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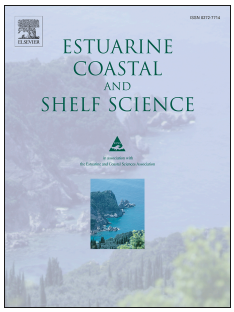

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Seasonal variability of the carbonate system and coccolithophore *Emiliania huxleyi* at a Scottish Coastal Observatory monitoring site

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Abstract

Lack of information about carbonate chemistry in inshore waters is a 'knowledge gap' in assessing the impacts of changing carbonate chemistry on the marine environment. Assessing the response of calcifying phytoplankton to this changing carbonate chemistry requires a greater understanding of temporal variation. This study provides a description of the variability of carbonate parameters at a monitoring site in the eastern coast of Scotland. Four-years of monthly data were analysed to assess the diversity, abundance and morphometrics of coccolithophores in relation to carbonate chemistry and environmental variables. The seasonality in carbonate parameters reflected the seasonal cycle in phytoplankton activity, with higher total alkalinity concentrations and pH and lower dissolved inorganic carbon concentrations during the growing season. The dominant coccolithophore at the site was *Emiliania huxleyi* which showed a clear seasonal pattern, being more abundant in mid-summer when warmer and nutrient-depleted conditions restricted the annual diatom bloom. This study revealed the presence of three morphotypes of *E. huxleyi*, type A, type A overcalcified (type AO) and type B, which were seasonally distributed throughout the year. The less calcified form was mainly observed in spring while heavily calcified morphotypes overlapped during summer. Autumn and winter months were dominated by the most calcified form (type AO). These results indicate that the seasonal pattern of *E. huxleyi* morphotypes was not related to the carbonate concentration at the site. This study reflects the strong interannual variability in carbonate chemistry and the complexity associated with coccolithophore calcification, and highlights the need of long-term data to understand the potential impact of ocean acidification on calcifying phytoplankton.

1. Introduction

Concentrations of atmospheric carbon dioxide (CO_2) are increasing at unprecedented rates due to anthropogenic activities (IPCC, 2014). More than a third of this CO_2 is taken up by the ocean (Sabine et al., 2004), causing an alteration to seawater carbonate chemistry and lowering its pH (Gattuso et al., 2015). This process, known as ocean acidification (OA) (Doney et al., 2009), is likely to have a significant impact on the phytoplankton community affecting processes such as photosynthesis, calcification and nitrogen fixation (Rost et al., 2008). Carbonate parameters are highly variable at both global and regional scales (Bates et al., 2014). This variability may be higher in coastal areas (Duarte et al., 2013), where many calcifying organisms inhabit, due to the combination of factors such as diurnal tidal cycles and terrestrial inflow. Most of the studies on the marine carbonate system have been performed in offshore areas. Both the OSPAR/ICES Study Group on Ocean Acidification (SGOA, ICES 2014) and the Global Ocean Acidification Observing Network (GOA-ON, Newton et al., 2015) identified particular gaps in data for coastal and inshore waters. The lack of carbonate chemistry measurements in coastal waters constrains the understanding of the potential impact of OA on the coastal ecosystem. Continued time-series observations are crucial to determine long-term trends and to assess the potential impact of OA (Bates et al., 2014; IPCC, 2014; Ostle et al., 2016). In this context, the Scottish Coastal Observatory (SCObs; Marine Scotland Science, 2016), operated by Marine Scotland Science (MSS), is providing baseline information about the seasonality and interannual variability of carbonate parameters in inshore waters in the western part of the northern North Sea (Bresnan et al., 2016).

Coccolithophores are an important component of the phytoplankton community and are present in the majority of the world's oceans (Tyrrell and Merico, 2004). This single-celled group is characterised by calcareous (calcite) scales named coccoliths, which surround the living cell to form an extracellular covering called a coccosphere (Winter et al., 1994; Sabine et al., 2004). Coccolithophores occupy the base of the oceanic food web, contribute significantly to marine primary production (Poulton et al., 2013) and are a crucial component in global biogeochemical cycles and Earth's climate system (Brown and Yoder, 1994). The process of pelagic calcite production by coccolithophores has a complex influence on the carbon cycle, driving either the CO_2 production, uptake, sequestration and export from the euphotic zone to the deep ocean (Rost and Riebesell, 2004).

Coccolithophore distribution and seasonality have been well studied worldwide over the last few decades (Ziveri et al., 1995; Beaufort and Heussner, 2001; Merico et al., 2006; Silva et al., 2008; Hinz et al., 2012; Narciso et al., 2016; among others). However, this group is still poorly documented in Scottish waters. Extensive blooms of coccolithophores have been recorded in the northern and western areas of the North Sea by satellite imagery (Holligan et al., 1993), but the sampling frequency of the few *in situ* observations (Van der Wal et al., 1995; Marañón and González, 1997; Head et al., 1998; Widdicombe et al., 2002; Charalampopoulou et al., 2011; Young et al., 2014; Rivero-Calle et al., 2015) do not allow coccolithophore seasonal variability to be assessed in the region.

Among coccolithophores, *Emiliania huxleyi* is probably the most abundant and widely distributed species (Tyrrell and Merico, 2004). Due to its intra-species variability (Read et al., 2013) and opportunistic behaviour (Winter et al., 1994) it can form large blooms in many of the seas and oceans under a wide range of environmental conditions (Tyrrell and Merico, 2004). Initially viewed as potentially sensitive to OA, many laboratory studies have focused on coccolithophores and particularly *E. huxleyi* as a proxy to assess the response of this group to future OA scenarios (Rost et al., 2008; Meyer and Riebesell, 2015). Some of these experimental studies have shown contrasting results for which extrapolation to natural conditions at sea is not straightforward (Ridgewell et al., 2009). This can be partially explained by logistical constraints and methodological issues, including short experimental timescales, the *E. huxleyi* strains used, length of time in culture and inter-strain genetic variability (Read et al., 2013; Blanco-Almejeiras et al. 2016). Some laboratory studies have led to conclusions that are not supportive of the suitability of *E. huxleyi* as a proxy species for monitoring the biological effects of OA (ICES, 2014). Field observations are thus critical to understand the natural seasonality and interannual variability of coccolithophores before OA driven changes can be understood in field and laboratory settings (Bates et al., 2014). Although observational studies are scarce, the relationship between carbonate chemistry and coccolithophore calcification has been assessed in different oceanographic regions with mixed results (Cubillos et al., 2007; Beaufort et al., 2011; Smith et al., 2012; Meier et al., 2014; Marañón et al., 2016). A recent study by Rivero-Calle et al. (2015) showed an increase in coccolithophores occurrence together with increasing CO_2 and temperature across the North Atlantic (including the North Sea). Young et al. (2014) showed the lack of relationship between coccolith calcification and carbonate chemistry in the northwestern European

continental shelf while, to our knowledge, no work on this topic has been performed in the north-western North Sea.

This study presents a description of carbonate parameters in inshore Scottish waters, providing one of the few sustained observations of seasonal and interannual variability of carbonate chemistry in coastal waters in the North Sea. In addition, four years of monthly samples were collected at the SCObs monitoring site at Stonehaven (off the North East of Scotland) to provide the first baseline description of coccolithophore diversity, seasonality and coccolith morphometrics in the region. A preliminary relationship with carbonate parameters and environmental variables was examined.

2. Material and methods

2.1. Sampling site

The Stonehaven monitoring site is part of the SCObs operated by MSS and has been in operation since 1997. This monitoring site is located 5 km offshore from Stonehaven in the north east of Scotland (56º 57.8´ N, 02 º 06.2´ W) (Fig. 1) and is approximately 50 m in depth. The hydrography is characterized by a coastal southward flow and strong tidal currents mixing the water column, resulting in thermal stratification during summer months being weak. Water samples have been collected on a weekly basis, weather permitting, for the determination of chlorophyll and inorganic nutrients (total oxidised nitrogen, phosphate, silicate, nitrite, ammonia), phytoplankton and zooplankton species composition, along with the physical parameters temperature, salinity and Secchi disc depth. Additional water samples have been collected since 2009 for the determination of the carbonate chemistry parameters (total alkalinity [TA] and dissolved inorganic carbon [DIC]). All datasets have been quality controlled. A summary of the quality control procedure for each parameter and further information about the Stonehaven monitoring site can be found in Bresnan et al. (2015, 2016).

2.2. Carbonate chemistry

Discrete water samples were collected at 1 m and 45 m for the determination of TA and DIC and stored in the dark at room temperature in 250 mL glass bottles (Schott Duran) poisoned with saturated $HgCl_2$ (50 µL) to prevent biological alteration during storage. Samples were analysed at the National Oceanography Centre Southampton (NOC). Analysis was performed using colorimetric and potentiometric open titration cell techniques. Samples were analysed

using the Versatile Instrument for Analysis of Titration Alkalinity (VINDTA 3C, Marianda, Germany) based on the procedures of Dickson et al. (2007). The instrument precision was assessed by repeated measurements on previously analysed samples (n>3) before each batch of sample analysis. The precision for all DIC and TA measurements was estimated as ± 1.5 µM/kg. The pH (total scale) and the calcite saturation constant (Ω_{cal}) were derived using CO2SYS (version 2.1; Pierrot et al., 2006). The dissociation constants of carbonic acid (pK_1 and pK_2) were taken from Millero et al. (2006), with an estimate error of ± 0.0054 for pK_1 and ± 0.011 for pK_2. Aragonite saturation has not been derived in this study since calcite is the carbonate form used by coccolithophorids to build their calcareous scales (Brownlee and Taylor, 2004). The gap in the 2011 data was due to logistical reasons.

2.3. Temperature and salinity

Niskin sampling bottles were used which were fitted with digital thermometers to record temperature at each sampling depth. Salinity samples were taken at 1 m and 45 m and stored in glass bottles which were dried and sealed to prevent salt crystal formation and water evaporation. Samples were analysed using a Guildline Portasal Salinometer Model 8410A previously standardised using International Association for Physical Sciences of the Ocean (IAPSO) standard seawater. The salinity results were recorded using the Practical Salinity Scale (UNESCO, 1981).

2.4. Nutrients

Water samples for inorganic nutrients were taken at 1, 5, 10 and 45 m and stored in glass bottles at -20 ºC until analysis. Total oxidised nitrogen (TOxN: nitrate plus nitrite), dissolved inorganic phosphorus (DIP) and dissolved silicate (DSi) concentrations were determined using a Bran-Luebbe QuAAtro continuous flow autoanalyser (Smith et al., 2014). Phosphate concentrations for the period March 2010-December 2011 were determined only to the first decimal place due to logistical reasons.

2.5. Chlorophyll

Water samples for chlorophyll and coccolithophore analysis were collected using a 10 m Lund tube, providing an integrated sample of the upper 10 m of the water column. Depending on the time of year, a sample volume of 500 mL to 2 L was filtered through a Whatman GF/F 47 mm filter paper (0.7 µm retention), using a vacuum of approximately 380 mmHg to avoid damaging the cells. The samples were stored at -80 ºC until analysis. Chlorophyll

concentration was determined by fluorometry using the method of Arar and Collins (1992) after extracting the pigments in buffered acetone for 24 h using a Turner AU fluorometer. The method includes an acidification step to correct chlorophyll *a* for the presence of phaeopigments. Since little difference has been found between corrected and uncorrected chlorophyll *a* concentrations at the site (Smith et al., 2007) and to align with OSPAR Joint Assessment and Monitoring Programme (JAMP) guidelines (OSPAR, 2012), uncorrected data have been used in this study.

2.6. Coccolithophores

A 250 mL subsample of water collected using the Lund tube was preserved with hexamethylenetetramine buffered formaldehyde (4% final formalin concentration) solution (Throndsen, 1978) and stored in amber glass jars in the dark until analysis using Scanning Electron Microscopy (SEM). Depending on the time of the year, a volume ranging between 5 and 20 mL of preserved sample was filtered through a 13 mm Nuclepore polycarbonate membrane with a 1.0 µm nominal pore size with vacuum pressure <100 mmHg. Filters were rinsed with buffered distilled water to remove salt and then air dried. Subsequently, filters were sputter-coated with gold/palladium and examined under a Zeiss EVO MA1O SEM at the Institute of Medical Sciences (University of Aberdeen). Coccolithophore cells and coccoliths were enumerated along perpendicular transects of equidistant areas of observation. At least 30 coccoliths per sample were measured. Identification of coccolithophores was performed using the morphological criteria detailed by Young et al. (2003). Coccospheres and coccoliths morphometrics of *E. huxleyi* (see online Supplementary material Fig. S1) were measured from SEM micrographs using Fiji (ImageJ) image processing package and categorized into morphotypes (Young et al., 2003). One sample per month between 2010 and 2013 was analysed using this method.

2.7. Statistical analyses

For each month, mean values of temperature and inorganic nutrients were calculated from samples collected at 1, 5 and 10 m in order to be compared with the upper 10 m integrated chlorophyll and coccolithophore data, and salinity and carbonate chemistry parameters (DIC, TA, pH and Ω_{cal}) collected at 1 m. Regression analyses were carried out to investigate the relationship between *E. huxleyi* abundance and morphometrics with single carbonate chemistry-environmental variables. Principal component analyses (PCA; Ramette, 2007) were performed to characterize seasonal patterns in *E. huxleyi* assemblages with

physicochemical properties. The first PCA was conducted on *E. huxleyi* total abundance data while a second analysis was conducted on *E. huxleyi* morphotypes abundance. The input variables for the PCAs were: *E. huxleyi* total cells/morphotypes abundance, temperature, salinity, chlorophyll, inorganic nutrients, Ω_{cal}, DIC and pH. A one-way ANOVA was used to assess differences among *E. huxleyi* coccolith morphometrics. The software package Statistica 7.1 (Statsoft, Inc. 1984-2005) was used for the statistical analyses.

3. Results

3.1. Carbonate chemistry

The carbonate system descriptors (DIC, TA, pH and Ω_{cal}) show a seasonal trend with interannual variability observed over the duration of the study (Fig. 2). Weekly concentrations of DIC were generally higher (up to 2,134 µmol kg^{-1}) and less variable during the winter months, decreasing (with a minimum of 2,013 µmol kg^{-1}) in the spring-summer period (Fig. 2a). Concentrations of TA, ranging between 2,210 and 2,309 µmol kg^{-1}, followed the inverse pattern to DIC with minimum concentrations observed during the winter months and maximum values in spring/summer (Fig 2b). The interannual comparison of DIC and TA highlighted variations in the duration of those periods. Differences between surface (1 m) and bottom (45 m) concentrations were observed between April-August in 2012 associated with less saline surface waters (Fig. 3b).

The derived pH (total scale) ranged between 7.88 and 8.25 during the course of the study. The pH was generally higher between April and July as the chlorophyll concentrations in the water column increased (Figs. 2c and 3f). An overall decreasing trend of pH (total scale) values was observed between December 2011 and December 2012 (Fig. 2c). Weekly derived calcite saturation (Ω_{cal}) varied between 2.1 and 4.2, indicating that seawater at Stonehaven was supersaturated with respect to calcite. Its distribution showed a general seasonal pattern with higher saturation states during late spring/summer months (May-September) and lower in winter-early spring (October-April) (Fig. 2d), influencing the DIC increase observed during that period. Overall, surface and bottom patterns were very similar for pH and Ω_{cal}. Similar to the other carbonate parameters, Ω_{cal} seasonality was clearly related to the cycles in photosynthesis/respiration.

3.2. Temperature and salinity

Weekly distributions of physical and chemical properties are shown in Fig. 3. Temperature (Fig. 3a) exhibited a strong seasonal cycle with increasing temperatures observed from March/April through to August/September when the temperatures maximised, reaching up to 14.1 ºC (2013) and 13.2 ºC (2010) at surface and bottom depths respectively. Decreasing values were observed between September/October and February/March, with minima of 4.4 ºC at surface and 4.7 ºC (2013) at bottom depths. Water temperatures were consistent through the water column, apart from 12 weeks during the spring and summer periods (April-August) where warmer surface-waters (up to 3 ºC) were observed, suggesting a weak stratification of the water column at these times. Salinity data, ranging between 33.29 and 34.99 at surface and between 34.25 and 34.99 at the bottom, revealed a higher interannual variability (Fig. 3b). Less saline waters were observed during the first months of the year and saltier waters in autumn, although the peak of salinity varied significantly among years. The highest salinities were in July/August 2010, October 2011, September 2012 (these being the highest salinity values since the time series began in 1997) and September/October 2013. Surface and bottom salinities also showed significant differences in some periods in 2010, 2011, and particularly in 2012 when the highest/lowest values in the study period were recorded (in April and October respectively).

3.3. Nutrients

Nutrient concentrations followed a seasonal pattern typical of higher latitudes (Figs. 3c-e). TOxN, DIP and DSi concentrations were minimal in late spring-summer (May-September), increasing rapidly during the autumn periods (October-November). Maximum nutrient concentrations were recorded during the winter months (December-March), with concentrations reaching a maximum of 10.9 µM of TOxN (2013), 0.7 µM of DIP (2010, 2012) and 8.3 µM of DSi (2010). Slight deviations from that pattern were observed in autumn 2012 and winter 2013, when nutrient concentrations increased and then decreased gradually. The period January-March 2013 also reflected important variations in weekly TOxN data. DIP concentrations were highly variable in spring-summer periods, and particularly in 2012 when some significant differences were observed between surface and bottom data. The interannual variability was also observed in the duration of the DIP and DSi-depletion periods, which were usually shorter than TOxN.

3.4. Chlorophyll

Weekly chlorophyll concentrations ranged between 0.2 and 5.1 µg L^{-1} during the study period. Its distribution varied at both seasonal and interannual time scales (Fig. 3f). Chlorophyll concentrations were low from October/November to March, while the highest concentrations of chlorophyll were observed in spring-early summer (May-July). This is a consequence of warmer and nutrient-depleted waters. Additional peaks in chlorophyll concentrations were observed during late summer (August) in 2010/2011, and early autumn (September-October) in 2012/2013 coinciding with an increase of nutrient concentrations (particularly DSi) (Figs. 3c-e).

3.5. Coccolithophore abundance and community composition

Six coccolithophore species were identified from SEM images including some disintegrated cells and free coccoliths; *Emiliania huxleyi*, *Syracosphaera spp.*, *Syracosphaera corolla*, *Coronosphaera mediterranea*, *Helicosphaera carteri HOL perforate*, and *Coccolithus pelagicus Braarudii spp.* (see online Supplementary material Fig. S2). The most common species in the coccolithophore community during the study period was *E. huxleyi* (Fig. 4a), with three different morphotypes observed; type A, type A 'overcalcified' (type AO) and type B (Supplementary material Fig. S2). *E. huxleyi* relative abundance ranged from 8% to 100% of total cell numbers (Fig. 4a). Only four samples, corresponding to July 2010, October 2010, July 2011 and June 2012, showed a relative contribution of *E. huxleyi* lower than 60% of total coccolithophore abundance. The occurrence of the other coccolithophore taxa was much lower (data not shown), being the most abundant species in the community only in specific samples. *C. mediterranea, H. carteri,* and *Syracosphaera spp.* contributed 50% (3,800 cells mL^{-1}), 24% (1,800 cells mL^{-1}) and 18% (1,400 cells mL^{-1}) respectively to total cell numbers in July 2011 (7,600 cells mL^{-1}); In June 2012 the genus *Syracosphaera* represented 80% (800 cells mL^{-1}) of total coccolithophore abundance (1,000 cells mL^{-1}).

3.6. Temporal patterns of E. huxleyi abundance and morphotypes

Due to the high relative contribution of *E. huxleyi* to the total coccolithophore community (>60% and often up to 100%), the temporal pattern in the distribution and abundance of total coccolithophores was very similar to that of *E. huxleyi* (Figs. 4a, 5a). The latter indicated a clear seasonal trend with higher concentrations during mid-summer (July-August) and low numbers in autumn-early spring (November-April). Coccolithophore cells were completely absent in most samples collected in spring between 2011-2013 (note that samples from spring 2010 were not available). *E. huxleyi* morphotypes showed a strong interannual variability in

their occurrence (Fig. 4b). The overall trend described a distinct seasonality with type B mainly observed in spring, type A increasing from early (June) to late summer (August) with type AO forms dominating from late summer (August) and into the winter (Fig. 5b).

3.7. Relationships to environmental variables.

The regression analysis showed significant relationships between single environmental variables, except for salinity, and coccolithophore abundance (Table 1). However, with the exception of temperature, in most of the cases the significance levels were low and the model explained low percentages of variability. No statistical relationships were obtained between the carbonate chemistry parameters and coccolithophore densities. The results of the PCA performed with the chemical, hydrological variables and *E. huxleyi* abundance are shown in Fig. 6 (a, b). Three principal components (PCs) were found to be significant, explaining 82% of the total variation within the data. The first component (PC1) accounted for 50% of the variability and was positively correlated with temperature, chlorophyll and calcite saturation while inorganic nutrients and DIC contributed negatively. Note that some of those variables were highly correlated (e.g. temperature and calcite saturation). This component clearly discriminated most of the samples collected in spring-summer and winter, with positive and negative scores respectively (Fig. 7a, b). Therefore, PC1 reflected the seasonal change in nutrient conditions and phytoplankton biomass in the water column. Salinity was the main variable contributing, in this case negatively, to the second component (PC2) (Fig. 6a) which represented 17% of the variance. Most samples collected in winter-spring had positive scores while most samples collected in summer-autumn had negative scores. PC2 separated samples collected under different salinity conditions (Fig. 7a). The third component (PC3) explained 14% of the variability and was mainly correlated with pH. However sample scores for PC3 did not distinguish any particular seasonality in the data (Fig. 7b). *E. huxleyi* abundance was positively correlated with PC1 and negatively with PC2. The PCA performed with *E. huxleyi* morphotypes abundance (Fig. 6c, d) showed similar results although explaining slightly less of the total variability (74%). In this case the sample scores did not allow discrimination of the seasonality within the data (Fig. 7c, d).

3.8. E. huxleyi morphometrics.

Coccosphere diameter ranged between 3.1 and 9.9 µm. Coccolith distal length (DL) and distal width (DW) varied between 0.5-3.3 µm and 0.2-1.9 µm respectively. The morphological parameters of *E. huxleyi* coccoliths showed significant differences among

morphotypes (one-way ANOVA, *P<0.001*); Type B had longer and wider coccoliths than heavier calcified forms (Type A and Type AO), while the latter did not show significant differences in plate measures (Fig. 8). Significant relationships were obtained between *E. huxleyi* DL-DW and chlorophyll, nutrients (except DIP), DIC and Ω_{cal} (Table 2). However no relationships between plate morphometrics with the environmental variables or carbon chemistry parameters were observed when morphotypes were analysed separately (Supplementary material Fig. S3).

4. Discussion

4.1. Carbonate system

This investigation presents the first baseline time series of carbonate chemistry data in Scottish coastal waters capturing the variability in these parameters on a weekly, seasonal and interannual scale. The weekly resolution of these data clearly reflects the strong variability in carbonate chemistry over short time scales (Johnson et al., 2013) that can be missed at lower sampling frequencies. An example of this is the influence of sporadic freshwater inputs on surface carbonate parameters at Stonehaven, particularly evident during 2012. Descriptions of the carbonate system in the literature are usually based on upper ocean observations (Takahashi et al., 2014; Bates et al., 2015, among others). However, strong vertical gradients in carbonate parameters can develop in seasonally-stratified waters (González-Dávila et al., 2010). At Stonehaven, although less pronounced at surface due to the influence of freshwater inflows, surface and bottom seasonal patterns are quite similar, probably as a consequence of the intense mixing at the site (Bresnan et al., 2016).

Seasonal variability of carbonate chemistry is usually a composite of biological and physical processes (Bates et al., 2014). The seasonal trends in carbonate parameters at Stonehaven reflect the seasonality in phytoplankton growth and biomass and concur with previous observations in the North Sea (Schiettecatte et al., 2007; Omar et al., 2010; Salt et al., 2013). Variations in TA and DIC around the spring-summertime period are primarily a consequence of primary productivity with the inorganic nitrogen (mainly nitrate) uptake by phytoplankton during the growing season (Bresnan et al., 2016). Dissolved organic matter produced by phytoplankton can also potentially contribute to TA (Kim and Lee, 2009). Marked changes in surface TA were also associated with sporadic freshwater inputs, maybe as a consequence of low TA riverine waters or organic matter inputs (Hoppe et al., 2012; Hydes and Hartman, 2012). Observed coccolithophore abundances were not sufficient to affect TA concentrations

(Wolf-Gladrow et al., 2007) and dismiss the impact of large coccolithophore blooms on TA during the study period. The intense photosynthetic activity during spring-summertime would also cause the reduction of dissolved CO_2 in the seawater, the decrease of hydrogen ions and hence becoming slightly more alkaline. Similarly the intensification of the respiration processes by non-photosynthetic organisms (e.g. zooplankton, bacteria and benthic invertebrates) during autumn would increase the release of CO_2, lowering the seawater pH (Ostle et al., 2016). Similar to the other carbonate parameters, Ω_{cal} seasonality was clearly related to the cycles in photosynthesis/respiration (Bresnan et al., 2016). Previous studies have highlighted the seasonal cycles in plankton community structure as the main factor controlling the seasonality in carbonate chemistry in coastal systems (Kitidis et al., 2012; Marrec et al., 2013). The succession between the spring-autumn blooms of phytoplankton and zooplankton respectively would dominate the transition of the trophic status (autotrophy vs. heterotrophy) in the system, leading to seasonal variations on carbonate parameters. In seasonally-stratified shelf seas, the breakdown of stratification in autumn typically causes the release of CO_2 from deeper waters (Thomas et al., 2008). The typical weak stratification of the water column at Stonehaven (Bresnan et al., 2015, 2016), would support a biology-controlled carbonate system dynamics at the site rather than the advection of high CO_2 deep waters.

4.2. Seasonal patterns of Emiliania huxleyi

Seasonal variations in coccolithophore assemblages at Stonehaven were dominated by changes in the abundance of *E. huxleyi*. The dominance of this species has been widely described in the worlds' oceans (Winter et al., 1994; Ziveri et al., 1995; Harlay et al., 2010; among others), particularly in high-latitude regions (Tyrrell and Merico, 2004) including the North Sea (Charalampopoulou et al., 2011). In temperate and subpolar oceans most *E. huxleyi* blooms occur during summer and early autumn (Holligan et al., 1993, 2010; Dylmer et al., 2015; Hopkins et al., 2015; among others), although blooms have also been described in spring (Ziveri et al., 1995; Baumann et al., 2000; Narciso et al., 2016). This seasonality is consistent with results from the Stonehaven monitoring site where a higher abundance of *E. huxleyi* was observed during mid-summer when higher temperature and nutrient-depletion conditions prevailed. This pattern is supported by the PCA analysis which clearly discriminated high and low productivity seasons, with *E. huxleyi* positively related to temperature and negatively to inorganic nutrients.

Results from this study are in agreement with previous studies on driving factors of *E. huxleyi* blooms. The latter are usually observed under stratified conditions in low productivity periods (Brand, 1994; Iida et al., 2012), although they can also occur in high turbulence (Ziveri et al., 1995) and nutrient-rich (Silva et al., 2008) situations. Blooms of *E. huxleyi* in the North Sea are consistent with that trend (Holligan et al., 1993; Van der Wal et al., 1995; Marañón and González, 1997; Head et al., 1998), with coccolithophore peak typically following the decline of the spring diatom bloom after high-nutrient (mainly nitrate and silicate; Marañón and González, 1997) conditions (Merico et al., 2006; Harlay et al., 2010). Despite the weakly stratified situation observed during the summer months at Stonehaven, the results from this study align well with that pattern. Maximum cell densities of *E. huxleyi* coincided with minima of DSi and low TOxN concentrations, which were generally greater than those reported as limiting for *E. huxleyi* growth (Eppley et al., 1969). In contrast, phosphate concentration does not seem to influence the release of *E. huxley*i blooms in the North Sea (Marañón and González, 1997). This pattern together with the typical phytoplankton seasonality at Stonehaven, with diatoms increasing in spring and decreasing in summer (Bresnan et al., 2015, 2016), seem to support the occurrence of high levels of *E. huxleyi* after the diatom seasonal bloom in the western North Sea.

The presence of *E. huxleyi* has been used as proxy of oceanographic conditions (Silva et al., 2008, 2013). In the North Sea the occurrence of *E. huxleyi* has been related to the inflows of water from the Atlantic and from the shelf west of Scotland, especially during the early summer (Holligan et al., 1993). The seasonal distribution of *E. huxleyi* was consistent during the study with maxima occurring in August 2010-2011 and 2013, when no sign of offshore influence (usually shown as an increase in salinity) on hydrographic conditions was detected. The peak of coccolithophores abundance observed in August 2012 extended until mid-September and was lower than the other study years, coinciding with the presence of the saltiest waters observed during this study. The latter probably indicates the advection of offshore waters into the monitoring site (Bresnan et al., 2015, 2016).

4.3. E. huxleyi morphotypes, carbonate chemistry and ocean acidification

The occurrence of *E. huxleyi* morphotypes characterised by different levels of calcification (Young et al., 2003) has been previously described in North Sea regions; with type A being usually most common (Young et al., 2014) although type B can be also present or even be the dominant form (Van Bleijswijk et al., 1991). This study has documented the presence of type

A, type AO and type B. It has also shown, although with a strong interannual variability, a repeated seasonality in the occurrence of different morphotypes: the less calcified form being more abundant in spring, heavily calcified types overlapping during summer and the overcalcified type dominating during the autumn and winter months. This pattern is consistent with the seasonal cycles described by Triantaphyllou et al. (2010) in the Aegean Sea and Smith et al. (2012) in the Bay of Biscay, with the most calcified forms dominating the *E. huxleyi* population in winter. In contrast, an opposite trend was obtained by Beaufort and Heusneer (2001) from sediment traps also in the Bay of Biscay. According to Smith et al. (2012), such discrepancies might be due to methodological biases affecting the traps.

The drivers of seasonal variation of *E. huxleyi* morphotypes are not straightforward. Experimental studies have suggested carbonate concentration as the most significant factor controlling coccolithophore calcification (Riebesell et al, 2000; Meyer and Riebesell, 2015). Global and regional observational studies, mostly on sediment and sediment trap samples, seem to support that conclusion (Beaufort et al., 2011; Meier et al., 2014). However, data from the Southern Ocean (Cubillos et al., 2007), northwestern Europe (Young et al., 2014) and tropical regions (Marañón et al., 2016) seem to indicate that coccolithophore calcification is independent of carbonate availability. In contrast to previous observations in the southern and western North Sea (Young et al., 2014), data from this study showed a significant relationship between coccolith morphometrics and carbonate parameters, indicating the presence of larger coccoliths (corresponding to the less calcified forms) under higher Ω_{cal}. This contradicts the general assumption that calcification and carbonate concentrations are positively correlated (e.g. Riebesell et al, 2000). These inconsistencies in the morphological response of *E. huxleyi* to seawater carbonate chemistry changes appear to be strain-specific (Langer et al., 2011). On the other hand, carbonate concentration was not a limiting factor for calcification during this study and the observational works described above. Recent research suggests that calcification is inhibited by the decrease of seawater pH rather than the seawater carbonate availability (Cyronak et al., 2016a,b; Waldbusser et al., 2016). However, in this study type AO and type B (the most and least calcified forms respectively) dominated the *E. huxleyi* population under either more and less acidic conditions (see October-November 2010, September-October 2012, May-June and December 2012) and no relationship was observed between coccolith morphometrics and pH. Furthermore, observations by Smith et al. (2012), revealing a higher abundance of most heavily calcified forms under more acidic conditions, directly confront the assumption that OA will affect negatively coccolithophorids

calcification (Rost et al., 2008; Meyer and Riebesell, 2015). Thus, this study seems to support the lack of influence of carbonate chemistry on coccolithophore calcification.

Other environmental variables have been described as factors influencing calcification in laboratory experiments (Båtvik et al., 2007; Bollmann et al., 2009; Fielding et al., 2009; De Bodt et al., 2010). Although temperature was not completely discarded by Smith et al. (2012), no statistical relationship with coccolith morphometrics was obtained. In agreement with *in situ* studies (Triantaphyllou et al., 2010; Beaufort et al., 2011), data from this study indicated a lack of salinity influence on *E. huxleyi* morphometry. The weak relationships with coccolith morphometrics do not suggest a strong influence of nutrient on calcification, although it might indicate different physiological requirements of each morphotype. The morphotype switch could also be a response to other variables not analysed in this work, including seasonal changes on grazing or infection patterns (Monteiro et al., 2016). The absence of a consistent calcification response to carbonate chemistry or any other environmental factor highlights the complexity of seasonal patterns of *E. huxleyi* and seems to support the hypothesis that changes in calcifying morphotypes are associated with shifts in the ecotype dominance rather than on variations of a single environmental factor (Cubillos et al., 2007; Read et al., 2013; Blanco-Almejeiras et al., 2016). The understanding of *E. huxleyi* seasonal variation could be improved by the genetic characterization of morphotypes (Smith et al., 2012). However, the relationship between morphotypes and genotypes remains unclear (Hagino et al., 2011). Seasonal patterns of morphotypes need to be considered when interpreting differences in calcification from cruise data collected at different times of year, since they might reflect the natural seasonality of *E. huxleyi* populations.

5. Summary and conclusions

This study presents a sustained description of the weekly, seasonal and interannual variability of the carbonate chemistry parameters in coastal waters off the north western North Sea. This study also highlights the diversity of the coccolithophore community as well as their monthly distribution in relation to environmental conditions. The annual changes in marine carbonate chemistry parameters reflect the seasonal cycles in phytoplankton activity during the period studied. Coccolithophore assemblage was dominated by *E. huxleyi*, the seasonality of which was mainly driven by temperature and the nutrient-depletion restricting the diatom bloom. Results from this study align with previous investigations suggesting that *in situ* calcification by coccolithophore is not affected by carbonate chemistry. The strong interannual variability

541 revealed by the year-to-year data also illustrates the complexity of the response of natural
542 assemblages to OA. This highlights the need for long term scale monitoring to distinguish
543 changes as consequence of anthropogenic activities from the natural seasonal and interannual
544 variability. Weekly time series can also help to interpret observations derived from sporadic
545 cruise samplings.
546

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557

558 **Supplementary materials**

559 Fig. S1. Micrograph showing the morphometric parameters measured on *E. huxleyi* coccoliths.
560 Fig. S2. Micrographs of coccolithophore species and *E. huxleyi* morphotypes observed at Stonehaven.
561 Fig. S3. Mean coccolith distal shield length versus mean carbon chemistry variables for each *E. huxleyi*
562 morphotype; Dissolved Inorganic Carbon (DIC) (a), Total Alkalinity (TA) (b), pH (c) and calcite saturation
563 coefficient (Ω_{cal}) (d).
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Figure captions

Figure 1. Location of the Stonehaven monitoring site (filled circle) and general circulation pattern of the Western North Sea (reproduced from OSPAR, 2000): North North Sea water (N.N.S.W), Fair Isle Current (F.I.C.), Dooley Current (D.C.), Scottish coastal water (S.C.W.). Bathymetry (from Gebco bathymetry) is also shown.

Figure 2. Weekly distribution of (a) DIC, (b) TA, (c) derived pH and (d) Ω_{cal} at 1 m (surface; filled circles) and 45 m depth (bottom; blank circles).

Figure 3. Weekly distribution of (a) temperature, (b) salinity, (c) TOxN, (d) DIP and (e) DSi at 1 m (surface; filled circles) and 45 m depth (bottom; blank circles), and integrated chlorophyll (f).

Figure 4. Monthly distribution of (a) total coccolithophores (filled circles) and *E. huxleyi* abundance (blank circles), *E. huxleyi* percentage (grey-shaded area) and (b) *E. huxleyi* morphotypes percentage.

Figure 5. Mean monthly (2010-2013) abundances of (a) total coccolithophores and (b) *E. huxleyi* morphotypes. Error bars are not plotted in order to facilitate the observation of mean data (the interannual variability can be observed in figure 4).

Figure 6. Structure of first three factors extracted from factorial analysis performed for *E. huxleyi* total (a-b) and morphotype (c-d) abundances.

Figure 7. Bi-plot of the scores for the first three factors of each sample used in the factorial analysis performed for *E. huxleyi* total (a-b) and morphotype (c-d) abundance. Scores were grouped seasonally according to the period of the year in which each sample was collected: winter (January-March), spring (April-June), summer (July-September) and autumn (October-December).

Figure 8. Box whisker plot of coccoliths distal shield length (a) and width (b) for each *E. huxleyi* morphotype.

945 **Tables**

946 **Table 1.** R^2, intercept and slope for linear regression of coccolithophore
947 abundance (cells·L^{-1}) and environmental variables: temperature (ºC), salinity,
948 chlorophyll (µg·L^{-1}), TOxN (µM N), DIP (µM), DSi (µM), DIC (µmol kg^{-1}), TA
949 (µmol kg^{-1}), pH and calcite saturation coefficient (Ω_{cal}). ns: p>0.05, *p ≤ 0.05,
950 **p ≤ 0.01, ***p ≤ 0.001.
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Variables	n	R^2	y-intercept	Slope	P
Temperature	38	0.030	-2901.14	407.97	***
Salinity	37	0.016	-40909.14	1223.8	ns
Chlorophyll	38	0.105	365.20	553.10	*
TOxN	37	0.177	1998.13	-288.93	**
DIP	37	0.157	2646.26	-4783.82	*
DSi	37	0.168	2415.4	-470.98	*
DIC	34	0.111	56390.85	-26.41	ns
TA	34	0.0005	-6710.04	3.42	ns
pH	34	0.004	12349.69	-1399.84	ns
Ω_{cal}	34	0.102	-3521.70	1484.42	ns

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953 **Table 2.** R^2, intercept and slope for linear regression of *E. huxleyi* coccolith morphological
954 variables (distal shield length –DL- and distal shield width –DW-; µm) and environmental
955 variables: temperature (ºC), salinity, chlorophyll (µg·L^{-1}), TOxN (µM N), DIP (µM), DSi
956 (µM), DIC (µmol kg^{-1}), TA (µmol kg^{-1}), pH and calcite saturation coefficient (Ω_{cal}). ns:
957 p>0.05, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
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		Distal length				Distal width			
Variables	n	R^2	y-intercept	Slope	P	R^2	y-intercept	Slope	P
Temperature	37	0.0005	9.296	0.159	ns	0.0001	10.017	0.0225	ns
Salinity	36	0.019	34.714	-0.077	ns	0.026	34.706	0.086	ns
Chlorophyll	37	0.157	-3.678	1.274	*	0.171	-2.969	1.271	*
TOxN	36	0.132	4.173	0.046	*	0.116	3.622	0.045	*
DIP	36	0.126	4.28	0.784	ns	0.128	3.745	0.827	ns
DSi	37	0.109	8.946	-1.576	*	0.109	7.849	-1.509	*
DIC	33	0.237	2229.09	-33.337	**	0.235	2203.66	-31.259	**
TA	33	0.001	2267.29	0.982	ns	0.003	2276.65	-1.156	ns
pH	33	0.001	8.096	-0.009	ns	0.002	8.095	-0.0102	ns
Ω_{cal}	33	0.229	0.866	0.551	**	0.195	1.419	0.479	**

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960 **Figures**

961 **Figure 1**

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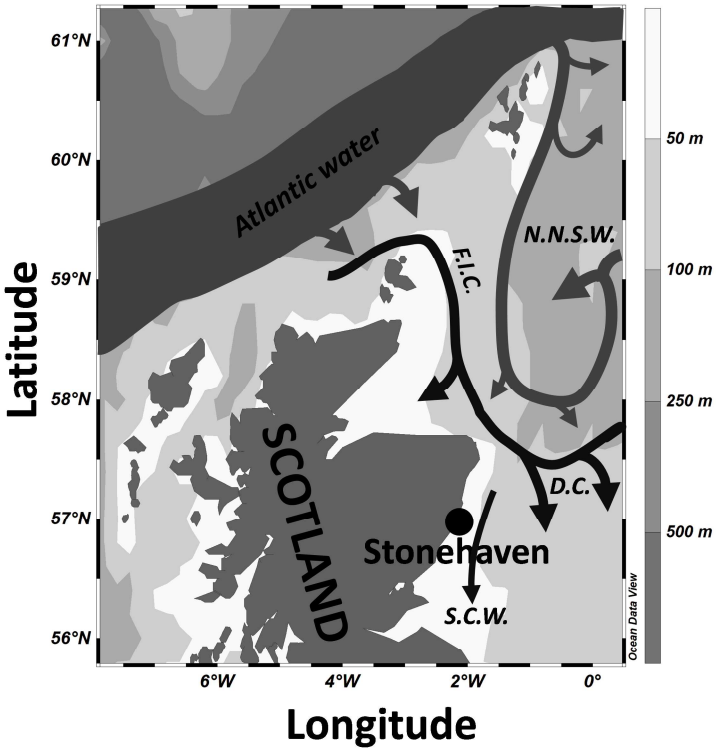

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980 **Figure 2**

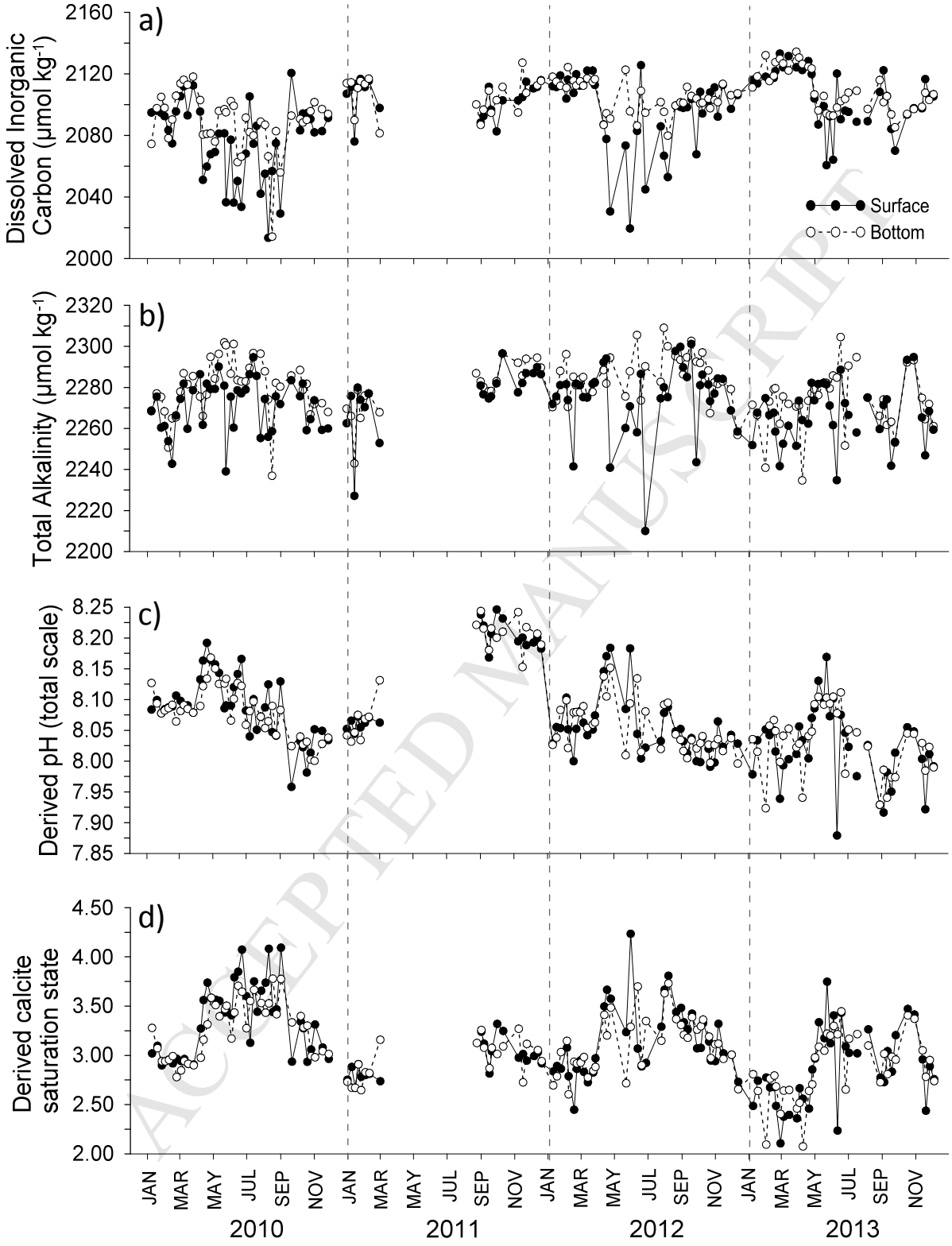

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Figure 3.

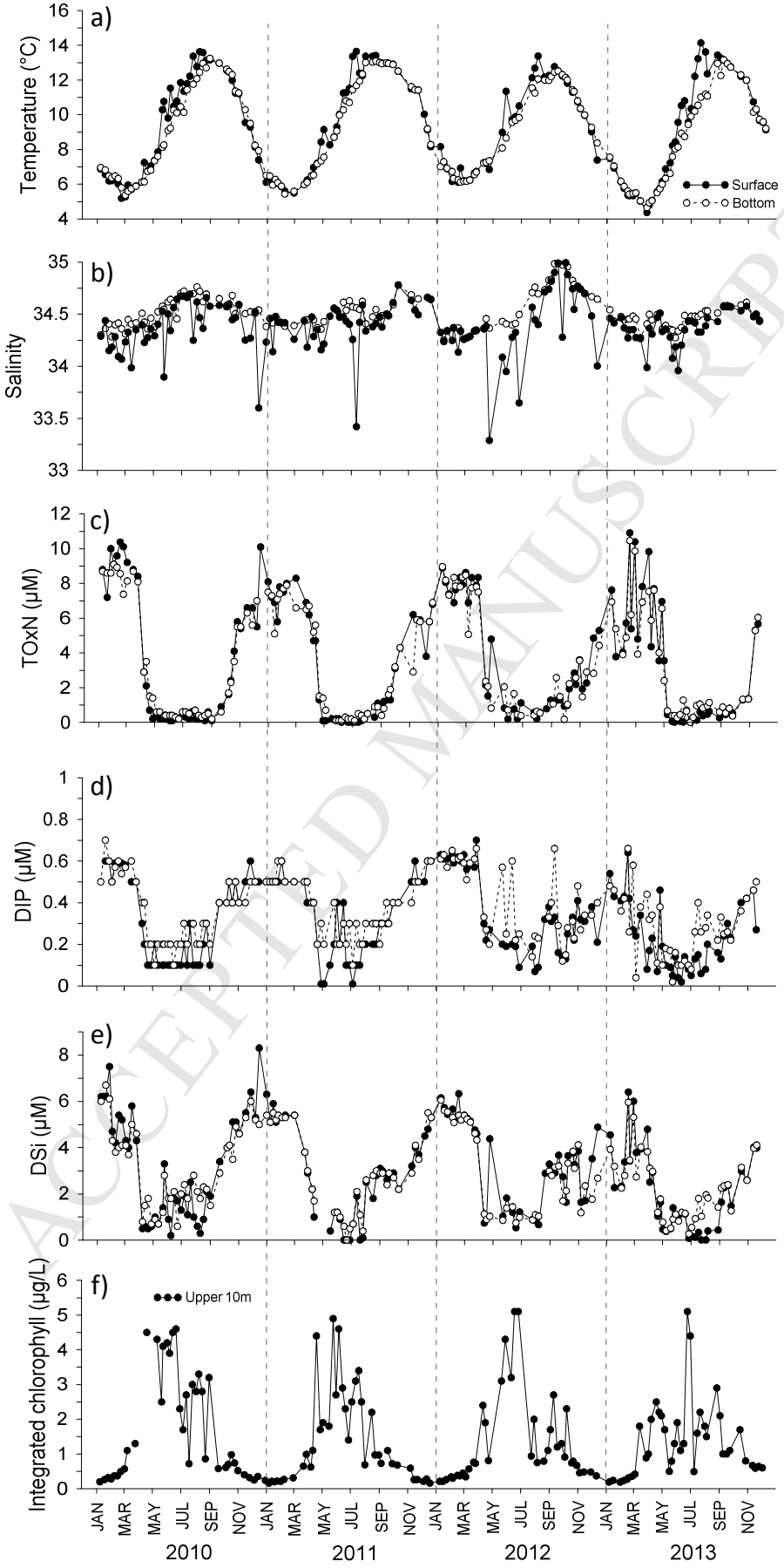

Figure 4.

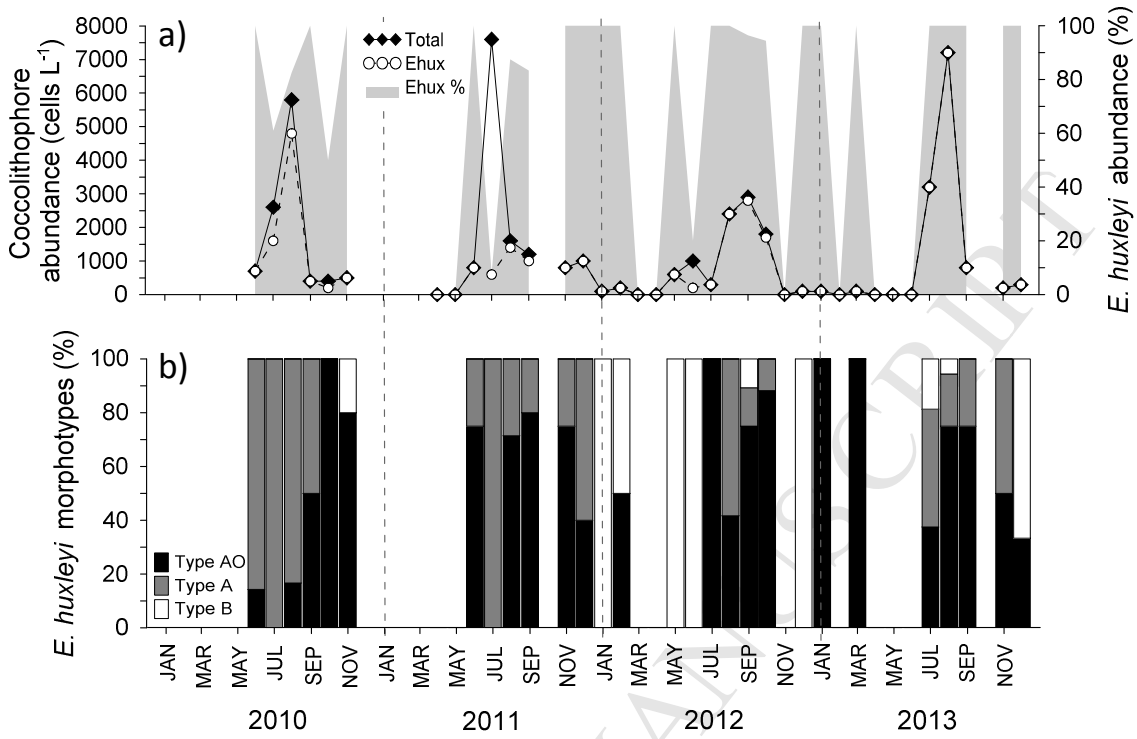

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Figure 5.

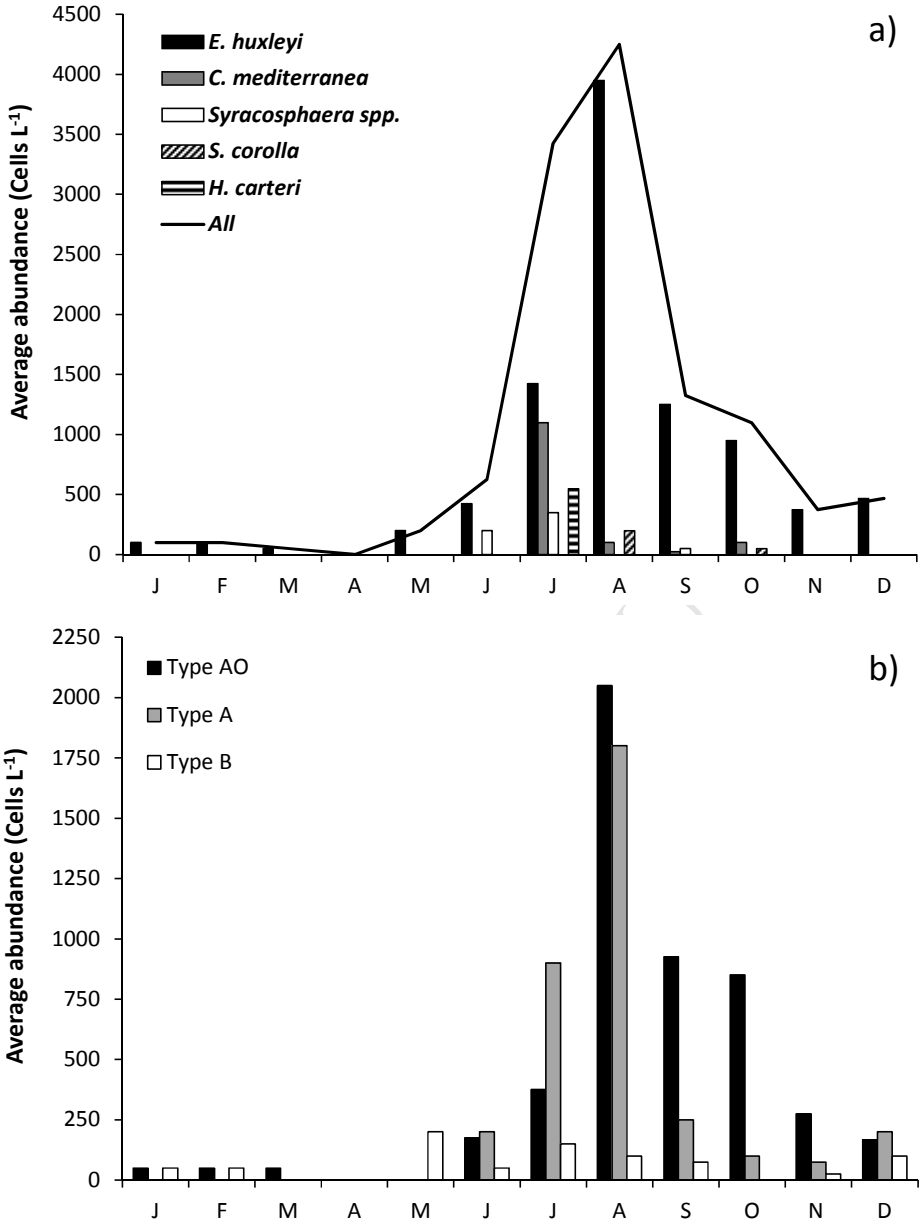
a)
E. huxleyi
C. mediterranea
Syracosphaera spp.
S. corolla
H. carteri
All
Average abundance (Cells L-1)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
J F M A M J J A S O N D
b)
Type AO
Type A
Type B
Average abundance (Cells L-1)
2250
2000
1750
1500
1250
1000
750
500
250
0
J F M A M J J A S O N D

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Figure 6.

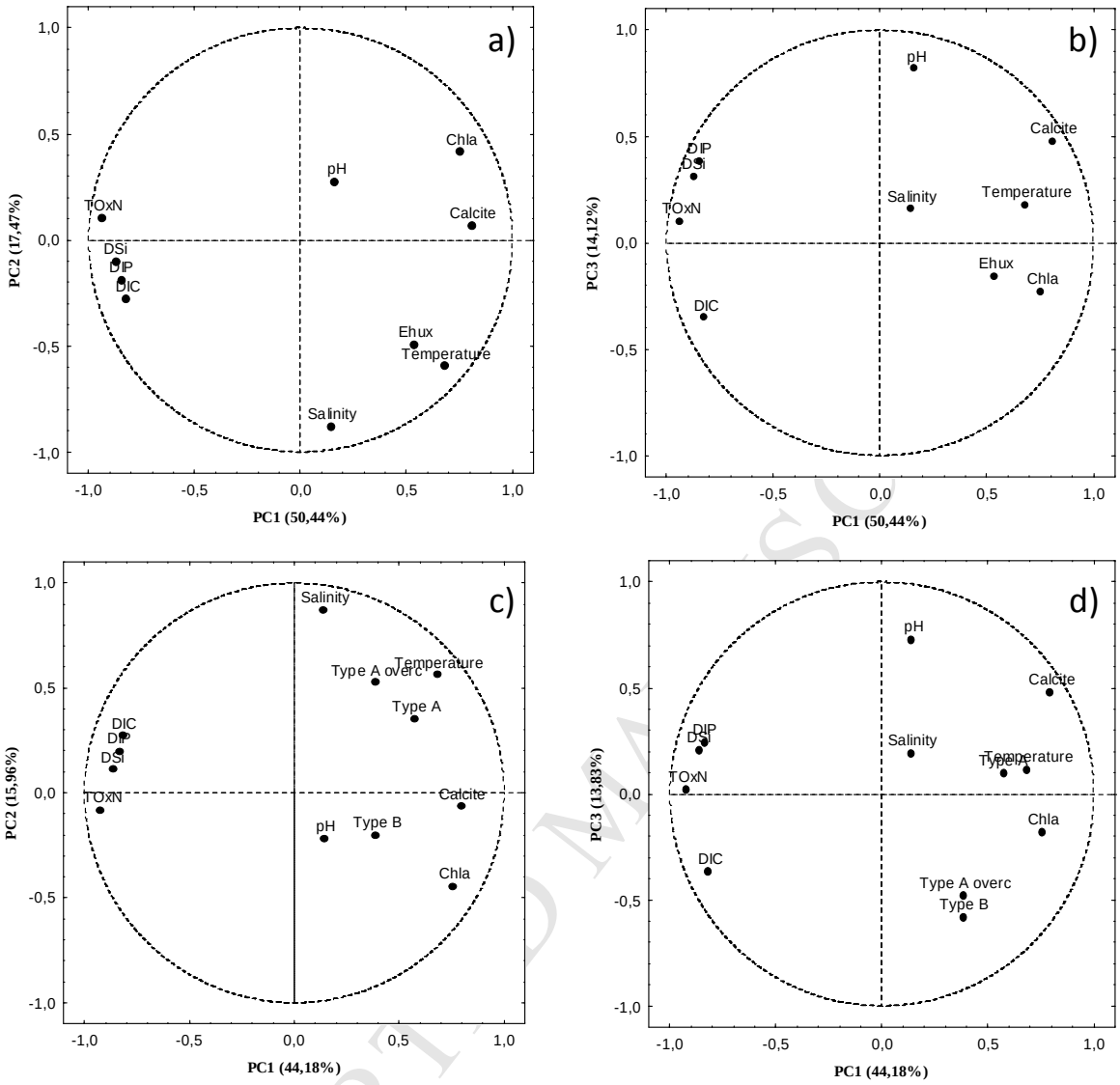

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Figure 7.

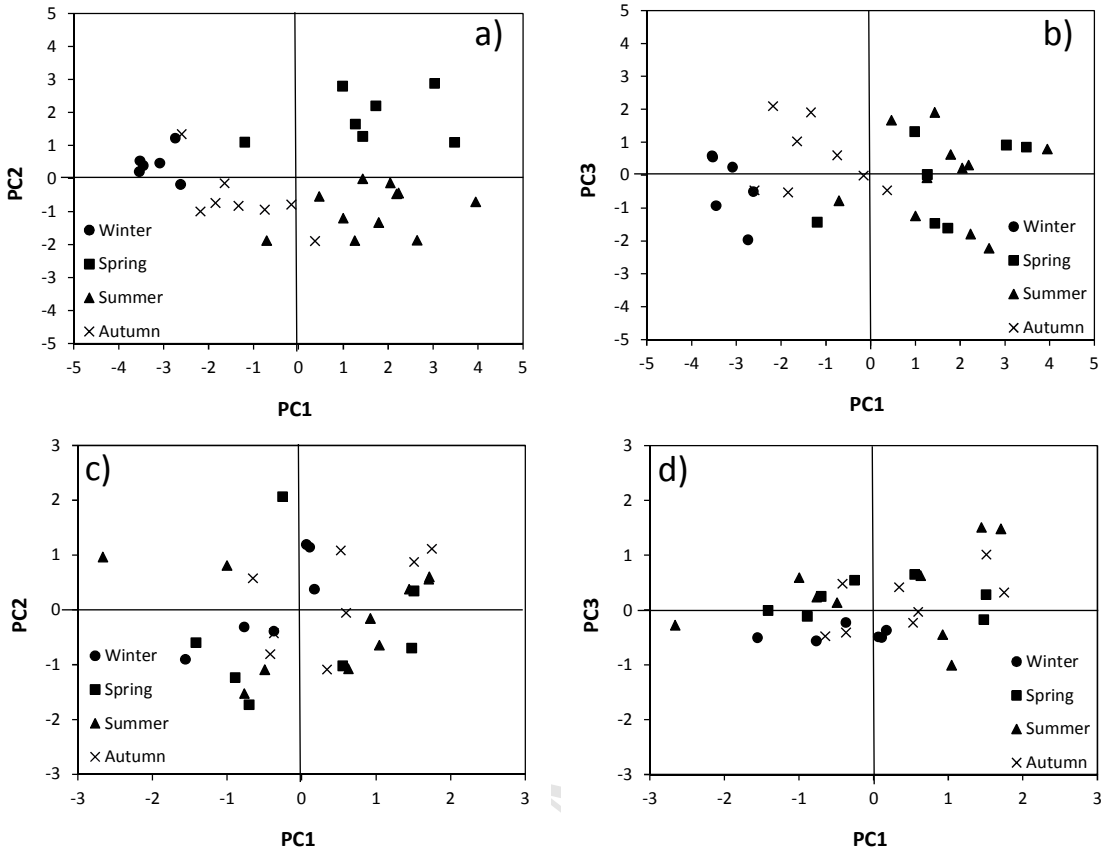

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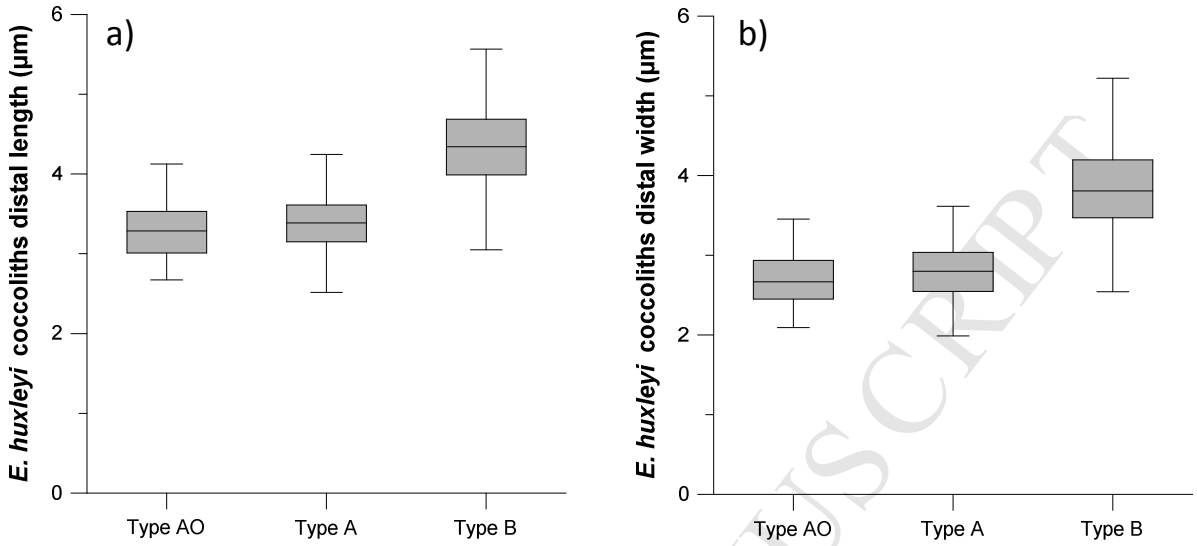
Figure 8.



Appendix. Supplementary material.

Figure S1. Micrograph showing the morphometric parameters measured on *E. huxleyi* coccoliths.

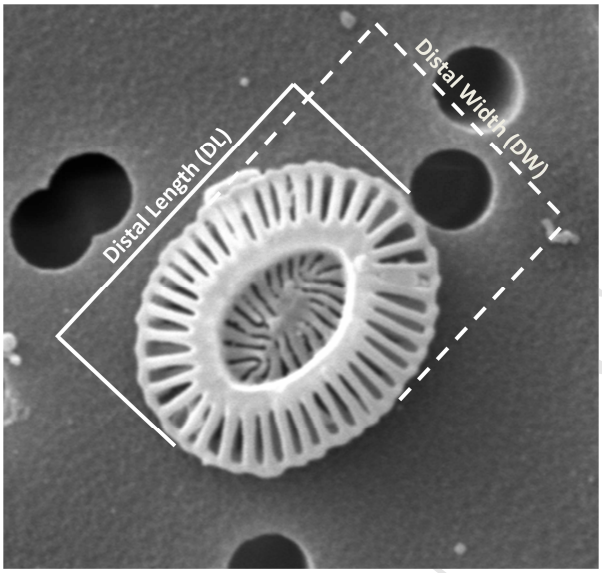
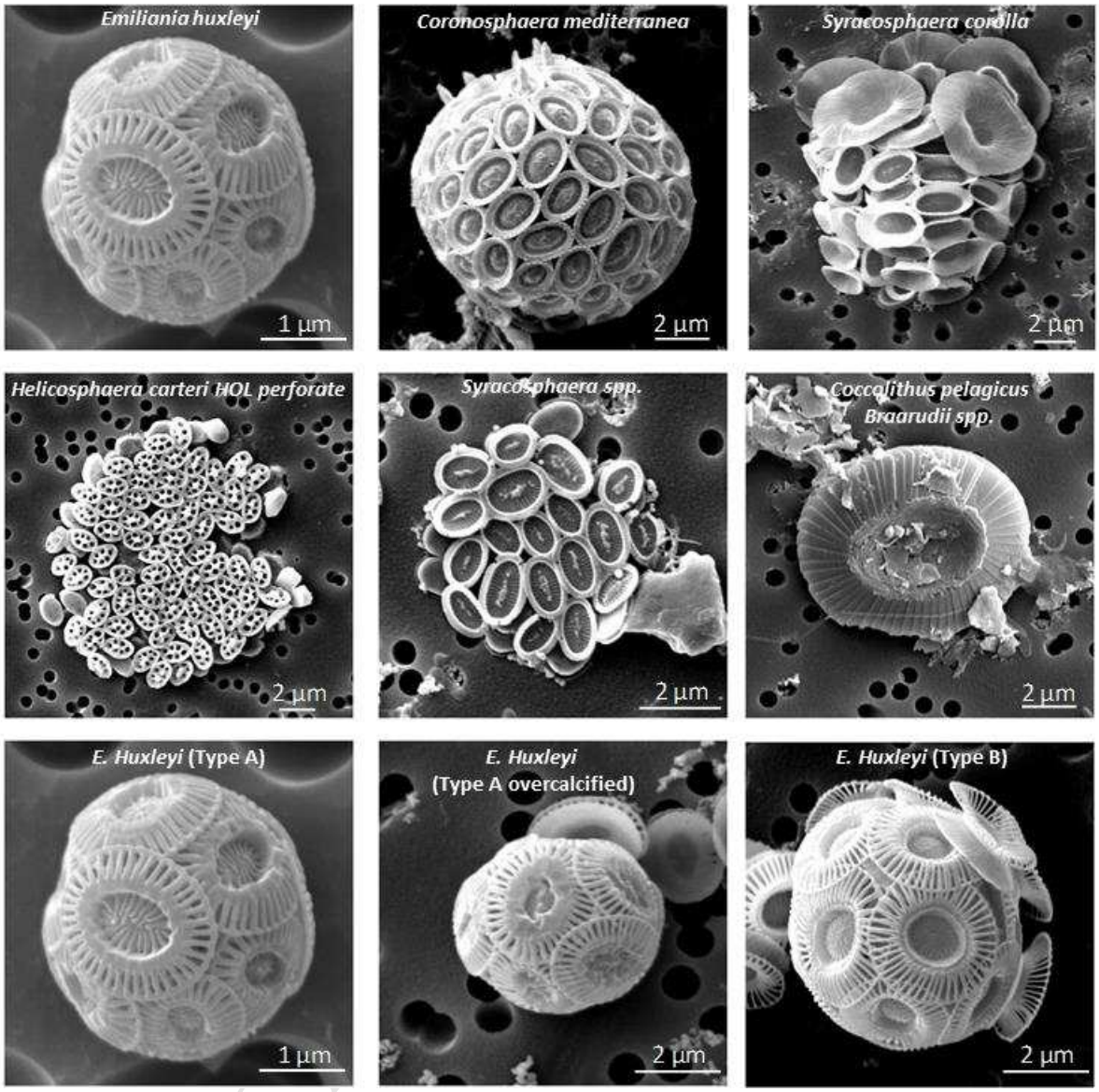
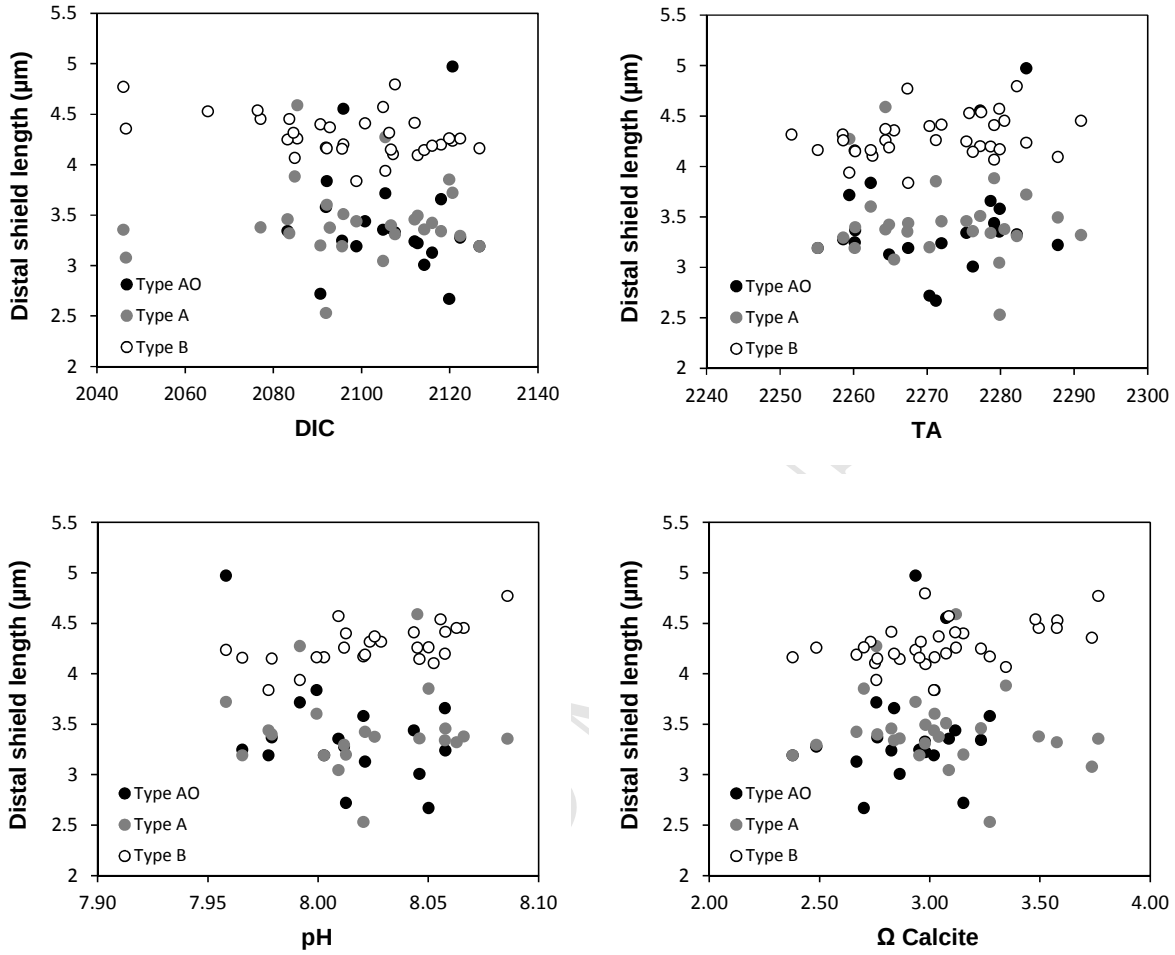


Figure S2. Micrographs of coccolithophore species and *E. huxleyi* morphotypes observed at Stonehaven.



1049 **Figure S3.** Mean coccolith distal shield length versus mean carbon chemistry variables for
1050 each *E. huxleyi* morphotype; Dissolved Inorganic Carbon (DIC) (a), Total Alkalinity (TA)
1051 (b), pH (c) and calcite saturation coefficient (Ω Calcite) (d).



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Highlights.

> There is a “knowledge gap” on carbonate chemistry in inshore waters.

> Stonehaven coastal carbonate system shows a strong variability at short-time and year-to-year scales.

> Occurrence of *E. huxleyi* morphotypes shows a repeated seasonal pattern.

> *E. huxleyi in situ* calcification seems not to be affected by carbonate chemistry.

> Seasonality in *E. huxleyi* morphotypes should be considered when interpreting sporadic cruises data.