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Abundance of a chlorophyll *a* precursor and the oxidation product hydroxychlorophyll *a* during seasonal phytoplankton community progression in the Western English Channel

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ABSTRACT

This study presents the first in-situ measurements of the chlorophyll *a* oxidation product, hydroxychlorophyll *a* as well as the chlorophyll *a* precursor, chlorophyll a_{P276} conducted over an annual cycle. Chlorophyll *a* oxidation products, such as hydroxychlorophyll *a* may be associated with the decline of algal populations and can act as an initial step in the degradation of chlorophyll *a* into products which can be found in the geochemical record, important for studying past climate change events. Here, hydroxychlorophyll *a* and chlorophyll a_{P276} were measured at the long-term monitoring station L4, Western Channel Observatory (UK, www.westernchannelobservatory.org) over an annual cycle (2012). Weekly measurements of phytoplankton species composition and abundance enabled detailed analysis of possible sources of hydroxychlorophyll *a*. Dinoflagellates, 2 diatom species, the prymnesiophyte *Phaeocystis* spp. and the coccolithophorid *Emiliania huxleyi* were all associated with hydroxychlorophyll *a* occurrence. However, during alternate peaks in abundance of the diatoms, no association with hydroxychlorophyll *a* occurred, indicating that the oxidation of chlorophyll *a* was dependant not only on species but also on additional factors such as the mode of mortality, growth limiting factor (i.e. nutrient concentration) or phenotypic plasticity. Surface sediment samples contained 10 times more hydroxychlorophyll *a* (relative to chlorophyll *a*) than pelagic particulate samples, indicating that more chlorophyll *a* oxidation occurred during sedimentation or at the sediment–water interface, than in the pelagic environment. In addition, chlorophyll a_{P276} correlated with chl-*a* concentration, thus supporting its assignment as a chl-*a* precursor.

1. Introduction

The patterns of phytoplankton population growth and decline are governed by environmental (Berges and Falkowski, 1998; Berman-Frank et al., 2004; Alonso-Laita and Agustí, 2006; Smyth et al., 2014) and biotic (Brussaard et al., 1995) factors. During population decline, phytoplankton cells may alter their phenotype, enter into a new life history stage (Rousseau et al., 2007), or die due to grazing (Walsh, 1983), viral lysis (Suttle et al., 1990; Brussaard, 2004) or senescence (Walsh, 1983). The cells' functionality and primary productivity during population decline depends on the mode of mortality or growth limiting factor; For example nutrient concentration (Elser et al., 2007; Marañón et al., 2014), temperature (Regaudie-de-Gioux and Duarte, 2012), irradiance (Strzepek et al., 2012) and seasonal ice-cover (Arrigo et al., 2014; Wang et al., 2014).

Alterations to the chlorophyll structure can take place inside phytoplankton cells, particularly when the cell is disrupted during mortality, for example via grazing, viral lysis or environmental stress (Head and Horne, 1993; Head et al., 1994; Walker and Keely, 2004; Bale, 2010; Bale et al., 2011). Allomers, which are the early oxidation products of chlorophyll, can occur in phytoplankton cells, and are thought to be precursors of sedimentary porphyrins (Baker and Louda, 1986; Keely, 2006). It has been

Abbreviations: PAR, photosynthetically active radiation; WCO, Western Channel Observatory; chl-*a*, chlorophyll *a*; HO-chl-*a*, 13^2-hydroxychlorophyll *a*; chl-a_{P276}, chlorophyll a_{P276}; HPLC, high performance liquid chromatography.

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hypothesised that chlorophyll allomers arise due to a build-up of reactive oxygen species inside the cell, when oxidative stress overwhelms the cell defence mechanisms; i.e. during periods of physiological stress which may result in loss of membrane integrity and cell death (Franklin et al., 2012). However, it is known from long term (up to 10 year) dark incubations of phytoplankton cultures, that allomerization of chlorophyll *a* can occur in anoxic conditions (Louda et al., 2011).

Chlorophyll degradation is often considered a generic stage in phytoplankton mortality, assessed by fluorescence in a bulk measurement e.g. by Turner fluorometry (Welschmeyer, 1994). However, some alteration products of chlorophyll *a* (chl-*a*) have previously been associated with specific phytoplankton fates; for example chlorophyllide *a* occurs in certain phytoplankton species (Jeffrey and Hallegraeff, 1987), and has been linked to senescence (Louda et al., 1998). Chlorophyll *a* alterations involving demetallation, i.e. the loss of magnesium, forming products such as pheophytin *a*, have been linked with zooplankton grazing (Currie, 1962) and algal senescence (Louda et al., 1998, 2002). Chlorophyll alteration products are also found in recent sediments and are part of the pathway of chlorophyll conversion into porphyrins, which are found in the geochemical record (Treibs, 1936).

Some allomers are only subtly changed from the parent molecule, have unaltered fluorescence properties, and therefore require high resolution HPLC methods for detection, however, they have been well documented in marine and lake sediments (Walker et al., 2002; Hodgson et al., 2003, 2006; Squier et al., 2004). The allomers 13^2-hydroxychlorophyll *a* (HO-chl-*a*) and Mg-purpurin-7 dimethyl phytyl ester have also been detected in pelagic marine particulate samples (Walker and Keely, 2004; Bale et al., 2015). Some chlorophyll allomers are commonly detected but not assigned during routine analysis of marine phytoplankton extracts, and are quantified together with chlorophyll *a* (Hooker et al., 2005). The production of allomers is therefore thought to be common in phytoplankton cells within a population, and may represent cells undergoing mortality. As HO-chl-*a* is an early alteration product of chlorophyll *a*, its concentration will typically scale with chl-*a* concentration in natural populations. It is hypothesised that during disruption to microalgal cells, as cell oxidative protection mechanisms breakdown, comparatively more HO-chl-*a* will be produced, hence the ratio of HO-chl-*a* to chl-*a* will increase. Measurement of HO-chl-*a* in the marine environment has been associated with the onset of phytoplankton bloom decline (Walker and Keely, 2004).

In UK shelf seas, changing environmental conditions throughout the year drive the progression of the phytoplankton assemblage through both promotion and limitation of growth. Monitoring the phytoplankton assemblage over a yearly cycle allows the measurement of allomers during the growth and decline phases of many different phytoplankton groups and individual species. When ambient conditions are conducive for growth, phytoplankton species will divide rapidly, creating blooms. These events are transient and phytoplankton populations will decline with altered conditions, like environmental limitation (Alonso-Laita and Agustí, 2006), viral infection (Jacobsen et al., 1996; Brussaard, 2004) or zooplankton grazing (Baudoux et al., 2008). The productive spring period in the Western English Channel (UK) is typically characterised by a rapid depletion of nitrate. The duration of the spring bloom is therefore controlled by nitrate availability (Smyth et al., 2010) and its species composition is typically dominated by chain forming diatoms (Southward et al., 2004; Widdicombe et al., 2010). Thermal stratification of the water column generally occurs in the summer period and a second bloom of smaller pennate or centric diatoms (e.g. *Pseudo-nitzschia* sp. or *Leptocylindrus* sp.) is usual (Widdicombe et al., 2010). A productive autumn period is typically induced in September by mixing of the water column, replenishing nitrate to the surface waters (Smyth et al., 2010).

The measurement of chlorophyll *a* allomers and other alteration products over an annual cycle may reveal patterns of occurrence linked to seasonal phytoplankton population turnover. Analysis of the population growth cycles is aided by the detection of chlorophyll a_{P276} (chl-a_{P276}), which has one additional double bond in the phytyl chain (i.e. didehydrophytyl) compared to chlorophyll *a* (Franklin et al., 2012) and is thought to be a precursor in chlorophyll *a* biosynthesis (Rüdiger, 2006; Bale, 2010). Chl-a_{P276} has been detected previously during analysis of phytoplankton cultures (Franklin et al., 2012). It therefore may be a useful proxy for phytoplankton population growth.

This work aimed to resolve the sources of HO-chl-*a* in water column particulates and surface sediment and is the first presentation of hydroxychlorophyll *a* and chlorophyll a_{P276} over an annual cycle. Changes in particulate pelagic allomer abundance (relative to chlorophyll *a*) were related to temporal changes in phyto- and microzooplankton community structure, nutrient concentration, temperature, and light levels.

2. Material and methods

2.1. Sampling protocol

Samples for this study were collected between the 9th January and the 18th December 2012 and analysed as part of the long-term oceanographic and marine biodiversity time series study at the Western Channel Observatory (WCO, www.westernchannelobservatory.org.uk), from station L4, in the Western English Channel, 13 km southwest of Plymouth Breakwater, England, UK (50°15.00′N, 4°13.02′W, Fig. 1). Water samples were collected weekly (weather permitting) using 10 L Niskin bottles mounted on a rosette and temperature was measured by weekly Seabird 19+ CTD casts, deployed from the Plymouth Marine Laboratory vessel, RV Plymouth Quest. Surface photosynthetically active radiation (PAR) was calculated from a hyperspectral irradiance sensor (Satlantic, Halifax, Canada) monitoring continuously and mounted on to an autonomous buoy, controlled using a StorX logger (Satlantic). Rainfall measurements from Camborne MET station (50°21.30′N, 5°3.00′W), ~80 km from station L4 (Fig. 1), were accessed from www.metoffice.gov.uk.

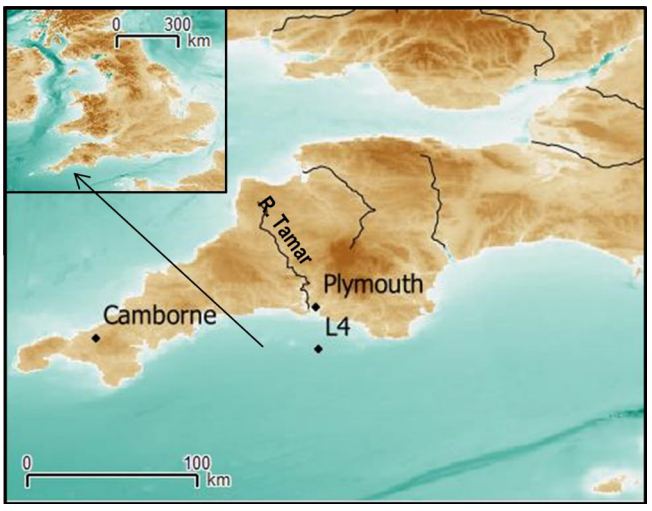

Fig. 1. Location of station L4 within the western English Channel, UK. Bathymetry map was derived from www.gebco.net.