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The combined effects of seasonal community succession and adaptive algal physiology on lipid profiles of coastal phytoplankton in the Western English Channel

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ABSTRACT

Lipids are key constituents of marine phytoplankton, and some fatty acids (key constituents of lipids) are essential dietary components for secondary producers. However, in natural marine ecosystems the interactions of factors affecting seasonal phytoplankton lipid composition are still poorly understood. The aim of this study was to assess the roles of seasonal succession in phytoplankton community composition and nutrient concentrations, on the lipid composition of the phytoplankton community. Fatty acid and polar lipid composition in seston was measured in surface waters at the time series station L4, an inshore station in the Western English Channel, from January to December 2013. Redundancy analyses (RDA) were used to identify factors (abiotic and biotic) that explained the seasonal variability in phytoplankton lipids. RDA demonstrated that nutrients (namely nitrogen) explained the majority of variation in phytoplankton lipid composition, as well as a smaller explanatory contribution from changes in phytoplankton community composition. The physiological adaptations of the phytoplankton community to nutrient deplete conditions during the summer season when the water column was stratified, was further supported by changes in the polar lipid to phytoplankton biomass ratios (also modelled with RDA) and increases in the lipid to chlorophyll *a* ratios, which are both indicative of nutrient stress. However, the association of key fatty acid markers with phytoplankton groups e.g. 22:6 *n*-3 and dinoflagellate biomass (predominant in summer), meant there were no clear seasonal differences in the overall degree of fatty acid saturation, as might have been expected from typical nutrient stress on phytoplankton. Based on the use of polyunsaturated fatty acids (PUFA) as markers of 'food quality' for grazers, our results suggest that in this environment high food quality is maintained throughout summer, due to seasonal succession towards flagellated phytoplankton species able to maintain PUFA synthesis under surface layer nutrient depletion.

1. Introduction

Autotrophic, mixotrophic and heterotrophic protists (hereafter simply termed "phytoplankton") form the essential foundation for marine food-web processes, contributing around half of the biosphere's net primary production (Behrenfeld et al., 2006). Cellular lipids account for 11–23% of the organic carbon in marine plankton (Wakeham et al., 1997). Lipid molecules (e.g. triacylglycerols, wax esters, sterol esters and polar diacylglycerols) are important biochemicals within phytoplankton cells and have numerous roles including energy storage, membrane structure and function, photosynthesis, metabolism, and cell–cell signalling (Guschina and Harwood, 2009; Murata and Siegenthaler, 1998; van Meer et al., 2008). Furthermore, lipids in phytoplankton contribute an essential and significant proportion of the total carbon flux through the trophic levels in marine ecosystems (Lee et al., 1971). For instance, zooplankton production and reproductive success can be limited both by the quality of fatty acids (e.g. chain length, degree of unsaturation) in their diet, and by the quantity of these fatty acids, which are often better food proxies than chlorophyll *a* or particulate organic carbon (Jonasdottir et al., 2009; Pond et al., 1996).

Fatty acids are often reported as the most abundant lipidic component of marine plankton as they are the building blocks for the majority of different lipid classes. Owing to genotypic differences in cellular lipid biosynthesis between different phytoplankton taxa (Alonso et al., 1994), the total fatty acid composition of natural marine phytoplankton is influenced by phytoplankton community succession in these assemblages (Dijkman and Kromkamp, 2006; Hayakawa et al., 1996). However, natural environmental variables such as light, temperature, nutrient availability, etc., have significant influences on the cellular composition of fatty acids and phytoplankton physiological status (Guiheneuf et al., 2009; Reitan et al., 1994; Roessler, 1990; Thompson et al., 1992). In coastal–shelf systems, abiotic variables may vary on wide-ranging time scales and influence the biochemical/lipid composition of cells. In addition, seasonal cycles and phytoplankton community succession will influence the lipid composition of the prevailing phytoplankton

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assemblage. Consequently, the interpretation of lipid dynamics in coastal marine environments is problematic.

The majority of the fatty acids in phytoplankton are constituents of polar lipids, including different classes of phospholipids (phosphatidylcholine PC, phosphatidylglycerol PG, phosphatidylethanolamine PE), glycolipids (monogalactosyldiacylglycerol MGDG; digalactosyldiacylglycerol DGDG; sulfoquinovosyldiacylglycerol SQDG) and betaine lipids. These molecules are vital functional components of cell membranes in phytoplankton for cell division and growth, and the head-groups of these molecules contain the elements nitrogen, sulphur or phosphorus. Indeed, phospholipids account for up to 28% of plankton phosphate requirements (Van Mooy et al., 2006), underpinning the fact that these polar lipids play an essential role in the cycling of carbon and nutrients in oceans. Moreover, because polar lipids are rapidly degraded upon cell death they are used as a marker of extant phytoplankton biomass and microbial production (Schubotz et al., 2009; Zink et al., 2008).

Contrasting evidence exists as to whether measurements of polar lipid classes and the fatty acids they contain are useful chemotaxonomic markers of plankton in the marine environment (Brandsma et al., 2012a; Brandsma et al., 2012b; Popendorf et al., 2011a; Van Mooy and Fredricks, 2010). However, a growing body of evidence suggests marine microorganisms modulate or remodel polar lipid profiles in response to environmental stressors e.g. nitrate and phosphate limitation, through substitution or up- or down-regulation in biosynthesis, demonstrated in both the natural environment and in laboratory cultures (Lu et al., 2013; Martin et al., 2014; Popendorf et al., 2011b; Van Mooy et al., 2009). These modulations in polar lipid biosynthesis suggests that in a dynamic environment, whereby conditions (e.g. nutrient availability) vary over weekly scales, polar lipid class concentrations in the seston may offer a sensitive marker of environmental effects on the biochemical make-up of phytoplankton cells. Modern developments in Liquid Chromatography–Mass Spectrometry permit the determination and quantification of individual polar lipid species, which are distinguished based on different combinations of fatty acids with variable chain length and degree of saturation associated with the glycerol moiety. Combined, polar lipid class/species quantification may present improved biomarkers of both taxonomic composition (beyond fatty acid analyses) and physiological responses of phytoplankton to variable environmental conditions (Popendorf et al., 2013; Rutters et al., 2002; Schubotz et al., 2009; Sturt et al., 2004). Despite their obvious biological importance and the growing number of studies on polar lipid class distribution in natural plankton communities (Popendorf et al., 2011a; Popendorf et al., 2011b; Schubotz et al., 2009; Van Mooy and Fredricks, 2010; Van Mooy et al., 2006) resolved temporal studies concerning the polar lipid class and polar lipid species composition and abundance in coastal marine waters are scarce. This lack of information is especially the case in seasonally stratified environments, where conditions vary greatly and nutrient depletion occurs (Brandsma et al., 2012b).

The sampling station L4, part of the Western Channel Observatory (http://www.westernchannelobservatory.org.uk/) is a seasonally stratified site 13 km SSW of Plymouth with a water depth of ~54 m (Harris, 2010; Smyth et al., 2010). Whilst classed as transitionally stratified it is dynamic and exposed both to local coastal processes including estuarine input and pronounced inter-annual variability (Atkinson et al., 2015). Nevertheless, weekly ongoing sampling since 1988, shows strong underlying seasonality in phytoplankton community succession associated with summer stratification and nutrient depletion (Widdicombe et al., 2010).

The aim of this study was to determine the relative influence of abiotic (with emphasis on nutrient levels) and biotic (taxonomic composition) drivers of lipid composition (fatty acid and polar lipids) in phytoplankton samples at this site over one year. Redundancy analyses (RDA) was used to identify the different environmental factors which explained the variability in the lipid composition of the phytoplankton community across the different seasons, and through examination of lipid to phytoplankton biomass ratios, which were taken to indicate physiological response to nutrient stress conditions.

2. Material and methods

2.1. Sample collection and storage

The samples used for this study all came from CTD bottles fired at 10 m depth (within the upper mixed layer when a thermocline was present) and they span January–December 2013, and were collected on a weekly basis weather permitting. Data for temperature, light and salinity were collected in situ at 10 m using sensors attached to a sampling rosette deployed from the sampling vessel (R/V Plymouth Quest). An overview of the suite of measurements made each week at the L4 site (50° 15′ N, 4° 13′ W) is provided at http://www.westernchannelobservatory.org.uk/.

2.2. Phytoplankton biomass and taxonomic identification

Phytoplankton samples were represented by a single weekly sample of 100 mL of seawater (collected from a 10 L Niskin bottle fired at 10 m depth on the CTD; see above) and were preserved with 2% Lugols iodine solution. After settling for >48 h, cells were identified and enumerated microscopically (Widdicombe et al., 2010). Cell volumes were calculated according to published equations (Kovala and Larrance, 1966) and then converted to carbon estimates (Menden-Deuer and Lessard, 2000). Biomass estimates pertain only to the broad functional groups (diatoms, autotrophic dinoflagellates, coccolithophores, phytoflagellates, heterotrophic dinoflagellates, ciliates, and cyanobacteria) since counts to taxa level were not always possible. The distinction of autotrophic and heterotrophic dinoflagellates was based on the presence or absence of chloroplasts, respectively (Tomas, 1996). Functional phytoplankton groups may contain several taxa e.g. phytoflagellates contain phytoplankton pertaining to taxonomic classes *prymnesiophyceae*, *prasinophyceae*, *dictyochophyceae* etc. Since fatty acid composition is known to be correlated to specific phytoplankton class taxa (Dalsgaard et al., 2003), photosynthetic marker pigments were also used to estimate relative biomass of specific phytoplankton taxa (see below). For the picoplankton, 250 mL seawater samples from 10 m depth and collected using the same CTD, were used to determine cyanobacterial abundance using flow cytometry (Tarran et al., 2001). Cyanobacteria counts were converted to biomass using cell volume and carbon calculations as described above.

2.3. CHN, fluorescence and diagnostic photosynthetic pigment marker analyses

To quantify total particulate organic carbon and nitrogen, triplicate seawater samples (250 mL; 10 m sampling depth collected using the CTD) were pre-filtered through a 200 μm mesh, filtered onto ashed glass fibre filters (Whatman GF/F, 25 mm), dried at 60 °C and acidified with hydrochloric acid prior to analysis. All carbon and nitrogen analyses were carried out on a Thermoquest FlashEA 1112 elemental analyser. Fluorescence measurements (indicative of chlorophyll *a* concentrations), were determined by filtering 100 mL of seawater through 25 mm GF/F filters in triplicate. Samples were extracted in 90% acetone overnight at 4 °C and then analysed using a Turner fluorometer (Welschmeyer, 1994).

For pigments, 1 L water samples from 10 m depth were filtered through 25 mm GF/F filters on board the Plymouth Quest, immediately after sampling from the CTD. The filters were snap frozen and stored in liquid nitrogen until analysis. Samples were extracted under dim light conditions on ice, in 2 mL of 90% acetone by sonication (Sonics Vibracell probe, 35 s 40 W), followed by a soaking period (total extraction time = 1 h). Extracts were clarified by centrifugation (Centaur 2, 4000 rpm, 5 min), and by filtration (0.2 μm, 17 mm Teflon syringe filters,