/mesocosm studies alongside observational studies and to use multivariate statistics to understand the complex interactions within the data.

Acknowledgements

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| Groups	Environmental variables	Units	Minimum	Maximum	Average	±SD	
	Sea floor depth (SFD)	m	38.5	4758.4	417.0	±	1093.7
	Temperature (Temp)	° C	10.3	16.2	13.6	±	1.2
Physical	Salinity (Sal)	dimensionless	28.7	35.8	34.5	±	1.6
	Mixed layer depth (MLD)	m	2.0	131.0	27.1	±	24.0
	Euphotic zone depth (Zeup)	m	15.5	58.0	27.3	±	8.1
	Mixed-layer average irradiance (Ed^{ML})	mol PAR m^{-2} d^{-1}	1.7	31.0	11.8	±	7.2
	Dissolved inorganic carbon (DIC)	$\mu mol\ kg^{-1}$	1920.8	2133.6	2079.1	±	39.7
	Total Alkalinity (A_T)	$\mu mol\ kg^{-1}$	2121.9	2352.0	2312.8	±	47.8
Carbonate chemistry	[†]Fugacity of carbon dioxide (fCO_2)	μatm	274.0	395.7	347.9	±	27.0
	[†]Bicarbonate (HCO_3^-)	$\mu mol\ kg^{-1}$	1760.8	1963.6	1897.9	±	38.4
	[†]Carbonate (CO_3^{2-})	$\mu mol\ kg^{-1}$	136.7	191.8	167.6	±	12.4
	[†]Calcite saturation state ($\Omega_{calcite}$)	dimensionless	3.3	4.5	4.0	±	0.3
	[†]Aragonite saturation state ($\Omega_{aragonite}$)	dimensionless	2.1	2.9	2.6	±	0.2
	[†]H^+	$\mu mol\ kg^{-1}$	6.5092E-09	8.8975E-09	8E-09	±	5E-10
	Nitrate and Nitrite (NO_3)	$\mu mol\ L^{-1}$	0.10	4.61	0.76	±	1.11
	Silicic acid ($Si(OH)_4$)	$\mu mol\ L^{-1}$	0.10	3.63	1.02	±	0.90
Nutrients	Phosphate (PO_4)	$\mu mol\ L^{-1}$	0.02	0.38	0.07	±	0.07
	Si*	dimensionless	-1.67	2.25	0.26	±	0.83
	N*	dimensionless	-2.84	3.67	-0.32	±	1.00

Table 1. The range of environmental variables over the NW European shelf cruise, categorised into forcing variable groups. [†] denotes calculated from dissolved inorganic carbon and total alkalinity.

| Statistical test/methods	Abbreviation
Principal components analysis	PCA
Cluster + Similarity Percentages	Cluster +SIMPER
Principal co-ordination analysis	PCO
Analysis of similarities	ANOSIM
Mantel Test	RELATE analysis
Distance-based linear model	DISTLM
Multiple linear regression model	LM
Generalized linear model	GLM

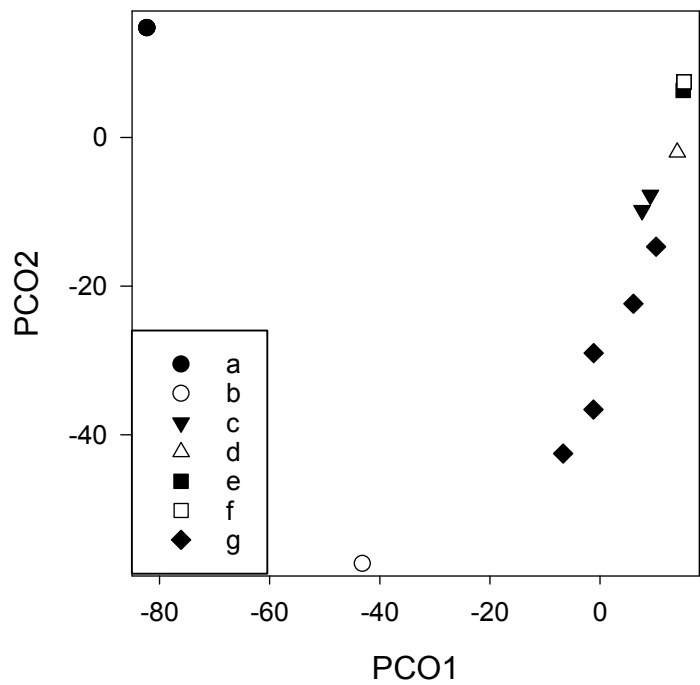Table 2. List of statistical test and methods used as outlined in detail in Section 2.4

D366 Stations
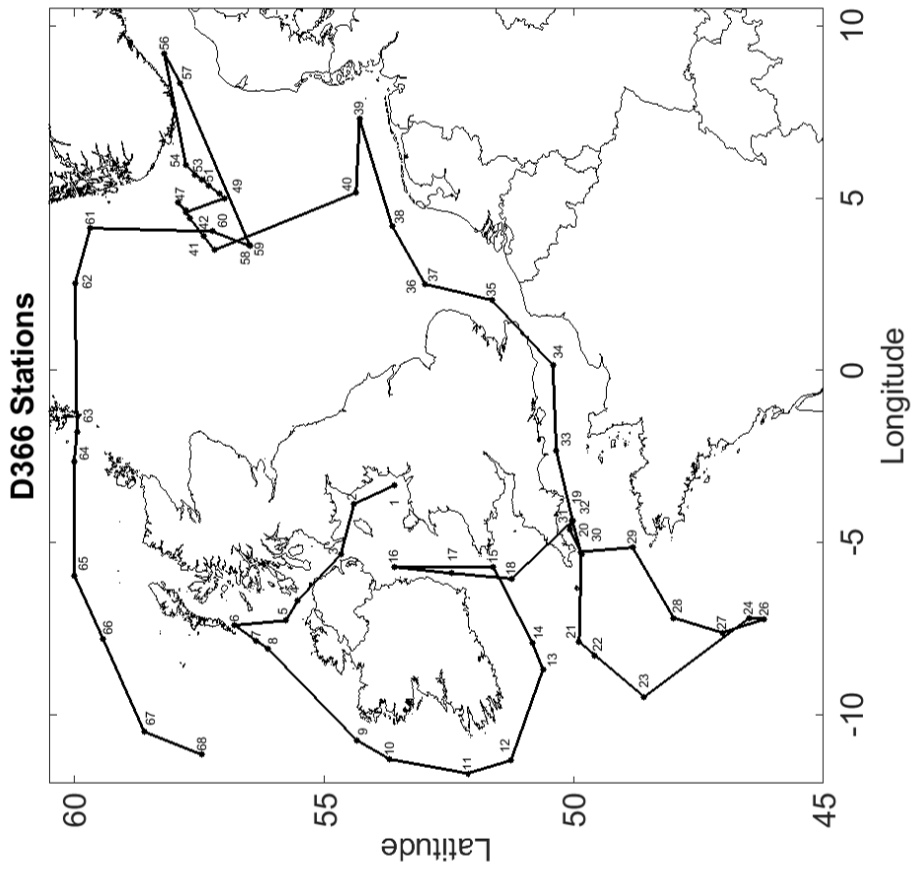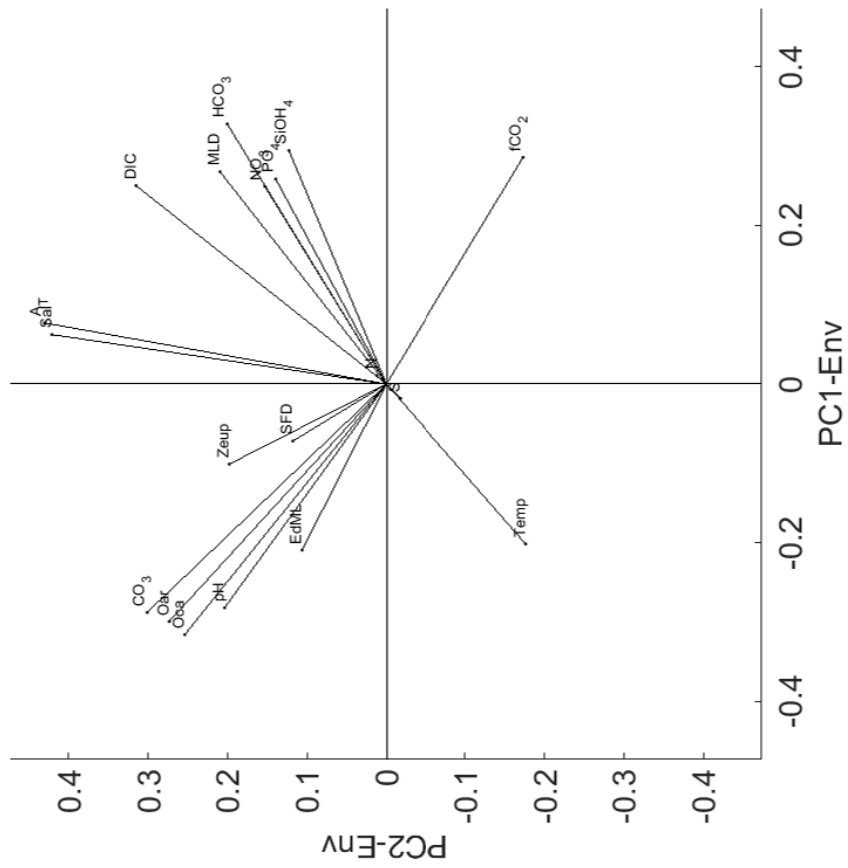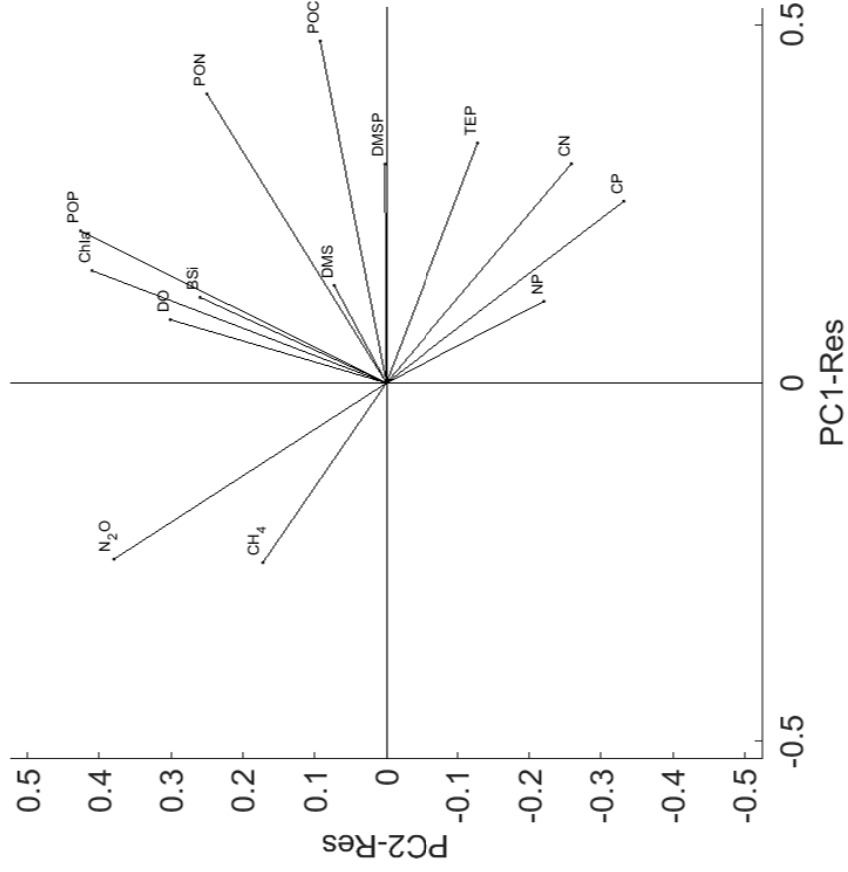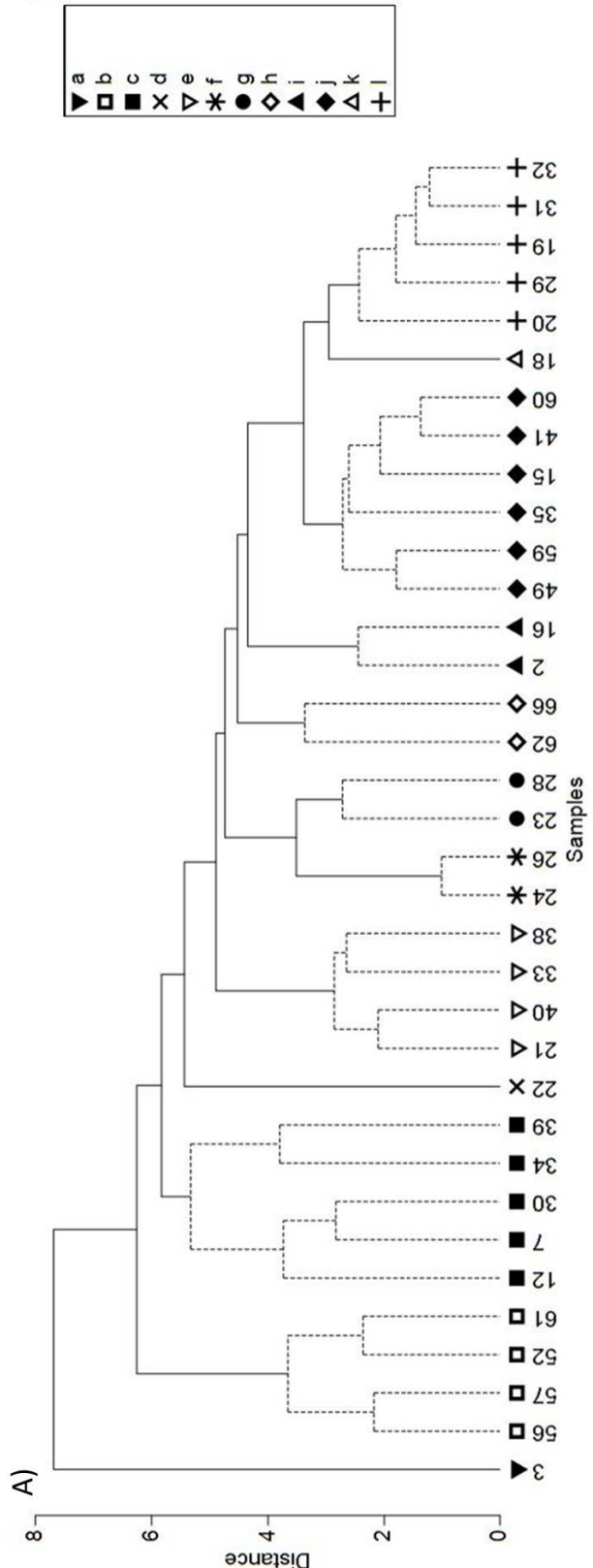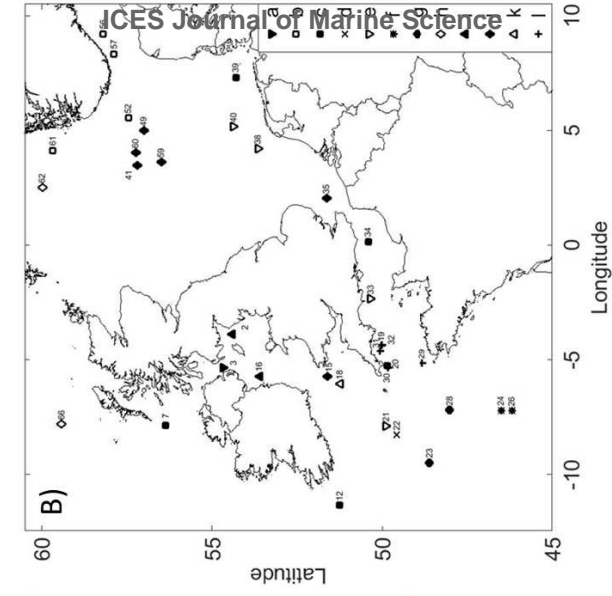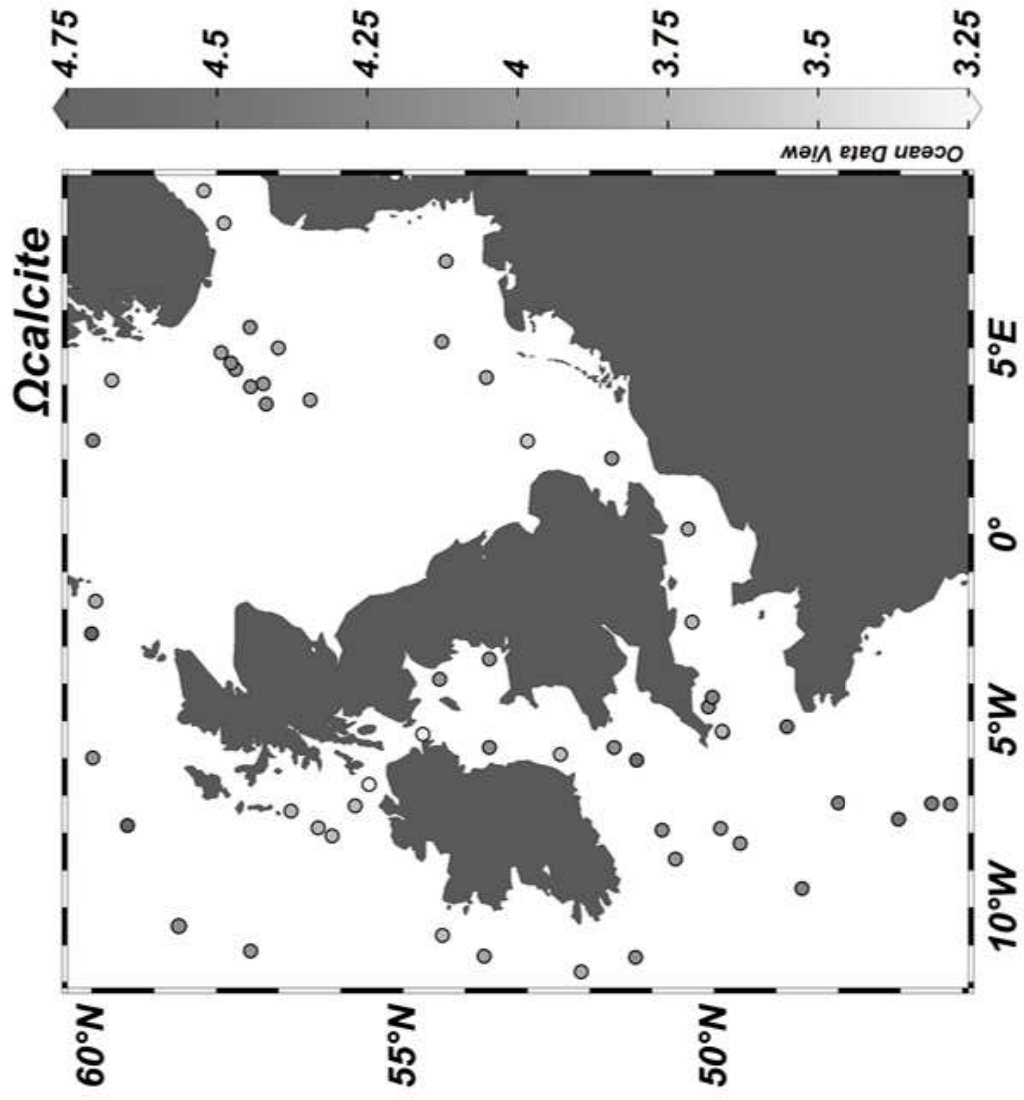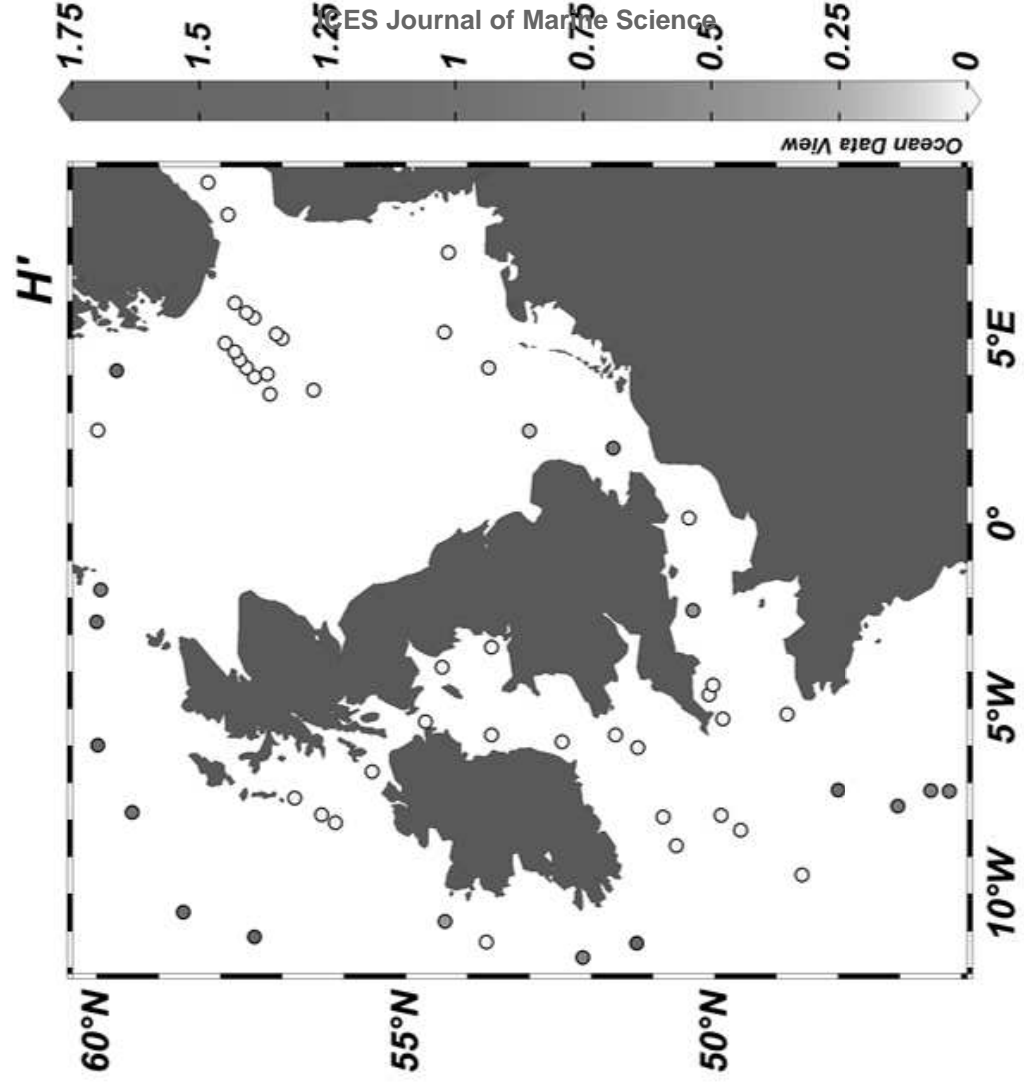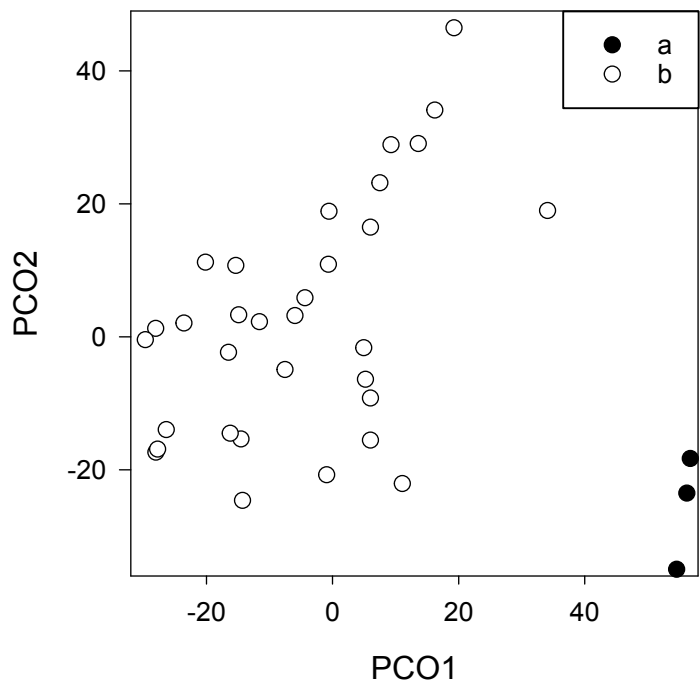
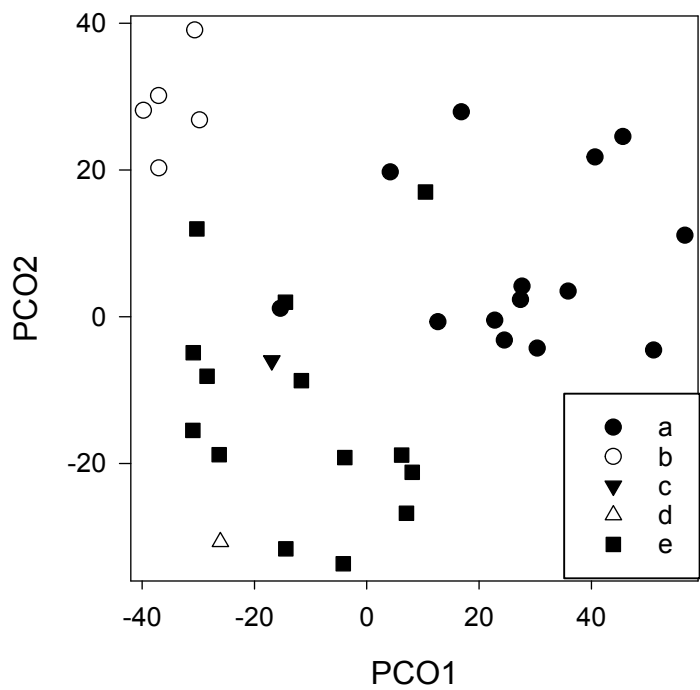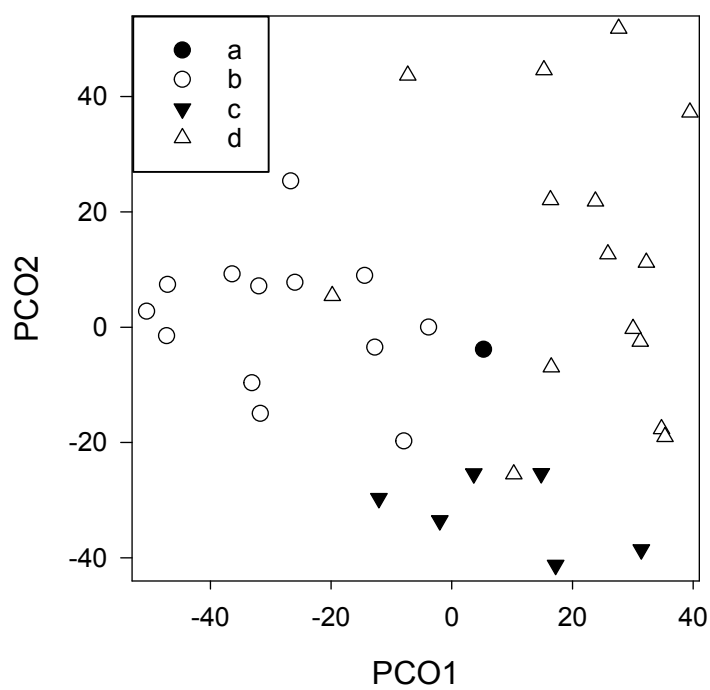A) Total phytoplankton community
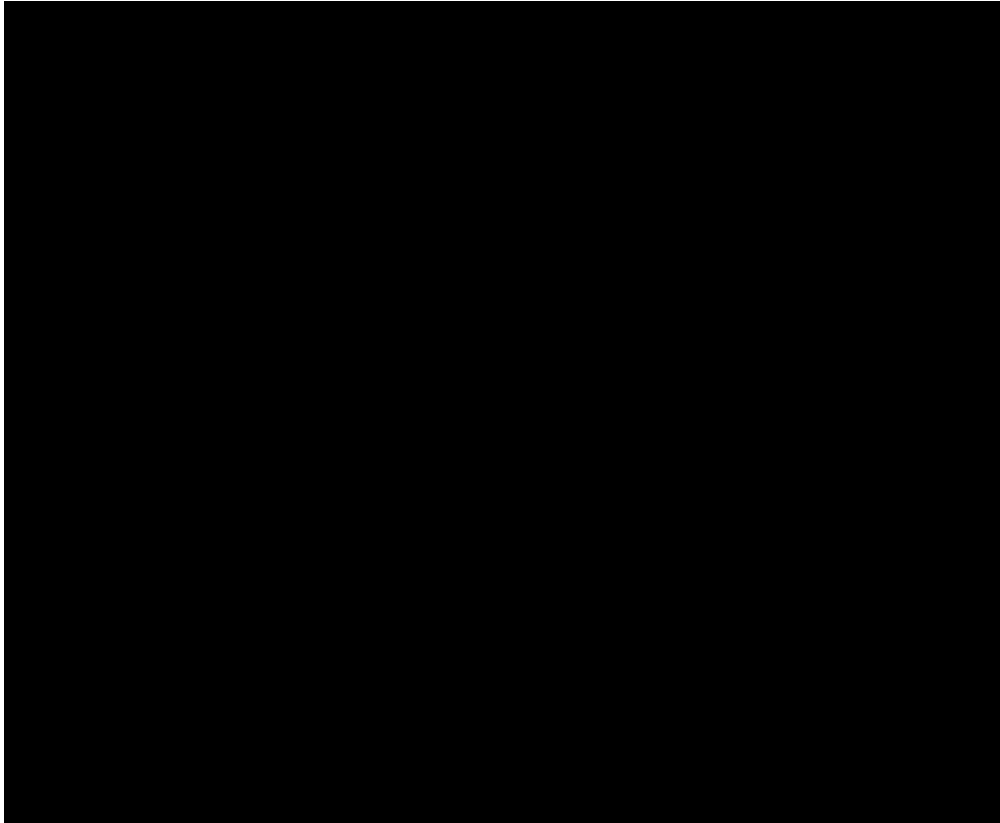B) Coccolithophores
C) Dinoflagellates
D) Diatoms

Figure 6. Schematic summarizing how the variance in phytoplankton community composition on the north-west European shelf is open between different forcing factors (hydrography, nutrients, carbonate chemistry): (A) total phytoplankton (without unidentified naked dinoflagellates), (B) coccolithophores, (C) dinoflagellates (without unidentified naked dinoflagellates) and (D) diatom community structure across the NW European shelf. Those environmental variables that are underlined within each group denote the subset of variables within each forcing group that explain the most variance of the community structure. Bold variables denote the best overall subset of variables across the 3 groups to explain the variance within the community structure.

400x329mm (150 x 150 DPI)