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Shelf sea subsurface chlorophyll maximum thin layers have a distinct phytoplankton community structure

Michelle L Barnett*, Alan E S Kemp, Anna E Hickman, Duncan A Purdie

Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton
Waterfront Campus, European Way, Southampton, SO14 3ZH, United Kingdom

*Correspondence to mb16g09@soton.ac.uk

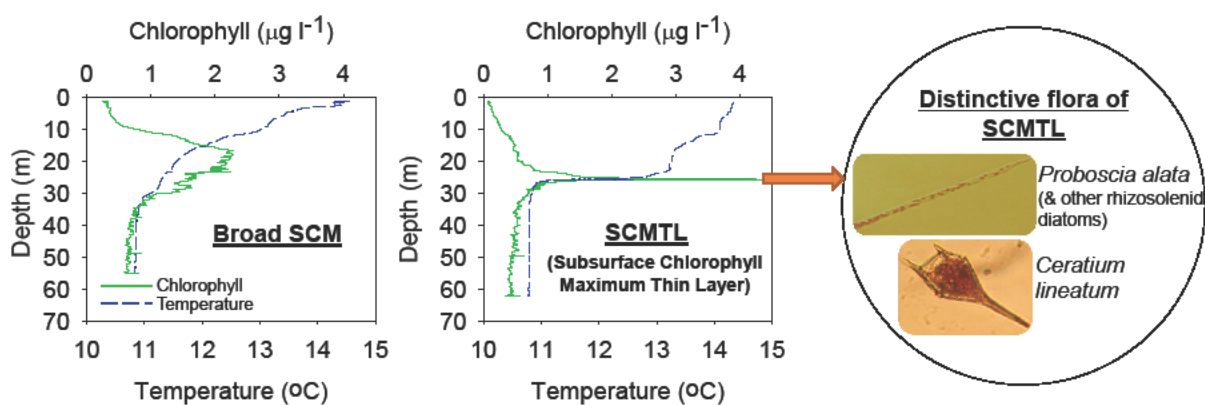
Abstract

The Western English Channel is a seasonally stratified temperate coastal sea where a subsurface chlorophyll maximum (SCM) is typically detectable within the seasonal thermocline. The SCM often develops as a thin layer (< 5 m) that may contain elevated concentrations of phytoplankton (subsurface chlorophyll maximum thin layer; SCMTL). During summer 2013 a study was conducted offshore of Falmouth, UK to assess spatial and short-term temporal variability in SCM thickness in relation to water column structure and physical conditions and to evaluate any associated changes in phytoplankton community structure. SCMTL were observed in 18 of 52 vertical profiles, typically characterised by higher chlorophyll concentrations than broader SCM. SCMTL were generally associated with a 'stepped' thermocline, likely representing the presence of one or more shallow mixed layers forming above/within the seasonal thermocline, and related to increased stratification and stability compared to broader SCM. *Pseudo-nitzschia* was almost exclusively the dominant diatom taxon in SCM, yet statistically distinct differences in community structure existed between SCMTL

and broader SCM. Within the phytoplankton, the distinction was largely due to a greater biomass of *Proboscia alata* and other rhizosolenid diatoms, and the dinoflagellate *Ceratium lineatum* in SCMTL, and a smaller population of the diatom *Chaetoceros* spp. There was also a distinction amongst heterotrophic dinoflagellates, with enhanced biomass of *Gyrodinium* spp. in SCMTL and a reduction in *Diplopsalis lenticula*. We propose that this observed difference resulted from promotion of phytoplankton better adapted to environmental conditions more specific to SCMTL compared to broader SCM. With more intense and prolonged stratification projected for the NW European shelf, there may be increased prevalence of SCMTL and the associated larger-sized specialised taxa, with implications for increased carbon export. This study adds to a growing body of evidence of the importance of SCMTL in coastal and shelf seas, and highlights the requirement for improved understanding of physical forcing, and the ecology and physiology of key taxa, particularly as predicted changes in stratification could alter the role of SCM phytoplankton in a future influenced by climate change.

Non-standard abbreviations: SCMTL – Subsurface chlorophyll maximum thin layer

Graphical Abst



Keywords: Western English Channel · Subsurface chlorophyll maximum · Thin layers ·

Thermocline · Stratification · Phytoplankton community structure

Subsurface chlorophyll maxima (SCM) are commonly identified in the seasonally stratified waters of temperate and high latitude coastal and shelf seas (Cullen, 1982, Holligan et al., 1984a, Martin et al., 2010). The introduction of continuous in vivo fluorometry fifty years ago (Lorenzen, 1966) allowed these structures to be recognised and increasing deployment of AUVs, towed undulators and autonomous profilers over the past two decades has demonstrated their ubiquity in shelf seas (Wang and Goodman, 2009, Martin et al., 2010, Sullivan et al., 2010). SCM are commonly observed within the seasonal thermocline that represents an interface at which both light availability and nutrient concentration are sufficient to support phytoplankton growth in a stratified water column (Holligan and Harbour, 1977, Holligan et al., 1984a, Sharples et al., 2001).

SCM vary widely in thickness, but in coastal and shelf seas they often occur as thin layers (SCMTL: < 5 m thick following the definition of Deksheniaks et al. (2001), with a horizontal extent of up to several kilometres and duration from a few hours to a few weeks (Bjørnsen and Nielsen, 1991, Deksheniaks et al., 2001, Durham and Stocker, 2012). Whilst methods for continuous measurement of chlorophyll in the subsurface have allowed for the consistent detection of SCMTL in a variety of coastal environments (Bjørnsen and Nielsen, 1991, Rines et al., 2010, Churnside and Marchbanks, 2015), they remain greatly under-sampled by conventional techniques, such as CTD rosette systems, so that their taxonomic composition is less well described.

Current trends of increasing ocean stratification due to warming (Bindoff et al., 2007) and localised freshening (Lyman et al., 2010) are predicted to develop further in future projections (Capotondi et al., 2012). Biogeochemical models predict a decrease in primary production and export of particulate organic carbon with increasing stratification (Steinacher et al., 2010). However, these models are highly simplified, not accurately replicating the phytoplankton community or the physics within the marine ecosystem, and their predictions have been challenged by studies conducted in stratified regions

of the modern ocean and by palaeoceanographic evidence (Kemp et al., 2006, Kemp and Villareal, 2013, Kemp and Villareal, 2018). SCM development is promoted in stratified waters, so it is important to improve our understanding of SCM ecology in the shelf seas, in particular because these regions of the marine environment are highly significant for global biogeochemical cycling and trophic dynamics (Muller-Karger et al., 2005, Jahnke, 2010, Simpson and Sharples, 2012). Moreover, shelf seas are of great socio-economic importance, since their phytoplankton form the base of food chains that support over 90 % of global fish catches (Pauly et al., 2002). Here we focus on a region of the NW European continental shelf, where more intense and persistent seasonal stratification has specifically been projected (Lowe et al., 2009, Sharples et al., 2013, Tinker et al., 2016).

There have been few detailed studies of SCM communities in the NW European shelf seas. Some SCM appear to be abundant in small cells ($< 20 \mu\text{m}$), such as $< 10 \mu\text{m}$ naked flagellates (Holligan et al., 1984b) and cyanobacteria (Hickman et al., 2009, Sharples et al., 2009), but on other occasions high abundances of much larger cells ($> 50 \mu\text{m}$), such as rhizosolenid diatoms have been reported (Holligan and Harbour, 1977, Weston et al., 2005). The occurrence of rhizosolenid diatoms, in particular, may be significant, since they are known to contribute massive flux to the sea floor (Sancetta et al., 1991, Kemp et al., 2000). It is therefore important to establish an understanding of community structure within SCMTL and broader SCM, as different taxa may have particular roles for biogeochemical processes, such as carbon transfer to depth, or to higher trophic levels and, consequently, for our predictions of how shelf sea ecosystems will respond to future climate change.

The Western English Channel has been routinely surveyed for over a century (Southward et al., 2005) and time series measurements have been made at a number of locations, most notably stations L4 and E1 (Fig. 1) via the Western English Channel Observatory (WCO) (Harris, 2010, Smyth et al., 2015). Weekly water samples from a depth of 10 m from L4 have formed the basis for several studies of phytoplankton community dynamics (Widdicombe et al., 2010, Smyth et al., 2015). However, there

has been little sampling of the SCM, although water column profiles of chlorophyll-fluorescence collected by the WCO from both L4 and E1 show that SCM are a recurrent feature of the summer stratification (at typical depths of 20 – 30 m), and studies of CO₂ fluxes have implicated the SCM in CO₂ uptake (Kitidis et al., 2012). Further afield, isolated studies have identified intense SCMTL in the southern Celtic Sea/ Western Channel (Sharples et al., 2001).

Motivated by these recent observations of a widespread SCM (SCMTL and broader SCM) in the Western English Channel, the aim of this paper is to present the first detailed study of SCMTL and broader SCM in the region. To test our main hypothesis that SCMTL community structure is distinct from that of broader SCM, we assess spatial and short-term temporal variation in phytoplankton community structure with regard to similarities/differences in community structure between SCMTL and broader SCM, and we compare to that of surface and bottom waters. We also investigate variability in vertical chlorophyll structure to evaluate controls on the occurrence of SCMTL compared to broader SCM, and relate their development to water column structure and physical conditions.

2. Methods

2.1. Sampling

The study was conducted offshore of Falmouth, UK (Fig. 1) during the summer of 2013, between the 24th of June and 4th of July, in seasonally stratified waters, with characteristic high sea surface temperatures (12.5 - 15°C; data not shown). The study area ranged from being in very close proximity to the tidal mixing front to over 30 km offshore, thus representative of a range of conditions. Note that we use the term tidal mixing front in reference to the frontal boundary that separated shallower mixed inshore waters from deeper stratified waters further offshore, as described by Holligan and Harbour (1977). Sampling strategy combined repeat sampling of individual stations, as well as a series of inshore – offshore transects (Fig. 1). Transects were completed in approximately 1.5 - 4 hours, with the exception of the 25th June transect completed in approximately 6 hours. Repeat stations were chosen

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based on past data revealing their location to be far enough away from the tidal mixing front to be permanently stratified in June/July, but also within a reasonable travel distance to enable regular sampling. These repeat stations were sampled on a daily basis (weather permitting) and in one instance an hourly basis. A SeaBird SBE19plus V2 conductivity, temperature, depth (CTD) probe mounted with a Wet Labs ECO FLNTU fluorometer was used to collect vertical water column profiles of temperature, salinity and chlorophyll-fluorescence at 52 stratified sites in the Western English Channel (Fig. 1). The configuration of the CTD package allowed for slow descent/ascent rates without slowing sensor responses, thus improving dynamic accuracy and allowing small scale structure to be resolved. Specifically, the CTD system was typically deployed at a descent/ascent rate of $0.01 - 0.1 \text{ m s}^{-1}$ (rate slowed on approach to SCM), with a data acquisition rate of 2 Hz. This CTD casting protocol was designed for very high resolution profiling, which in combination with a generally calm sea state allowed SCMTL as thin as 0.04 m to be resolved (Fig. S1) (thickness was measured at half maximum intensity of the chlorophyll peak – see 2.5, below). In addition, CTD upcasts and downcasts were consistently similar, indicating no significant disruption of SCM chlorophyll structure by the CTD. Niskin bottles used to sample this small vertical extent of the water column were 50.8 cm tall and the approximate midpoint of these bottles was aligned with the fluorometer, so we could be confident, with the CTD casting strategy, that the bottle samples included material from the narrow peaks.

Current velocity was measured with a hull mounted RDI Workhorse 600 kHz Acoustic Doppler Current Profiler (ADCP) and wind speed measurements were taken from the WCO L4 autonomous buoy situated at $50^{\circ} 15.000 \text{ N}$, $004^{\circ} 13.000 \text{ W}$ (PML 2013), approximately 40 km from the sampling area. Vertical profiles of buoyancy frequency, a measure of stratification, were computed from CTD data using SBE data processing software, where the buoyancy frequency was calculated using the Fofonoff adiabatic levelling method (Bray and Fofonoff, 1981).

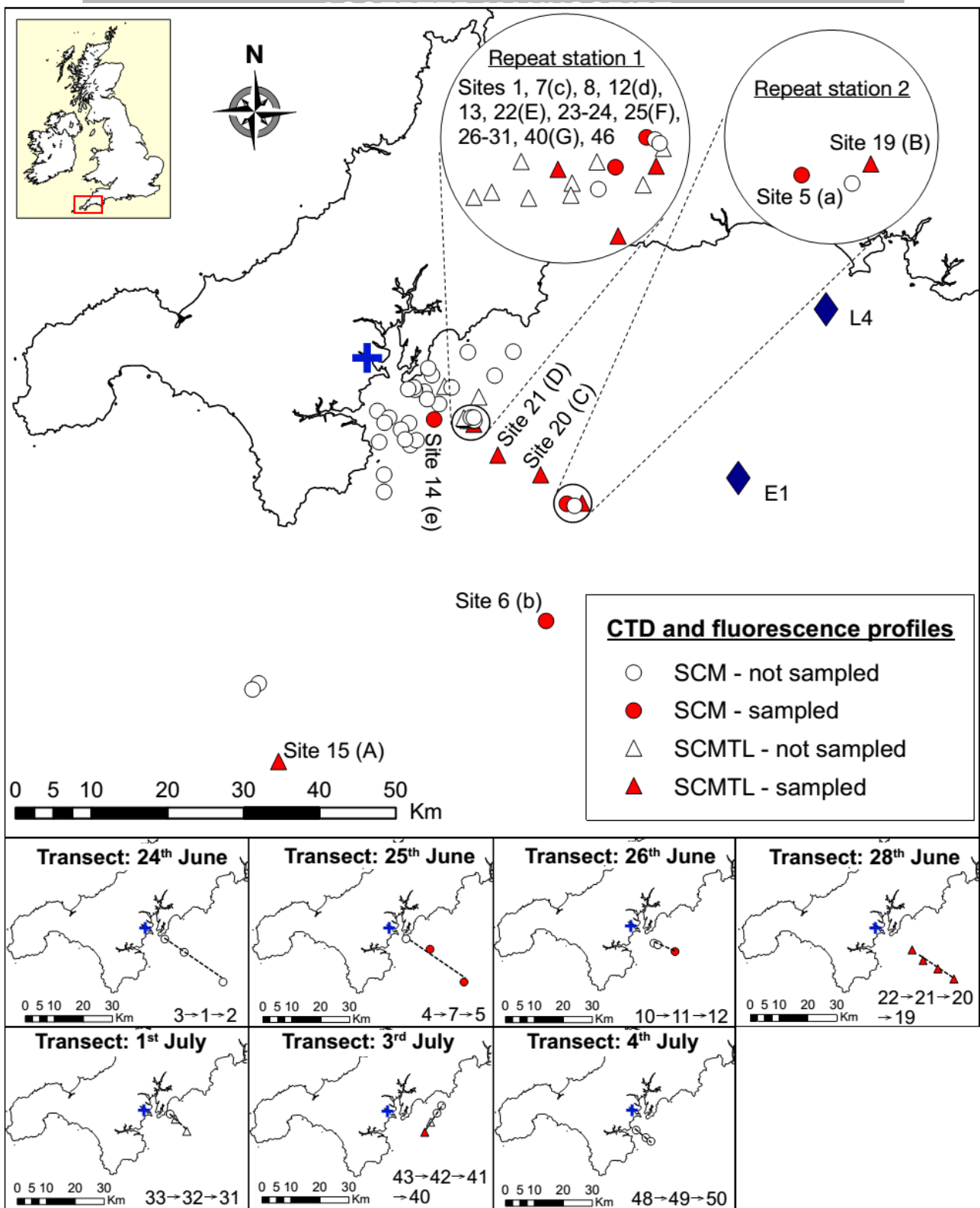


Figure 1. Study area in the Western English Channel where sampling occurred between the 24th of June and the 4th of July 2013. The blue cross indicates the location of Falmouth and the WCO stations L4 and E1 are marked with blue diamonds. Circles indicate where broader SCM were detected and triangles indicate where SCMTL were detected. Red symbols indicate sites where discrete samples analysed for phytoplankton were collected and white symbols represent sites where only a CTD profile and ADCP data was collected. Repeat sampling stations 1 and 2 are circled and all sites that were sampled for phytoplankton analysis are labelled with their site number and sample ID. Site numbers (1 to 52) were allocated in chronological order to the 52 stratified sites profiled during the study, and sample IDs were allocated to sites where

discrete samples were collected for phytoplankton analysis. An upper case sample ID indicates sampling of a SCMTL and a lower case ID indicates sampling of a broader SCM. Transects travelled during the sampling period are shown in the miniature transect maps, where a dashed black line indicates a transect travelled and site numbers included in each transect are given in order of distance from shore.

Water samples were collected from the SCM at 12 sites (Fig. S1) typically using a CTD Niskin rosette system (6 x 5 L Niskin bottles), but in one case using a custom made horizontal sampler consisting of three 50 ml syringes spaced approximately 20 cm apart, acting as a multiple 'slurp gun' (analysis performed on water sample from the middle syringe). The slurp gun was similar in design to the gradient sampler of Bjørnsen and Nielsen (1991), and was mounted on the CTD rosette frame. Water samples were also collected from the surface and bottom waters at 4 of the 12 sites (Fig. S1) using the CTD Niskin rosette system. Water samples were analysed for chlorophyll concentration, the Fluorescence Induction and Relaxation (FIRE) parameter of F_v/F_m , a measure of photosynthetic efficiency (Kolber et al., 1988), and for phytoplankton identification, enumeration and biomass determination.

2.2. Determination of chlorophyll concentration

Samples for chlorophyll analysis were collected by filtering 50 ml of water sample through 25 mm Whatman GF/F filters immediately after collection. To assess the relative contributions of $< 10 \mu\text{m}$ and $> 10 \mu\text{m}$ phytoplankton to total chlorophyll, water samples were passed through Whatman track-etched polycarbonate membrane with a pore size of $10 \mu\text{m}$ before being passed through a GF/F filter. These filters were stored in a $-20 \text{ }^\circ\text{C}$ freezer until analysis, which was conducted as soon as possible on return to the lab to avoid error associated with pigment degradation at $-20 \text{ }^\circ\text{C}$ (Graff and Rynearson, 2011). Chlorophyll was extracted in 90 % acetone via sonication and concentrations determined using a Turner Designs 10AU fluorometer (Welschmeyer, 1994). Chlorophyll values measured on the water samples were used to calibrate the fluorometer mounted on the CTD. Determining chlorophyll concentrations also allowed identification of where water samples were collected within the SCM, confirming all samples were collected from/very near to the depth of maximal chlorophyll, i.e. relative

sampling depth within SCM was consistent (Fig. S1). Therefore, we could be confident that any differences in community structure observed within SCM of different sampling casts were not simply a function of the relative depth of sampling within the SCM.

2.3. Assessment of photophysiology using FIRE measurements

Measurements of Fv/Fm were collected for SCM, bottom water and surface samples by decanting 5 ml (which was first dark adapted by storing in the dark for at least 25 minutes) into a cuvette that was analysed in the Satlantic bench top FIRE instrument. The FIRE protocol given in Bibby et al. (2008) was followed. Thirty unique iterations from the same sample were averaged, the sample delay was set at 1000 msec and the gain set at between 50 – 70 % of the sensor's saturation. Raw FIRE data was processed to generate the parameters of Fm and Fo (maximum and minimal fluorescence yield) based on the biophysical model of Kolber et al. (1988) using MATLAB R2013a. The parameter of Fv/Fm was calculated using equation 1, which includes blank correction, for which 0.7 µm filtered seawater was analysed at the start and end of each day:

$$\frac{Fv}{Fm} = \frac{(Fm_{sample} - Fm_{blank}) - (Fo_{sample} - Fo_{blank})}{(Fm_{sample} - Fm_{blank})} \quad (1)$$

2.4. Phytoplankton identification, enumeration and biomass determination

Samples for phytoplankton analysis were collected by decanting 50 ml of water sample into a darkened glass bottle and preserving in acidic Lugol's iodine to a final concentration of 1 % (analysis thus did not consider coccolithophorids). For analysis, 10 ml of preserved sample was settled in a sedimentation chamber for 24 hours and cells then identified and counted using a Brunel SP951 inverted trinocular light microscope (Utermöhl, 1958). Individual cells were counted in all cases, whether they be part of a colony/chain or solitary. Also, cells were counted irrespective of trophic status because although phytoplankton are traditionally regarded to derive nutrition through obligate photoautotrophy, some dinoflagellate and ciliate taxa are known to be mixotrophic or heterotrophic, and are routinely included

in phytoplankton counts (Olenina et al., 2006, Widdicombe et al., 2010). Therefore, the use of the term phytoplankton encompasses photosynthesising microscopic algae, including mixotrophs, and heterotrophic dinoflagellates and ciliates. Numerically dominant taxa/groups (> 50 cells per ml) were counted along a single middle transect under 100x or 250x magnification depending on cell size. Cryptophytes (> 8 μm) and unidentified small naked dinoflagellates (10 – 20 μm and 20 – 25 μm) were also counted along a single middle transect at 250x magnification. All other cells \geq 10 μm were counted at 100x magnification during examination of the entire chamber base plate. A complete list of phytoplankton taxa/groups identified by microscopy is presented in Table S1, with the authority provided where appropriate (Guiry and Guiry, 2018). It was not possible to identify most nano-phytoplankton < 10 μm or any pico-phytoplankton, so the chlorophyll concentrations of the < 10 μm and > 10 μm phytoplankton fractions of all samples analysed for phytoplankton were determined.

Cells were identified to a species level where possible, but when species could not be differentiated accurately with optical microscopy, cells were identified to the genus level, e.g. *Chaetoceros* spp. (thought to be mostly *Chaetoceros brevis* and *Chaetoceros debilis*). Any remaining unidentified diatoms were grouped as pennate or centric according to size (Table S1). Unidentified dinoflagellates and ciliates were also grouped according to size and with reference to cell wall structure where appropriate (e.g. 10 – 20 μm and 20 – 25 μm naked dinoflagellates, 10 – 30 μm armoured dinoflagellates, and small (< 20 μm), medium (20 – 40 μm) and large (> 40 μm) aloricate ciliates). Some genera were classified into size categories, including *Pleurosigma*, *Thalassiosira*, *Protoperidinium* and *Rhizosolenia* (Table S1). In the case of *Rhizosolenia*, small diameter cells appeared to be mainly *Rhizosolenia setigera*, and medium and large diameter cells appeared to be a mix of *Rhizosolenia imbricata* and *Rhizosolenia styliformis*. Note that we use the term rhizosolenids more generally in the text to encompass genera within the family Rhizosoleniaceae, including *Rhizosolenia*, *Proboscia*, *Guinardia* and *Dactyliosolen*.

Cell biovolume was calculated for each taxon/taxon size category/group using geometric shapes and formulae of Olenina et al. (2006). Dimensions of at least 30 cells per taxon/taxon size category/group (only less when rare) were measured with the open source software 'ImageJ'. Cell biovolume was converted to cell carbon biomass using the carbon - biovolume relationships of Menden-Deuer and Lessard (2000).

2.5. Definition of Subsurface Chlorophyll Maximum Thin Layers and chlorophyll intensity ratio

To distinguish a SCMTL from a broader SCM three specific criteria were used, which were based upon three requirements developed by Dekshenieks et al. (2001) and subsequently adopted by other reviews (Durham and Stocker, 2012). These were: (1) The vertical thickness of the thin layer is less than 5 m, where thickness was measured at half maximum intensity of the chlorophyll signal; (2) The peak chlorophyll concentration is at least three times greater than the background intensity values, where background values were defined as values taken from the bottom mixed layer; and (3) The thin layer is present as a persistent feature, existing in at least two repeated CTD casts. To determine if a SCM adhered to the second requirement the ratio of peak chlorophyll concentration to background chlorophyll concentration was determined and is referred to hereafter as the chlorophyll intensity ratio.

2.6. Statistical analysis

Two multiple linear regression analyses were performed using SigmaPlot 12.5 software to identify predictors of the SCM characteristics thickness and chlorophyll intensity ratio from a database of environmental data that included the physical forcing parameters of buoyancy frequency, current velocity and wind speed. Phytoplankton community structure was investigated using PRIMER v6 software (Clarke and Warwick, 2001, Clarke and Gorley, 2006). Statistical analysis was conducted on carbon biomass data as biomass provides a more accurate representation of community structure than abundance when the community consists of taxa spanning a range of different sizes, and because biomass is a more biogeochemically relevant property (Paasche, 1960). Biomass data for each

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taxon/taxon size category/group identified within the Lugol's preserved water samples (Table S1) was standardised by dividing individual biomass values by the total biomass (inclusive of autotrophs, mixotrophs and heterotrophs) identified for a given sample, then normalised by performing a square root transformation to moderate the influence of dominant taxa/groups (e.g. *Pseudo-nitzschia*) on similarity between samples. To explore similarity of community structure among SCMTL and broader SCM a cluster analysis with SIMPROF (Similarity Profile Analysis; significance level at 0.05) was performed, using the Bray-Curtis index as the measure of similarity. SCM that were most similar and significantly different from other SCM were grouped into clusters, and a SIMPER (Similarity Percentage Analysis) was performed to investigate similarities within clusters and dissimilarities between clusters. A second SIMPER analysis was conducted to further investigate the community structure dissimilarity apparent between SCMTL and broader SCM, whereby all SCMTL samples > 68 % similar were grouped to compare to broader SCM samples that were > 68 % similar. A post hoc analysis of similarity (one-way ANOSIM) was applied to determine the level of separation between SCMTL and broader SCM samples (given by global R value, where values close to 0 indicate no separation and values close to 1 indicate high separation), and a non-metric multi-dimensional scaling (nMDS) plot was used to visually display the separation between samples. Samples with greater community resemblances were spatially closer than ones that were less similar. The stress level of the nMDS plot is a measurement of how accurate a representation the ordination is, where a value below 0.2 is considered to indicate a good fit (Zuur et al., 2007).

3. Results

3.1. Distribution and characteristics of SCMTL and broader SCM

A total of 52 profiles were collected between 24th June and 4th July, with 18 profiles constituting SCMTL meeting the criteria outlined above (Fig. 1 and S1; details provided in Table S2). Broader SCM ranged in thickness from the 5 m threshold to over 20 m, while SCMTL ranged down to less than 10 cm, with most SCMTL being less than 3 m (12 of 18 SCMTL observed) (Fig. 2 and S1). Maximum

chlorophyll concentrations ranged from $1.1 - 4.5 \mu\text{g L}^{-1}$ for SCMTL and $0.9 - 3.6 \mu\text{g L}^{-1}$ for broader SCM (Fig. S1; data in Table S2), while chlorophyll intensity ratios ranged from 4.9 to 10.7 for SCMTL and were generally between 1.3 and 10.5 for broader SCM. Despite similar ranges, 72 % of SCMTL intensity ratios were > 8 , whereas 70 % of intensity ratios for broader SCM were < 8 (Fig. 2). The depth of maximal chlorophyll for all SCMTL and broader SCM was shallower than 35 m, with the majority of broader SCM (30 of 34) being 10 – 25 m deep and the majority of SCMTL (12 of 18) being 20 – 25 m deep (Fig. 3). SCM depth generally varied according to water column depth, with shallower SCM occurring in shallower water columns and vice versa (Fig. S1; data in Table S2).

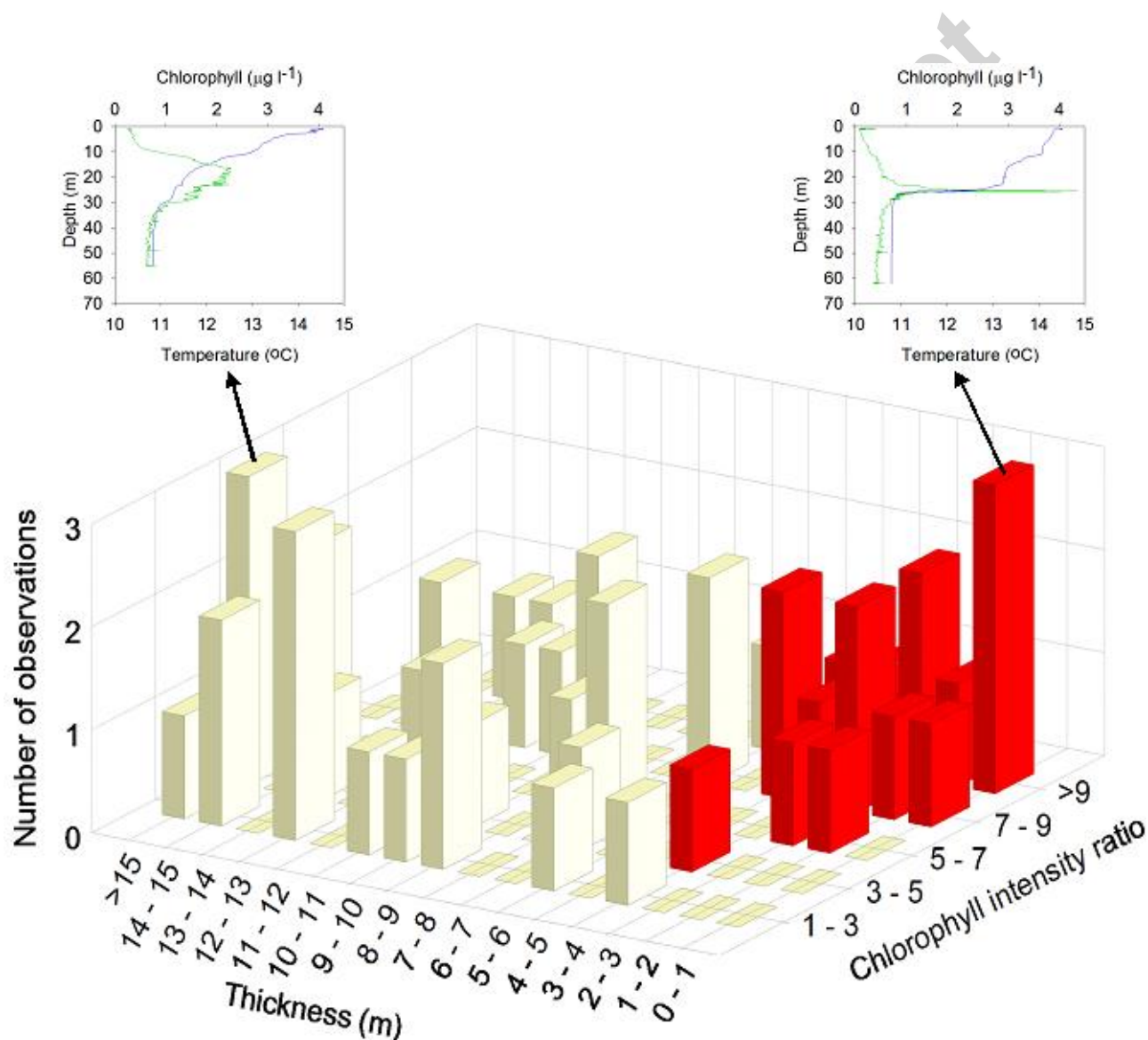


Figure 2. Chlorophyll intensity ratio and thickness of chlorophyll maxima at all 52 stratified sites profiled in the Western English Channel. Red bars represent SCMTL and yellow bars represent all other/broader SCM. Depth profiles featured are example ‘end members’:- an intense and thin SCMTL, and a less intense, broad SCM.

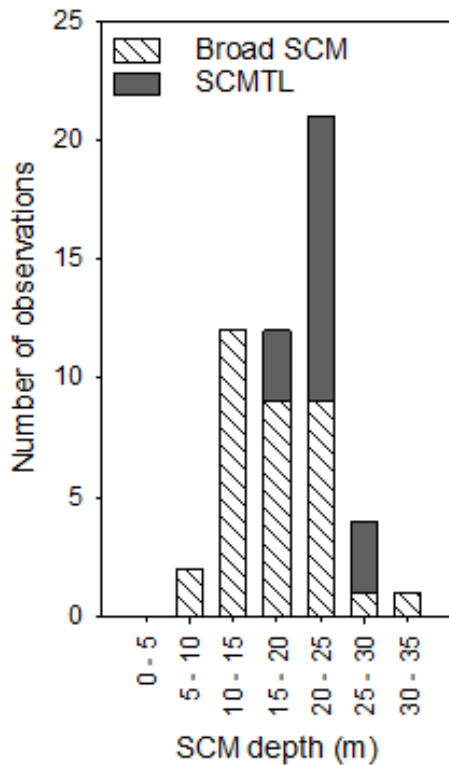


Figure 3. Depth of chlorophyll peak (at maximal intensity) at all stratified sites profiled during study of the Western English Channel. The hatched section of the stacked bars represents broad SCM and the block grey section represents SCMTL.

3.2. SCM relation to water column structure and physical forcing

3.2.1. Association with the thermocline

All SCMTL and broader SCM were closely associated with the base of the thermocline (Figs. 4, 5, 6 and S1) (also the pycnocline since density variability was dominated by temperature; data not shown). The thermocline took on three main forms, which will be referred to as (1) Gradual (one thermocline present, exhibiting a temperature change of < 1 °C over 3 m); (2) Simple steep (one thermocline present, exhibiting a temperature change of > 1 °C over 3 m); and (3) Stepped (two thermoclines present separating three layers of mixed water, where the lower thermocline typically exhibited a temperature change of > 1 °C over 3 m) (Fig. 4). The difference in temperature between the surface and bottom waters at each site (an indication of the level of stratification) ranged from 0.73 °C to 4.00 °C (Fig. S1; data in Table S2). SCMTL were most commonly associated with a stepped thermocline (14 of 18 SCMTL observed), and larger differences in temperature (14 of 18 SCMTL profiles had a surface to bottom temperature difference of > 3 °C). Whereas broader SCM were mostly associated

with a gradual thermocline (22 of 34 broader SCM observed) spanning smaller differences in temperature (30 of 34 broader SCM profiles had a surface to bottom temperature difference of < 3 °C). A gradual thermocline was often observed at sites furthest inshore, i.e. in closest proximity to the tidal front. Additionally, chlorophyll peaks with higher chlorophyll concentrations, whether they were SCMTL or broader SCM, were associated with a greater surface to bottom temperature difference (Figs. 5, 6 and S1; data in Table S2).

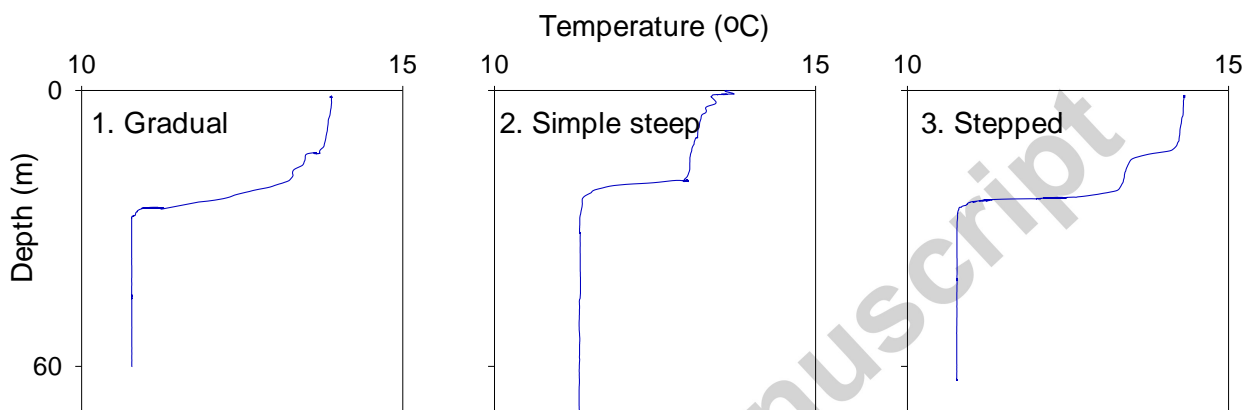


Figure 4. Example profiles illustrating the types of thermocline observed: 1. Gradual (site 22 profiled on the 28th June); 2. Simple steep (site 6 profiled on the 25th June); 3. Stepped (site 25 profiled on the 29th June).

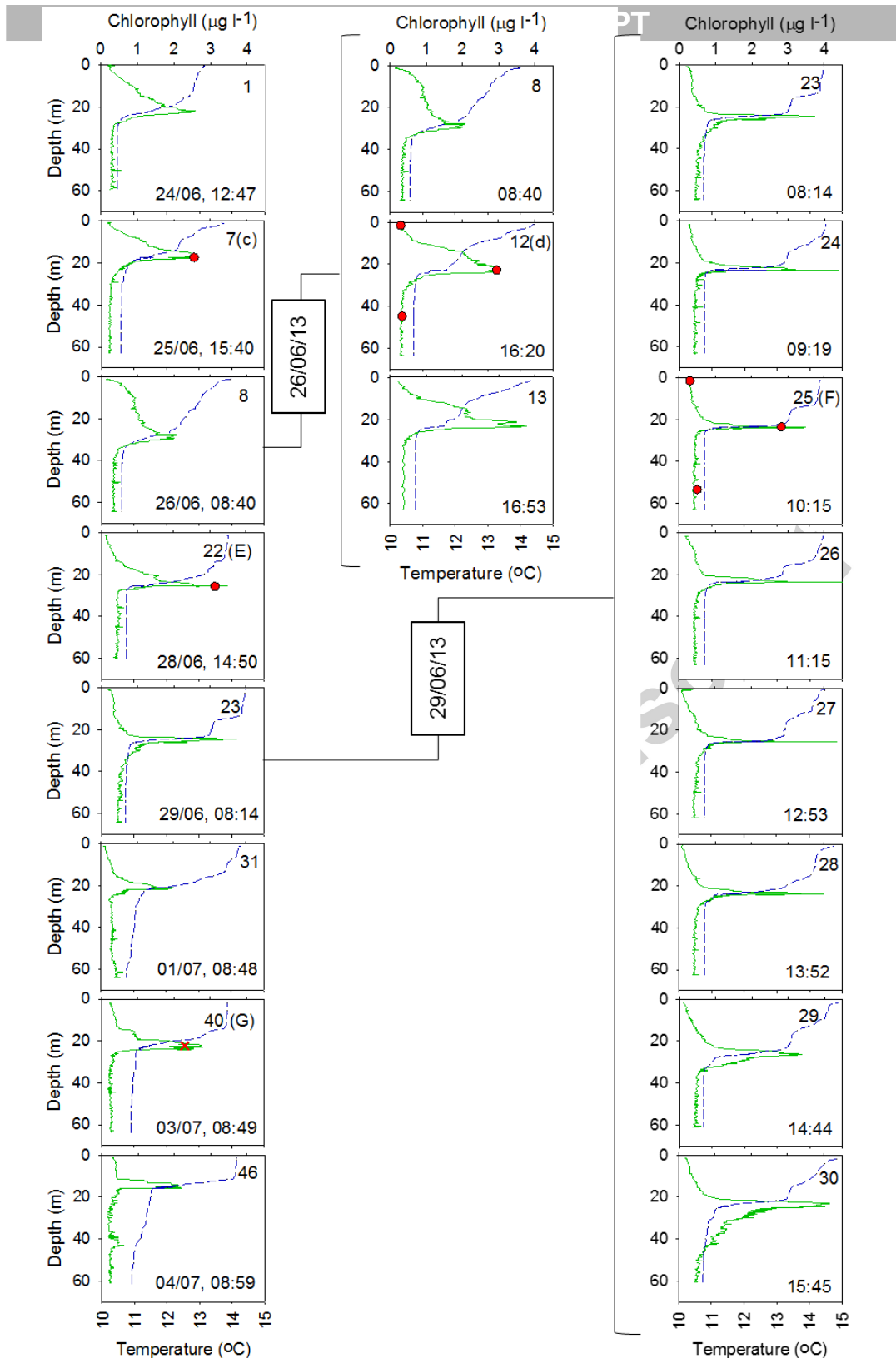


Figure 5. Temperature and chlorophyll profiles collected at repeat station 1 (profile dates and times given). Profiles in column one show water column temperature and chlorophyll structure from day to day during the survey, profiles in column two were all collected on the 26th June and profiles in column three were all collected on the 29th June at hourly intervals. Site numbers given in the top right hand corner of each plot, with sample IDs included in brackets, where an upper case ID indicates a SCMTL and a lower case ID indicates a broader SCM. The green line represents chlorophyll concentration determined from CTD chlorophyll-fluorescence, the blue dashed line represents temperature, red circles where Niskin water samples were collected and the red X where a slurp gun sample was collected.

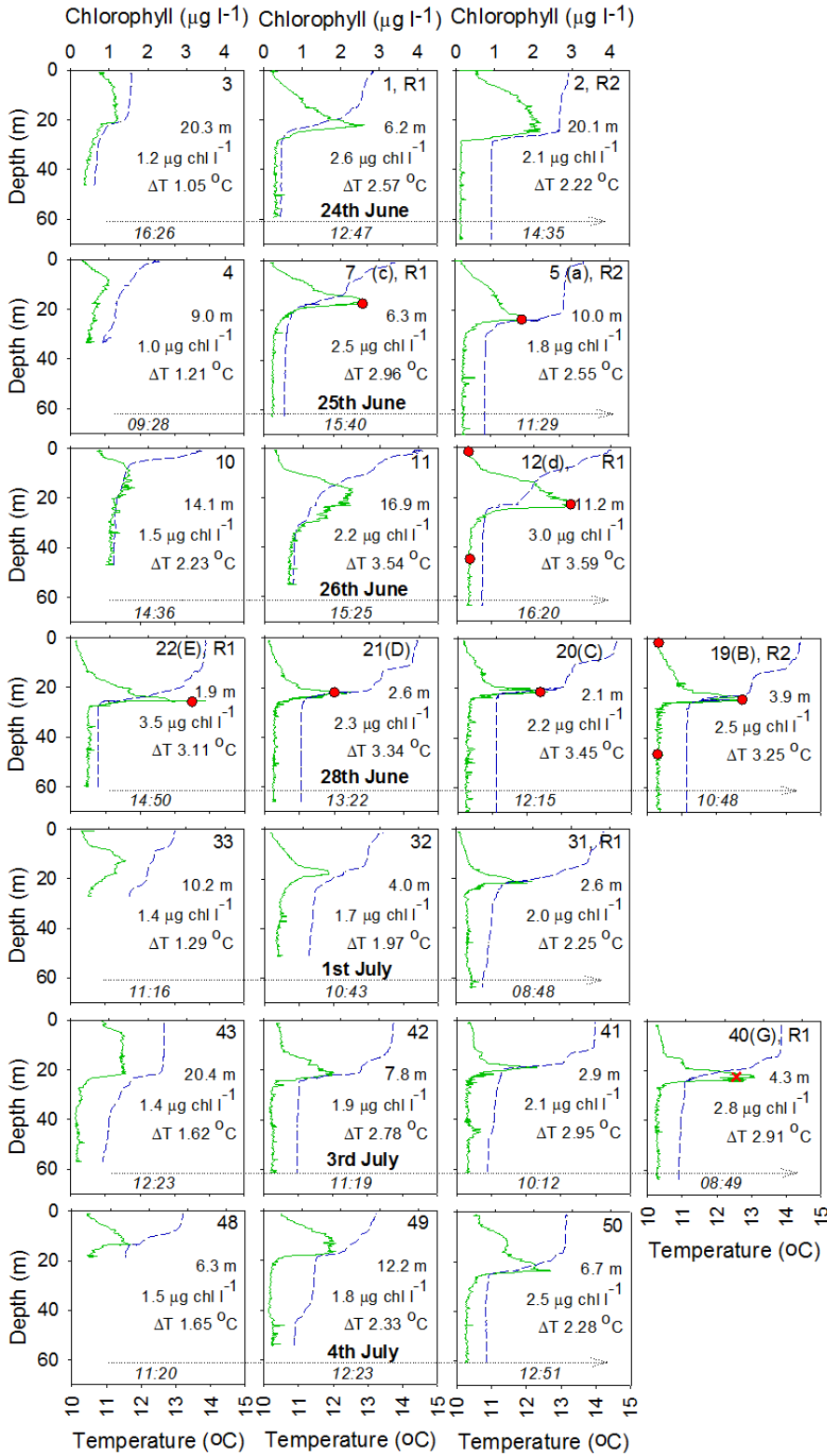


Figure 6. Temperature and chlorophyll profiles of stratified sites profiled along seven transects travelled during the survey period as indicated in Fig. 1. Site numbers given in the top right hand corner of each plot, with sample IDs in brackets, and profiles at repeat station 1 (R1) and repeat station 2 (R2) are indicated. Upper case sample IDs indicate SCMTL and lower case IDs indicate broader SCM. SCM thickness, SCM maximum chlorophyll and surface to bottom temperature difference (ΔT) for each profile is also given. Plots for each transect are shown in order of location from shore, moving offshore as arrows indicate. Therefore, these profiles represent a range of conditions, from near frontal to well established stratification. Dates and profiling times for each site are given. The green line represents chlorophyll concentration determined from CTD chlorophyll-fluorescence, the blue dashed line represents temperature, red circles where Niskin water bottle samples were collected and the red X where a slurp gun sample was collected.

At repeat station 1 (Fig. 1; sampled frequently from 24th June to 4th July) thermocline form was quite variable from day to day over the survey period, being a gradual, simple steep and stepped thermocline, with apparent progression in that order (Fig. 5). Thermocline depth (at base of thermocline) ranged from approximately 15 m to 27 m and the difference in temperature between the surface and bottom waters ranged from 2.57 – 4.00 °C (Fig. 5; Table S2). By contrast, an hourly time series over 8 hours at repeat station 1 on 29th June (site 23 – 30) showed the thermocline to remain stepped, at a depth of approximately 24 m, with a surface to bottom water temperature difference ranging only 3.52 – 4.00 °C (Fig. 5; Table S2). At repeat station 2 (Fig. 1) thermocline structure was also variable, taking on a steep and stepped form, ranging in depth from approximately 20 m to 30 m, with a surface to bottom temperature difference ranging between 2.22 – 3.25 °C (Fig. 6; Table S2). At both repeat stations the characteristics of the chlorophyll peak changed along with the characteristics of the thermocline, with sharper SCM associated with a stepped thermocline and a greater surface to bottom temperature difference.

3.2.3. Inshore – offshore transects

The depth and strength of the thermocline varied moving inshore to offshore. Along each transect travelled it was generally the case that the thermocline deepened as the water column depth increased, and the furthest inshore sites consistently had the lowest surface to bottom temperature differences (Fig. 6; Table S2). Chlorophyll structure varied along transects also, where chlorophyll peaks of the furthest inshore sites had the lowest chlorophyll concentrations and greater peak chlorophyll concentrations were generally associated with larger surface to bottom temperature differences (Fig. 6).

3.2.4. SCM relation to strength of stratification (buoyancy frequency)

All 52 profiles exhibited a buoyancy frequency maximum located within the thermocline, which ranged from 0.0001 to 0.0042 rad² s⁻². The magnitude of buoyancy frequency and SCM chlorophyll

intensity ratio were positively correlated (Fig. 7a), while a negative correlation was evident between maximum buoyancy frequency and SCM thickness (Fig. 7b). Multiple linear regression analysis demonstrated buoyancy frequency to be a significant predictor of chlorophyll intensity ratio ($p < 0.001$) and thickness ($p < 0.001$) (Table 1). Maximum buoyancy frequency values for profiles that exhibited a SCMTL were always above $0.001 \text{ rad}^2 \text{ s}^{-2}$ and reached as high as $0.0042 \text{ rad}^2 \text{ s}^{-2}$, indicating SCMTL occurred when stratification was strongest (as previously recognised through analysis of surface to bottom water temperature difference, a basic metric for stratification strength).

3.2.5. SCM relation to current velocity

Water column averaged current velocity averaged over the duration of the CTD profiling process (~ 15 minutes) ranged from 0.06 to 0.73 m s^{-1} , with no relationship with chlorophyll intensity ratio (Fig. 7c) or thickness (Fig. 7d), suggesting current velocity was not a key influencing factor in the development of SCMTL. However, current velocity data acquired at repeat station 1 over the hourly time series on the 29th June did reveal SCMTL to be thinnest ($0.04 - 2.2 \text{ m}$) during lower current velocities ($0.16 - 0.31 \text{ m s}^{-1}$), and broadest (4.5 m) during maximum current velocities of the time series ($0.38 - 0.43 \text{ m s}^{-1}$) (Table S2). Additionally all SCMTL observed at repeat station 1 occurred approaching and during the first three days of neap tides (Table S2). These observations indicate low current velocities were not a requirement for SCMTL, but do suggest some influence of current velocity over SCM structure.

3.2.6. SCM relation to wind speed

Wind speeds obtained 30 – 90 mins before each CTD profile ranged from $1.54 - 13.88 \text{ m s}^{-1}$. Wind speed was not a significant predictor of chlorophyll intensity ratio (Fig. 7e) or thickness (Fig. 7f). However, all SCMTL occurred when wind speed was less than 8 m s^{-1} , suggesting lower wind speeds may have been more favourable for the development of SCMTL.

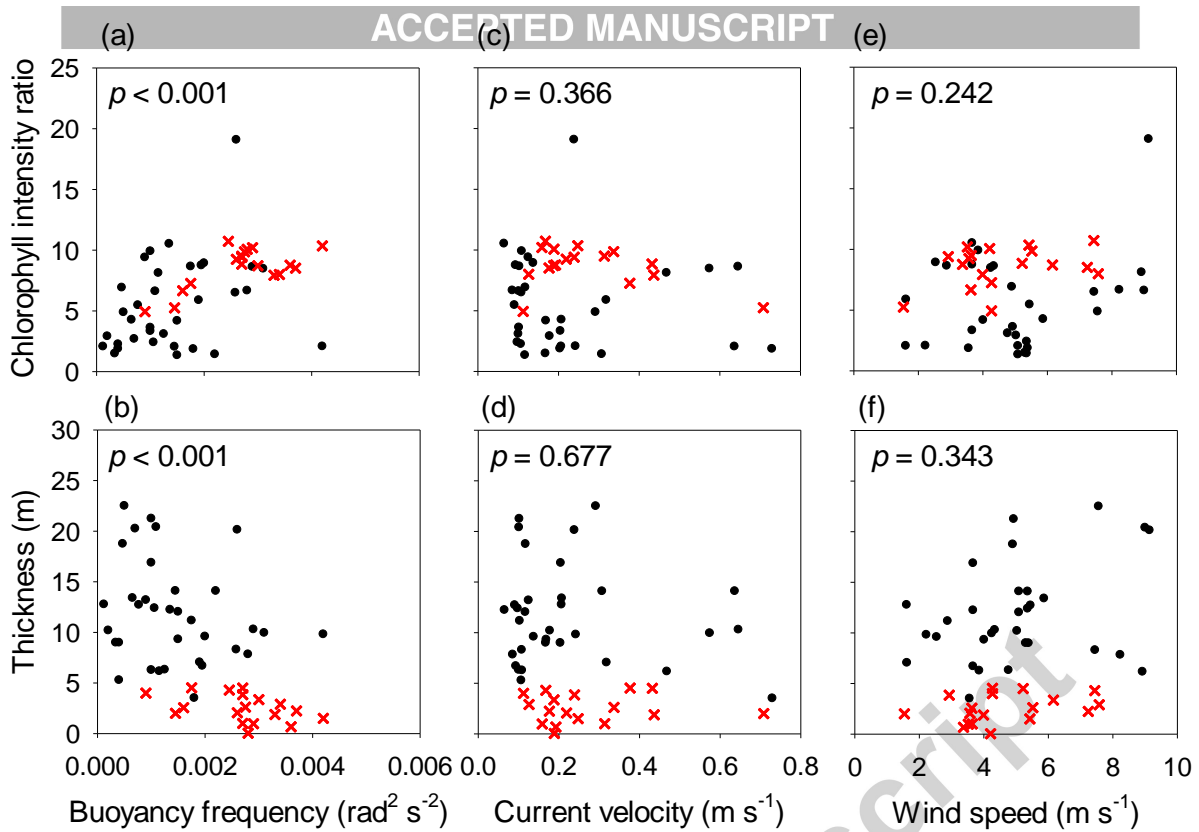


Figure 7. Relationship of SCM chlorophyll intensity ratio and thickness with buoyancy frequency (a) & (b), current velocity (c) & (d), and wind speed (e) & (f). Red crosses represent SCMTL, black circles represent other/broader SCM. p values determined by multiple regression analysis are given identifying significant predictors of intensity ratio and thickness (further details of multiple linear regression analyses given in Table 1). For panels (a), (b), (e) and (f) $n = 52$, and for panels (c) and (d) $n = 50$ (Table S2 for details of missing values).

Table 1. Significant physical forcing predictors of the SCM characteristics chlorophyll intensity ratio and thickness, as determined by multiple linear regression analysis.

	<i>Coeff.</i>	<i>T</i>	<i>P</i>
<u>Chl intensity ratio</u>			
$R^2 = 0.29$			
Buoyancy Frequency	1746	4.26	< 0.001
<u>Thickness</u>			
$R^2 = 0.30$			
Buoyancy Frequency	-2719	-4.01	< 0.001

3.3. Phytoplankton community structure and photophysiology

3.3.1. Overall water column community structure

At the four stratified sites where surface, SCM and deep waters were sampled, community structure was broadly similar throughout the water column (Fig. 8; sites 12, 14, 19, 25; sample IDs d, e, B, F). The community was a mixed assemblage, primarily of diatoms and dinoflagellates, with > 70 % of the biomass contributed by just seven taxa/groups. These were the diatoms *Pseudo-nitzschia* spp. and *Chaetoceros* spp., with *Proboscia alata/Rhizosolenia* spp. locally important, and the dinoflagellates *Ceratium lineatum*, *Protoperidinium* spp., *Diplopsalis lenticula* and small (10 – 25 µm) naked dinoflagellates (genus/species not identified) (Fig. 8). Within the diatoms, *Pseudo-nitzschia* spp. were generally dominant except in the furthest offshore sites where rhizosolenids dominated. Within the rhizosolenid diatoms, *Proboscia alata* (3 – 8 µm diameter) was dominant, with significant amounts also (mainly in the offshore sites) of *Rhizosolenia* spp., most likely *R. styliformis* and *R. imbricata*. Size-fractionated chlorophyll measurements confirmed that pigmented cells > 10 µm contributed most of the chlorophyll in all samples: specifically, between 64 – 80 % in SCM samples (65 – 79 % for broader SCM and 64 – 80 % for SCMTL), 62 – 79 % in bottom water samples, and 52 – 72 % in surface samples.

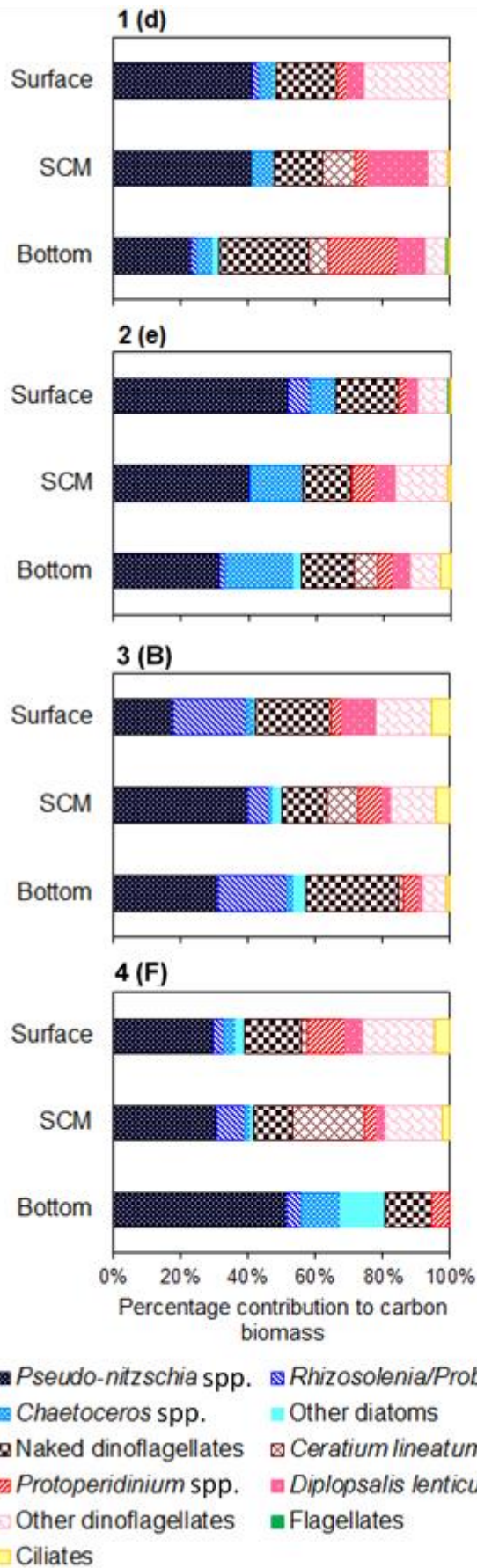


Figure 8. Community structure within the surface, SCM and bottom waters at four stratified sites (letters in brackets are sample IDs; Table S2): Percentage contribution of diatoms, dinoflagellates, flagellates and ciliates to carbon biomass identified by microscopy, where diatoms are indicated by blue colouration and dinoflagellates by red colouration (NB naked dinoflagellates are small (10-25µm) naked dinoflagellates not identified to genus/species).

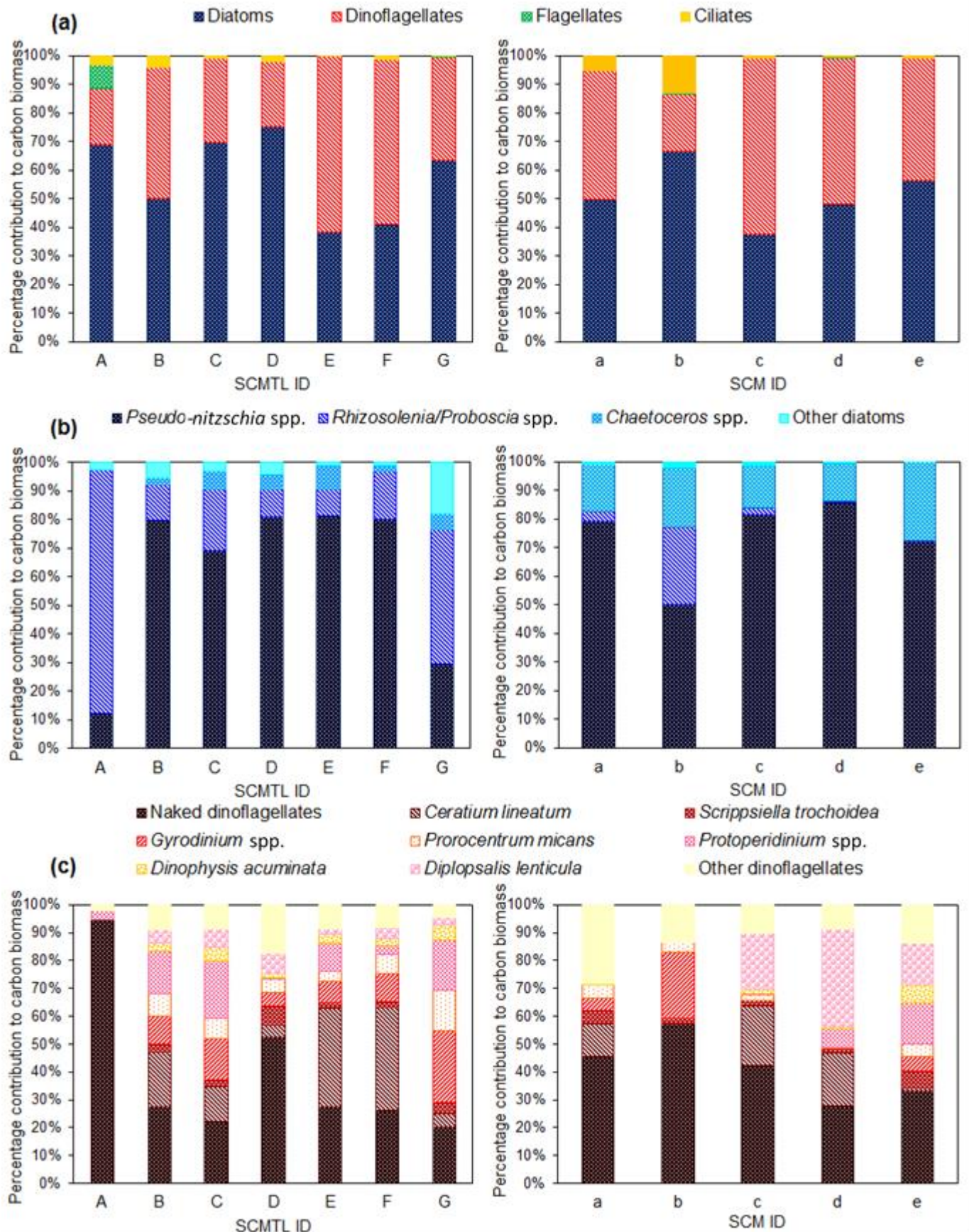


Figure 9. Community structure within SCMTL and broader SCM: Percentage contribution of (a) the diatoms, dinoflagellates, flagellates and ciliates to total carbon biomass identified by microscopy; (b) diatom taxa/groups to total diatom carbon biomass; (c) dinoflagellate taxa/groups to total dinoflagellate carbon biomass (NB naked dinoflagellates refer to small (10 - 25 μ m) naked dinoflagellates that were not identified to genus/species).

3.3.2. Overall water column photosynthetic community health

To establish photosynthetic functionality of the community sampled within the SCM, surface waters and bottom waters we assessed Fv/Fm values (Table S3). All Fv/Fm values were ≥ 0.28 indicating that there was photosynthetic functionality at all three depths sampled in the water column. More specifically, Fv/Fm increased from surface waters (mean and standard deviation of 0.34 ± 0.04 ; samples taken from 1.5 – 2 m depth) to the SCM (0.45 ± 0.03 for all SCM) and bottom waters (0.44 ± 0.06), indicating that the photosynthesising proportion of the community had a greater photosynthetic energy conversion efficiency in the SCM and bottom waters than in the surface.

3.3.3. SCMTL community structure

The community within SCMTL was predominantly diatoms and dinoflagellates, which contributed 23 – 75 % and 20 – 76 % of carbon biomass respectively (Fig. 9a). Ciliates (Fig. S2 o1 - o5) and flagellates (predominantly cryptophytes; Fig. S2 n) combined contributed less than 4 % of biomass, with the exception of SCMTL A (furthest offshore) where they contributed 11.6 % (Fig. 9a).

The diatom population was generally dominated by *Pseudo-nitzschia* spp. (Fig. S2 b1 - b3), typically contributing over 68 % of diatom carbon biomass (Fig. 9b). The exceptions were SCMTL A and G, where *Proboscia alata/Rhizosolenia* spp. (Fig. S2 a1 - a2) dominated, contributing 85.2 % and 47.0 % respectively, with *Pseudo-nitzschia* spp. only contributing 12.2 % and 29.4 % respectively. In SCMTL B – F *Proboscia alata/Rhizosolenia* spp. contributed 9 - 22 % of diatom biomass. Other rhizosolenid diatoms, namely *Guinardia flaccida* and *G. delicatula*, were, barring one occurrence, found only in SCMTL samples, but in low abundance (< 2.5 % of diatom biomass), with the exception of SCMTL G where *G. flaccida* contributed 16.8 % to diatom biomass. *Chaetoceros* spp. (Fig. S2 c1 - c4) was also a noteworthy contributor, responsible for up to 8.4 % of diatom biomass (Fig. 9b).

A mix of autotrophs, heterotrophs and mixotrophs made considerable contributions to dinoflagellate carbon biomass within SCMTL (Fig. 9c). Small (10 – 25 µm) naked dinoflagellates (genus/species not identified) (Fig. S2 j1 - j3) contributed 20.3 – 94.7 %, making the highest biomass contribution of the dinoflagellate population within SCMTL A – D. *Ceratium lineatum* (Fig. S2 d) and *Gyrodinium* spp. (Fig. S2 m) generally contributed substantially also, typically 4.5 – 37.0 % and 4.6 – 25.6 % respectively. *Ceratium lineatum* was the most dominant taxon in SCMTL E – F and *Gyrodinium* spp. in SCMTL G. Other key contributors of dinoflagellate biomass were *Protoperidinium* spp. (up to 20.8 %), *Prorocentrum micans* (up to 14.5 %), *Diplopsalis lenticula* (up to 7.6 %), *Scrippsiella trochoidea* (up to 7.0 %) and *Dinophysis acuminata* (up to 5.3 %) (Fig. 9c; Fig S2).

3.3.4. Comparison of community structure between SCMTL and broader SCM

The community within broader SCM was also a mixed assemblage primarily of diatoms and dinoflagellates, contributing 37.4 - 66.6 % and 19.8 – 61.7 % respectively. Flagellate and ciliate biomass contribution was low, between 0.06 – 0.44 % and 0.7 – 13.2 % respectively (Fig. 9a).

The overall diatom assemblage in broader SCM was similar to that of SCMTL with *Pseudo-nitzschia* spp. dominant, contributing 50.1 – 85.6 % of diatom biomass, but with different relative contributions of *Chaetoceros* spp. and *Proboscia alata/Rhizosolenia* spp. (Fig. 9b). The key difference was that rhizosolenids were a substantial component of SCMTL (9.4 – 89.3 %) but had a minimal presence in broader SCM (0.4 – 3.7 %) (except for broader SCM b, furthest offshore, where rhizosolenids contributed 28.7 %). By contrast *Chaetoceros* spp. contributed more in broader SCM (13.3 – 27.5 %), with minimal presence in SCMTL (0 - 8.4 %) (Fig. 9b). The diatom *Guinardia* spp. was almost exclusively found in SCMTL (Table S1).

The dinoflagellate composition of broader SCM was also broadly similar to that of SCMTL (Fig. 9c), but with some key differences. Small (10 – 25 µm) naked dinoflagellates made a considerable

contribution to dinoflagellate biomass, between 27.7 – 57.3 %, commonly making the largest biomass contribution of the dinoflagellate community (broader SCM a – c, e). *Ceratium lineatum*, *Scrippsiella trochoidea*, *Prorocentrum micans*, *Dinophysis acuminata*, *Protoperidinium* spp., *Gyrodinium* spp. and *Diplopsalis lenticula* remained important contributors of carbon biomass, but the contribution of some of these dinoflagellates was quite different in broader SCM compared with SCMTL. Most notably, when present *Diplopsalis lenticula* made a contribution of 14.9 – 35.2 % to dinoflagellate biomass in broader SCM compared with only 2.2 - 7.6 % in SCMTL, and *Ceratium lineatum* generally contributed more in SCMTL, responsible for 4.5 – 37 % of dinoflagellate biomass, compared with 1.3 – 21.4 % in broader SCM (Fig. 9c).

3.3.5. Statistical analyses of SCMTL and broader SCM community structure

Cluster analysis combined with SIMPROF analysis of carbon biomass from SCMTL and broader SCM distinguished six significant clusters of samples ($p < 0.05$; Fig. 10). A SIMPER analysis identified the taxa/groups responsible for the similarity within clusters (full results in Table S4). Samples from SCMTL A and G, and broader SCM b were all individually distinct and therefore placed in their own clusters. Cluster 1 only included the sample from SCMTL A, sampled at the most south westerly and furthest offshore site, approximately 40 – 50 km distant from all other sites sampled for phytoplankton. Cluster 4 only included the sample from SCMTL G, sampled at repeat station 1 on 3rd July. Cluster 6 only included the sample from broader SCM b, sampled at the second most southern site of the study, approximately 30 km from repeat station 1. Cluster 2 included samples from SCMTL B, E and F located at or within 15 km of repeat station 1, and had an average similarity of 82.0 %, with the largest contributions to group similarity made by *Pseudo-nitzschia* spp. (18.1 %), *Ceratium lineatum* (11.7 %) and 10 - 20 μm naked dinoflagellates (10.4 %). Cluster 3 included samples from SCMTL C and D, part of the 28th June transect, both located within 10 km of repeat station 1, and had an average similarity of 73.5 %, almost half of which was contributed by *Pseudo-nitzschia* spp. (27.2 %), *Proboscia alata* (10.5 %) and 10 - 20 μm naked dinoflagellates (10.4 %). Cluster 5 included the samples from broader SCM

a, c-e located at or within 15 km of repeat station 1, and had an average similarity of 72.2 %, a majority contributed by only *Pseudo-nitzschia* spp., (22.5 %) 10 - 20 µm naked dinoflagellates (13.3 %) and *Chaetoceros* spp. (9.5 %).

Community structure of cluster 1 and 6 was most dissimilar from the other clusters, between 62.5 - 70.6 % and 41.5 - 45.8 % respectively, and 50.8 % dissimilar from each other. Dissimilarity between cluster groups 2 through 5 was 29.1 - 43.2 % (Table S4). The SIMPER analysis also identified the taxa/groups responsible for the dissimilarity between clusters (Table S4).

From the cluster analysis with SIMPROF, phytoplankton community structure dissimilarity between SCMTL in clusters 2, 3, 4 and broader SCM in cluster 5 was apparent at a similarity level of 68 % (Fig. 10). An ANOSIM analysis comparing groups containing samples that were > 68 % similar identified groups to be well separated, with a global R of 0.77, and confirmed a significant difference in community structure between SCMTL and broader SCM ($p = 0.001$). An nMDS plot provided a 2D spatial representation of the separation between SCMTL and broader SCM based on phytoplankton biomass values. A stress level of 0.01 indicated the ordination to be an accurate representation of similarity among samples (Fig. 11). SIMPER analysis identified the largest contributors to dissimilarity between these SCMTL and broader SCM to be *Proboscia alata* (8.7 %), *Diplopsalis lenticula* (7.6 %), *Ceratium lineatum* (6.3 %), *Gyrodinium* (5.2 %) and *Chaetoceros* (5.0 %) (Table 2; full list in Table S4). Further statistical investigation assessing the purely autotrophic community identified a similar distinction in community structure between SCMTL (B - F) and broader SCM (a, c - e), with 35 % of the dissimilarity owing to increased *Proboscia alata* biomass but reduced *Chaetoceros* spp. biomass in SCMTL compared to broader SCM (Fig. S3 and Table S5).

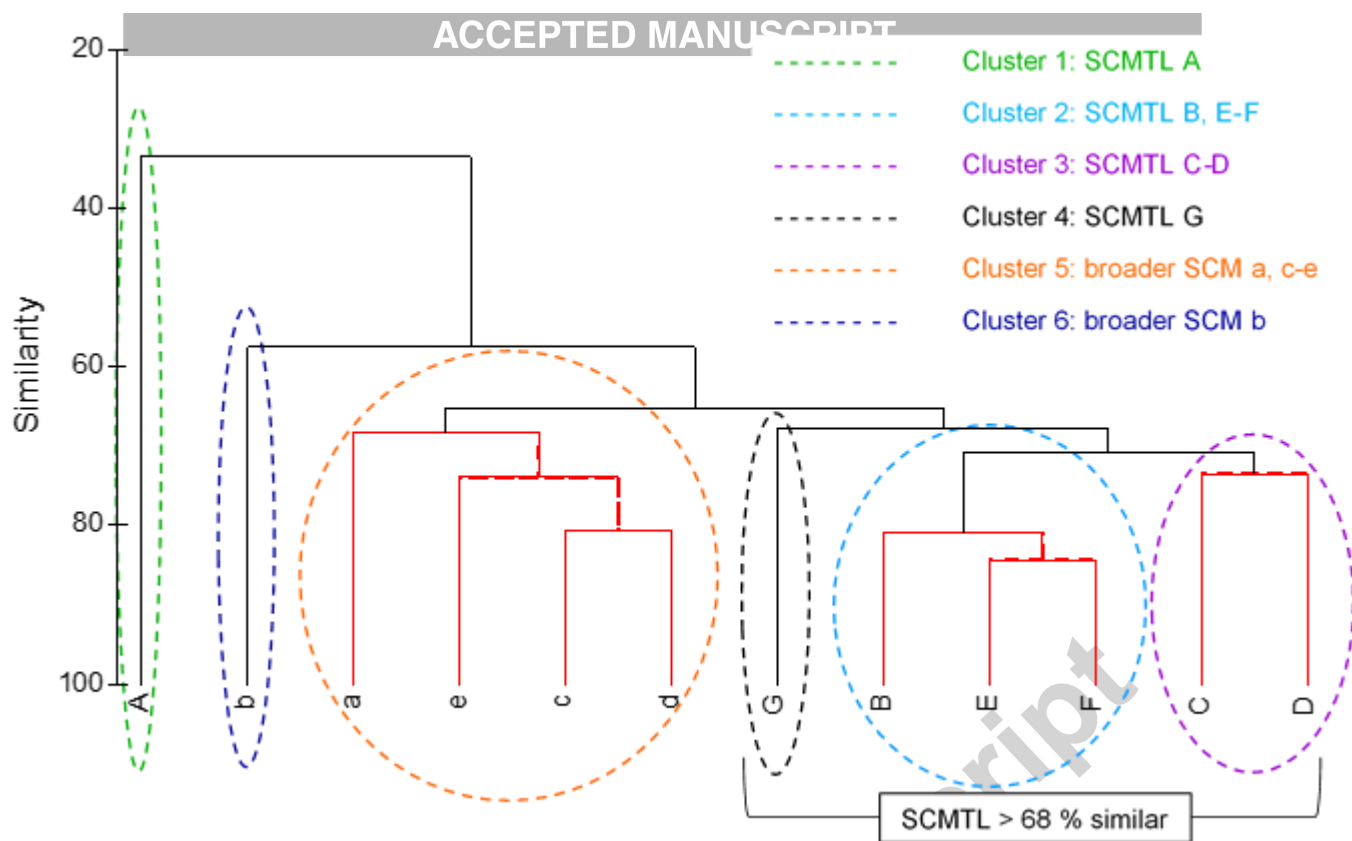


Figure 10. Cluster analysis of community structure within SCMTL and broader SCM sampled during the study, based on carbon biomass values. Red branches indicate no significant difference ($p > 0.05$) in community structure between linked SCMTL/broader SCM as determined by SIMPROF analysis. SCMTL that were $> 68\%$ similar are indicated.

3.3.6. Temporal changes in community structure at repeat station 1

At repeat station 1, an initially broad SCM observed between the 24th to the 26th June transitioned to a SCMTL thereafter (Fig. 5), and an associated temporal variation in community structure was identified. From the 28th to the 29th June (SCMTL E – F within cluster 2) the community was not statistically different, but by the 3rd of July (SCMTL G) a significant shift in community structure had occurred. This was largely due to a further increase in the *Proboscia alata* population (accounted for 14.1 % of the dissimilarity) and the development of a notable population of the large rhizosolenid diatom *Guinardia flaccida* (accountable for 13.3 % of the dissimilarity) (Fig 9 and 10; Table S4). Assessment of the autotrophic population alone identified the same significant shift in community structure caused by increases of *Proboscia alata* and *Guinardia flaccida* biomass (Fig. S3 and Table S5).

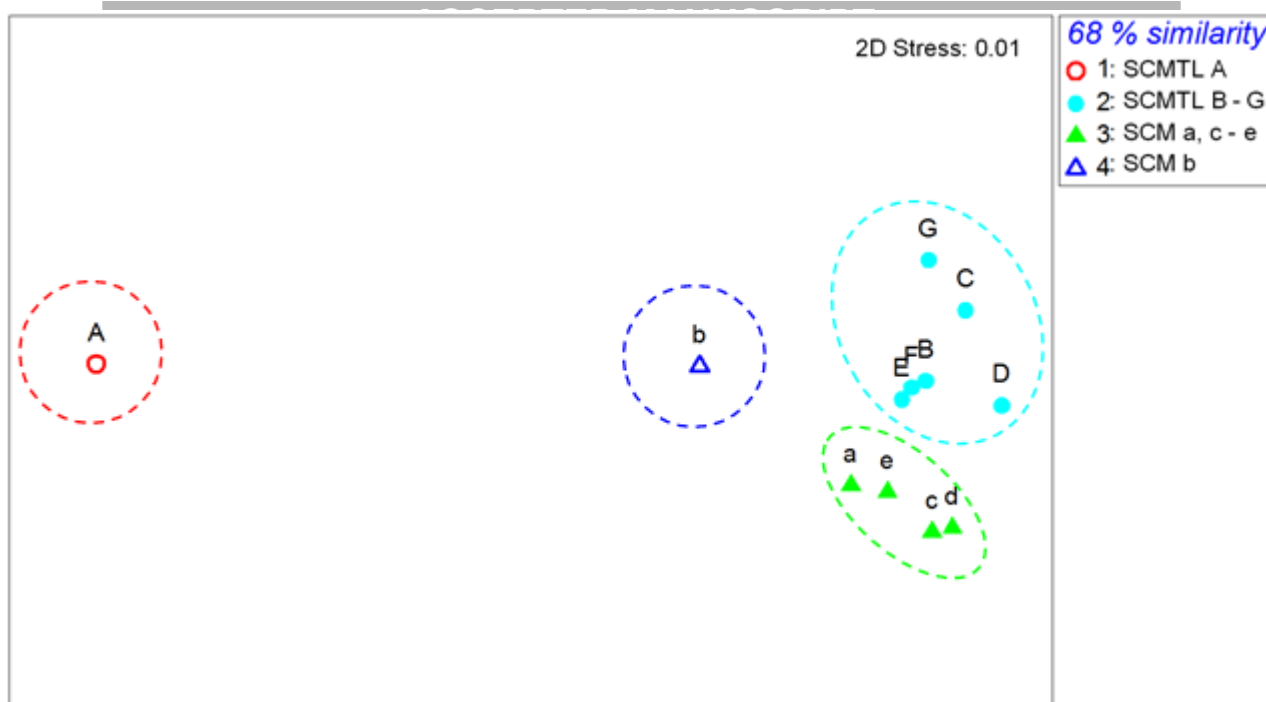


Fig. 11. nMDS plot representing the similarity in community structure between SCMTL and broader SCM samples at a 68 % similarity level based on carbon biomass values. Circular data points represent SCMTL and triangular data points represent broader SCM.

Table 2. The top five greatest contributors to the significant dissimilarity in community structure between SCMTL (cluster 2, 3 and 4) and broader SCM (cluster 5), as determined by a SIMPER analysis. Average percentage dissimilarity between SCMTL and broader SCM samples, and percentage contribution of these taxa to this dissimilarity is given. + or – symbols indicate if the taxon contributed more or less in SCMTL compared to broader SCM. Average cell volumes of the different taxa are also indicated. The full table of results provided by SIMPER analysis is given in Table S4.

Phytoplankton taxa (avg. cell volume, μm^3)	Dissimilarity (%)	
	SCMTL vs broader SCM	
<i>Proboscia alata</i> (9600)	8.73	+
<i>Ceratium lineatum</i> (15200)	6.25	+
<i>Gyrodinium</i> spp. (15195)	5.24	+
<i>Chaetoceros</i> spp. (650)	5.03	–
<i>Diplopsalis lenticula</i> (14900)	7.55	–
Cumulative contribution (%)	32.80	
Average dissimilarity (%)	34.87	

4.1. SCM relation to water column structure and physical forcing

All SCM occurred within the thermocline (Figs. 5, 6, S1), consistent with previous findings in the Western English Channel (Pingree et al., 1978, Holligan et al., 1984b, Sharples et al., 2001), as well as further afield (Deksheniaks et al., 2001, Sullivan et al., 2005, Hickman et al., 2012). The temperature structure appeared to have a governing influence over the nature of the SCM. SCMTL were generally associated with a stepped thermocline (14 of 18 instances), located at the sharp lower step, and with greater surface to bottom temperature differences. Stepped thermoclines (Fig. 4-3) may form when a sustained episode of strong winds is succeeded by a prolonged calm period, followed by a renewed windy period that mixes the uppermost warm waters and produces the new surface mixed layer and associated (upper) thermocline (Beer, 1983). Alternatively, stepped thermoclines may form in response to internal waves that propagate along the thermocline density interface, and induce shear and mixing (Orlanski and Bryan, 1969, Dale et al., 2006). The lower (main/seasonal) thermocline represents a layer of sustained stratification that has been stable for a significant time interval and, when temporarily protected from wind mixing by the presence of the shallower thermocline above, may promote the development of the SCMTL. Lateral intrusions in the thermocline have been described (Pedersen, 1994, Richardson et al., 2003, Steinbuck et al., 2010), which can also result in a stepped thermocline. However, there was no additional evidence within our dataset to support a lateral intrusion. Broader SCM were generally associated with more gradual or gentler temperature gradients (Fig. 4-1), indicative of weaker stratification with dispersal of phytoplankton over a broader depth range (Donaghay and Osborn, 1997). This relationship between thermal structure and chlorophyll is particularly evident at repeat station 1, where daily sampling demonstrated how SCM structure evolved as thermocline structure transitioned over time (Fig. 5).

Profiling along inshore-offshore transects (Fig. 6) revealed that the broadest SCM of the transects generally formed near to the tidal mixing front and at the sites furthest offshore from the tidal mixing

front, associated with smaller surface to bottom temperature differences. In the vicinity of the tidal mixing front, waters were shallower and therefore there would be a greater influence of tidally generated turbulence on stratification (Pingree, 1975), thus accounting for the weakest stratification observed, and by association, the broader and least intense SCM. The temperature profiles suggest that the tidal mixing front approached repeat station 1 during spring tides, and retracted again during neaps due to spring-neap adjustment (Pedersen, 1994, Sharples, 2008). The strongest stratification and most likely conditions for SCMTL formation close to repeat station 1 thus occurred on approach to and during neap tides. The outermost sites of the survey area were less influenced by the front and perhaps relatively more strongly affected by ambient weather conditions, particularly the prevailing southwesterly winds (Pingree, 1980, PML, 2013). Increased wind driven turbulence could account for the slightly weaker stratification and broader SCM at these sites.

All SCM were associated with a vertical buoyancy frequency maximum (Fig. 7a-b), where turbulent mixing would be reduced relative to the surface and bottom mixed layers (Fernando, 1991, Simpson and Sharples, 2012). SCMTL were specifically associated with stronger stratification (Fig. 7a-b), providing further evidence for this requirement for SCMTL formation. These findings are consistent with several studies that have used turbulence probes to show that chlorophyll maxima do not form in regions of the water column where turbulent mixing is high, but rather SCM occur at depths between turbulent energy dissipation maxima (Sharples et al., 2001, Alldredge et al., 2002, Wang and Goodman, 2009). The vertical extent of turbulence minima within the water column could therefore have played a role in governing the thickness of SCM, with SCMTL developing when the region of minimal turbulent energy dissipation became particularly focused.

Other possible mechanisms by which turbulence may control SCM include (1) chlorophyll dispersal by turbulent vertical diffusion (Donaghay and Osborn, 1997, Cowles et al., 1998); (2) increased phytoplankton settling velocity promoted by intensification of turbulence (Ruiz et al., 2004), causing

accumulation of cells in low turbulence regions below a turbulence maximum; and (3) enhanced nutrient supply from the bottom mixed layer to the thermocline driven by turbulence, promoting chlorophyll synthesis (Sharples et al., 2001, Rippeth et al., 2009, Williams et al., 2013).

In shelf seas two main sources of turbulence are wind and tides (Pingree, 1975). To gauge the relative influence of these sources of turbulence on the SCM, the relationships between current velocity and wind speed to SCM chlorophyll intensity ratio and thickness were investigated (Fig. 7c-f). No relationship between wind speed and SCM thickness or chlorophyll intensity ratio was found. However, all SCMTL occurred when wind speeds were less than 8 m s^{-1} , perhaps suggesting that SCMTL form and persist in weaker winds, potentially preventing any significant erosion of the main thermocline and dispersion of the chlorophyll peak. This finding is in agreement with that of McManus et al. (2012), who reported the most persistent SCMTL during periods of strong stratification and light winds. No relationship was identified between current velocity and SCM thickness or chlorophyll intensity ratio, although it is acknowledged that a longer measurement period would be required to capture the variability over a semi-diurnal tidal cycle (Rippeth, 2005). Nevertheless, the time series data at repeat station 1 did demonstrate some influence of current velocity on SCMTL structure and formation. During the hourly time series on the 29th June there was a general trend of increasing SCMTL thickness with increasing current velocity (Table S2). A similar relationship was also observed in the southern Celtic Sea/ Western Channel by Sharples et al. (2001), who documented a thin layer to persist over current velocities of approximately $0.2 - 0.6 \text{ m s}^{-1}$, but increase in thickness from approximately < 1 to 4 m with increasing velocity. Internal waves have been documented to modulate the thickness of SCM also (Steinbuck et al., 2010), but data available were not suitable for the assessment of effects of possible internal waves on SCM characteristics.

4.2. Phytoplankton community structure and photophysiology

The community within surface waters, the SCM and deep waters for four stratified sites consistently had photosynthetic functionality (Table S3) and was broadly similar in composition, with > 35 % of biomass consistently contributed by the diatoms *Pseudo-nitzschia* spp., *Chaetoceros* spp. and rhizosolenids (locally), and the dinoflagellate *Ceratium lineatum* (Fig. 8). Thus, the SCM phytoplankton population could have been seeded from surface phytoplankton that sank to the thermocline at the onset of stratification. However, higher Fv/Fm measurements for SCM samples compared to surface samples (Table S3) suggest that the phytoplankton within the SCM were not senescent cells settling and accumulating, but rather were photosynthetically healthier than those in the surface. The SCM was therefore likely a region of enhanced productivity and growth. This finding is consistent with many studies that have demonstrated phytoplankton can thrive within a chlorophyll maximum (Parslow et al., 2001, Sullivan et al., 2010, Hickman et al., 2012), through being photophysiologicaly well adapted to the low light SCM environment (Moore et al., 2006, Hickman et al., 2009). Below the thermocline, Fv/Fm values similar to that measured for SCM samples in combination with strong tidal flows and associated turbulence (Pingree, 1975, Pingree, 1980, Simpson and Sharples, 2012), indicates the presence of photosynthetically active cells that had been recently mixed out of the SCM.

Phytoplankton growth within the SCM was likely sustained/promoted by vertical flux of nutrients from bottom waters (Sharples et al., 2001, Rippeth et al., 2009, Williams et al., 2013). Maintenance of these phytoplankton within the thermocline may have been facilitated by alterations in cell buoyancy associated with the local environmental conditions (Alldredge et al., 2002) and/or modulation of cell sinking velocity associated with the transition in turbulent energy levels between surface waters and the thermocline (Ruiz et al., 2004). In addition, phytoplankton buoyancy regulation and motility may have played an important role in maintenance of the SCM. Diatoms were prevalent in the SCM and, although non-motile, are capable of regulating their buoyancy, with near-neutral buoyancy evident in many taxa (Waite et al., 1997, Erga et al., 2015), and positive buoyancy observed in rhizosolenids

(Moore and Villareal, 1996) and in some coastal taxa life stages (Waite and Harrison, 1992).

Dinoflagellates were also key in the SCM community, thus convergent swimming may have contributed to SCM development and intensification (Durham and Stocker, 2012, Cullen, 2015). It has been suggested that motility may explain the observed co-existence of dinoflagellates and diatoms within SCM, as it can act to compensate for some of the more modest physiological disadvantages of dinoflagellates compared to diatoms (Ross and Sharples, 2007). Specifically, motility can provide dinoflagellates with the means to respond to resource gradients within the thermocline and avoid entrainment into bottom waters.

In detail there were some significant differences between the communities of the SCMTL as compared with those of the surface, deep and broader SCM. For example, the rhizosolenid diatom *Guinardia spp.* was not identified in surface or bottom water samples, and in fact typically only occurred within SCMTL. These differences are discussed in more detail below. Spatial variation in overall phytoplankton community structure was limited, but indicated some changes offshore. Most notably, the two most southerly sites sampled, site 15 (SCMTL A) and site 6 (broader SCM b), were 60 – 70 % and 40 – 50 % dissimilar to other SCMTL and broader SCM, with a reduction in *Pseudo-nitzschia* and an increase in rhizosolenids being the principal differences. Although limited to two sites, this may suggest transition from a coastal flora to that of the more open shelf.

4.3. The ubiquity of *Pseudo-nitzschia*

Pseudo-nitzschia spp. was generally the most dominant diatom taxon in SCMTL and broader SCM sampled during the study (typically >70 % of diatom biomass; Fig. 9b), the main exception being the furthest offshore site (A). This cosmopolitan genus is commonly reported within SCM in many different coastal locations globally (Trainer et al., 2012), and is recorded annually in the Western English Channel (Holligan and Harbour, 1977, Widdicombe et al., 2010, Downes-Tettmar et al., 2013). *Pseudo-nitzschia* species local to the Western English Channel include *Pseudo-nitzschia*

pungens and *Pseudo-nitzschia delicatissima*, both of which can produce the neurotoxin domoic acid (Mos, 2001), a potential means of refuge from predation (Durham and Stocker, 2012). This capability to deter grazing along with other adaptations of the genus, including high division rates (e.g. 1.20 div d⁻¹ (Pan et al., 1993)) and phenotypic plasticity (Marchetti et al., 2006) may explain the observed dominance of *Pseudo-nitzschia*.

4.4. Key differences in community structure between broader SCM and SCMTL

The phytoplankton community structure within SCMTL (group 2/cluster 2, 3 and 4) was statistically distinct from that identified for broader SCM (group 3/cluster 5) (Figs. 9, 10, 11; Table 2 and S4). This distinction was caused by a significant difference in the biomass of a mix of autotrophic diatoms, and mixotrophic and heterotrophic dinoflagellates in SCMTL relative to broader SCM.

4.4.1. Autotrophs

The most significant differences in the diatom floras of the SCMTL were the increased abundance of *Proboscia alata*, and reduced significance of *Chaetoceros* spp. (Table 2 and Table S5). Temporally, our repeat station (R1) surveys indicated further increases of *Proboscia alata* biomass with persistence of the SCMTL. Of the rhizosolenids, *Proboscia alata* was the most significant contributor, but there were important contributions also by *Rhizosolenia* spp. (mainly *R. imbricata* and *R. styliformis*), particularly in more offshore sites (A, B, C). On one occasion at repeat station 1 (G) *Guinardia flaccida* also contributed substantially. Elsewhere *Guinardia* spp. were typically only found in the SCMTL, albeit as a minor component.

4.4.2. Mixotrophs and heterotrophs

Within the dinoflagellates, the principal difference was the relative importance of *Ceratium lineatum* and *Gyrodinium* spp. in the SCMTL and of *Diplopsalis lenticula* within the broader SCM (Table 2). *Ceratium lineatum* is primarily photosynthetic, although mixotrophy through phagotrophy of ciliates

has been documented in other species from the same genus (Smalley et al., 2003). Species within this genus are strong swimmers, with swimming speeds of up to $280 \mu\text{m s}^{-1}$ documented (Nielsen, 1991, Baek et al., 2009). *Ceratium lineatum* is also a relatively large dinoflagellate, armoured with an elongate spine and horns, and is avoided as prey by small copepods (Verity and Paffenhofer, 1996). *Gyrodinium* spp. and *Diplopsalis lenticula*, on the other hand, are heterotrophic with a preference for spherical prey typically autotrophic dinoflagellate/flagellates (Hansen, 1992), and diatom prey (Naustvoll, 1998) respectively. *Gyrodinium* spp. have been found to require relatively high prey concentrations in order to grow ($1000 - 4000 \text{ cells ml}^{-1}$) (Hansen, 1992), consistent with higher SCMTL concentrations. On the other hand, *Diplopsalis lenticula* growth has been recorded in prey concentrations of just 30 cells ml^{-1} (Naustvoll, 1998). *Gyrodinium* spp., similar to *Ceratium* spp., are strong swimmers, with speeds in excess of $200 \mu\text{m s}^{-1}$ documented (Kamykowski et al., 1992), but peridinioid dinoflagellates like *Diplopsalis lenticula* have slower swimming speeds, typically in the range of approximately $60 - 150 \mu\text{m s}^{-1}$ (Levandowsky and Kaneta, 1987, Kamykowski et al., 1989, Kamykowski et al., 1992). *Protoperidinium* spp., another heterotrophic dinoflagellate and documented to have a high grazing potential (Gribble et al., 2007), with a predilection for medium to large phytoplankton (Buskey, 1997, Menden-Deuer et al., 2005, Gribble et al., 2007), was also a more persistent minor component of the SCMTL.

4.4.3. Scenarios for the distinction in community structure between SCMTL and broader SCM

The principle differences in SCMTL and broader SCM community structure identified (as summarised in Table 2) could reflect two scenarios: (1) promotion or selection of different phytoplankton within SCMTL compared to broader SCM (species succession) relating to environmental conditions associated with SCMTL; or (2) local lateral advection introducing ‘external’ phytoplankton to the SCM community. In our study area, possible mechanisms for lateral advection relate to frontal dynamics. For example, instabilities associated with the along-front jet can lead to entrainment of water from the mixed side of the front into the thermocline, potentially providing a lateral supply of

nutrients and phytoplankton (Pingree, 1978, Pingree et al., 1979, Mork, 1981). However, such a mechanism is unlikely to be a major driver of the distinction in community structure observed because SCMTL typically occurred at locations further offshore and away from the front. On the other hand, phytoplankton within SCMTL appeared to be thriving, suggesting that they have adaptations suited to this niche that promote growth, as well as offsetting potential inhibiting factors, such as enhanced self-shading (Shigesada and Okubo, 1981, Carter et al., 1987) and increased grazing pressure associated with the more concentrated nature of SCMTL.

4.5. Adaptations of the SCMTL taxa

All three taxa (*Proboscia alata*, *Ceratium lineatum* and *Gyrodinium* spp.) that made a significantly increased contribution to the community within SCMTL compared to broader SCM were large, with relatively high aspect ratios. If photosynthetic cells were horizontally orientated within SCMTL (Malkiel et al., 1999, Talapatra et al., 2013), they could have enhanced their light absorption (McFarland et al., 2016, Nayak et al., 2018) and by association potentially their growth. Large size also makes ingestion difficult for many predators (Smetacek, 2001, Hamm and Smetacek, 2007), and large chains, such as those formed by *Proboscia alata* (Sukhanova et al., 2006), can reduce encounter rates and cause avoidance by small grazers (Beardall et al., 2009). *Ceratium lineatum* is known to be avoided by calanoid copepods (Verity and Paffenhofer, 1996) and localised episodes of anoxia have been attributed to *Ceratium* species when in high biomass (Trainer et al., 2010), a potential mechanism for limiting predation pressure when aggregated in a SCMTL.

Ecologically, both *Proboscia alata* and *Rhizosolenia styliformis* are known to be characteristic of the ‘Fall Dump’ diatom taxa that are adapted to grow and accumulate significant biomass in summer stratified conditions (Kemp et al., 2000). *Proboscia alata* has previously been recorded as a dominant component of SCM in the North Sea (Weston et al., 2005) and Celtic Sea (Gribble et al., 2007).

Furthermore, intense annually recurrent summer blooms of *Proboscia alata* similarly associated with

the thermocline at depths of 20 – 30 m are documented in a possibly analogous hydrographic setting with similar water depths on the Bering Sea shelf (Sukhanova et al., 2006). Typically, rhizosolenid mechanisms for growth at depth in stratified waters include the ability to grow in low light conditions (Goldman and McGillicuddy, 2003) and/or buoyancy regulation (Moore and Villareal, 1996, Woods and Villareal, 2008).

A further attribute of *P. alata* and other rhizosolenids, and *Ceratium lineatum* is that they have adaptations suited to environments with intermittent nutrient supply. Established diatom traits including the vacuole that enables nutrient storage (Dortch, 1982, Raven, 1997) and luxury uptake of nutrients (Sunda and Huntsman, 1995), could further enable *P. alata* to exploit the episodic nitrate supply from the bottom layer to the thermocline characteristic of shelf seas (Sharples et al., 2001, Rippeth et al., 2009, Williams et al., 2013). *P. alata* nutrient acquisition could also be enhanced in chains compared to single cells (Beardall et al., 2009). The ecology of *Ceratium lineatum* is not reported in detail, but the close relative *Ceratium furca* may undertake luxury nutrient uptake and is also known to feed through phagotrophy only in nutrient limited conditions (Baek et al., 2008), both strategies that would allow populations to subsist, pending nutrient input. Finally, based on documented swimming speeds previously detailed, both *Ceratium lineatum* and *Gyrodinium spp.* may have superior swimming abilities relative to *Diplopsalis lenticula*, which could have allowed the former to respond more rapidly to desirable environmental cues.

4.6. SCM phytoplankton in a changing climate

Future climate projections show increased intensity and duration of stratification in UK shelf seas (Lowe et al., 2009, Holt et al., 2010, Tinker et al., 2016). Increased stratification intensity will reduce the diapycnal nutrient flux (Sharples et al., 2013) and hence nutrient concentrations of open ocean waters transported onto the shelf (Holt et al., 2012), which could likely effect phytoplankton growth and primary production. Similarly, increased temperature could also affect growth and primary

production among other metabolic processes (Eppley, 1972, Raven and Geider, 1988, Moisan et al., 2002). Freshening, and changes in wind intensity and circulation have also been predicted to occur across the shelf (Lowe et al., 2009, Holt et al., 2016, Tinker et al., 2016), all of which could also influence SCM communities. The effects of climate change on the shelf seas are therefore subject to complex interactions and feedbacks among changing factors (Lowe et al., 2009, Tinker et al., 2016). Our results suggest that increased stratification could lead to increased incidence of SCMTL and the associated larger celled diatoms and dinoflagellates (Table 2). Such changes could alter trophic interactions, and potentially contribute to enhanced carbon flow to higher trophic levels and increased carbon transfer to the sediment since rhizosolenid diatoms, in particular, are known to be efficient exporters of carbon in stratified regions (Sancetta et al. 1991; Kemp et al. 2006; Kemp and Villareal 2013). Furthermore, a recent critique of current views of ocean productivity proposes a wider range of diatoms are adapted to grow in stratified waters (Kemp and Villareal, 2018). Some projections propose a shift to smaller cells in more stratified oceans (Finkel et al., 2010), but our results suggest, rather, that larger celled phytoplankton may be selected for with greater stratification.

5. Conclusion

Subsurface chlorophyll maximum thin layers (SCMTL) were identified in 18 of the 52 water column profiles collected over an 11 day period in 2013 in the Western English Channel, adding to a growing body of evidence for the recurring and persistent nature of these features in coastal and shelf seas. SCMTL were as thin as 10 cm, and typically had higher maximum chlorophyll concentrations and chlorophyll intensity ratios than broader SCM, suggesting that SCMTL are distinctive, both by vertical scale and by chlorophyll concentration. Water column structure and physical forcing had an apparent governing influence on the chlorophyll structure of SCM. SCM were closely associated with the thermocline, with SCMTL most associated with the sharp lower step of a strong stepped thermocline. A relationship between SCMTL and stronger stratification (greater buoyancy frequencies), suggests this factor to be a prerequisite for the development and persistence of SCMTL.

This study also presents the first detailed investigation of the comparative phytoplankton community structure within SCMTL, broader SCM and the surrounding water column in the NW European shelf seas. Where the whole water column was sampled, phytoplankton community composition was broadly similar with depth. However, there were some key differences in community structure within SCMTL compared to broader SCM. The distinction in community structure was largely due to a greater population of *Proboscia alata*, other rhizosolenid diatoms, *Ceratium lineatum* and *Gyrodinium* spp., and a smaller population of *Chaetoceros* spp. and *Diplopsalis lenticula* in SCMTL, suggested to relate to environmental conditions more specific to SCMTL. Our results suggest that enhanced stratification in the future could lead to increased prevalence of SCMTL, which may in turn promote higher abundances of these larger-sized specialised SCMTL diatoms and dinoflagellates. This may influence trophic dynamics, and the resulting biogeochemical implications may be significant. An improved understanding of SCMTL phytoplankton will therefore be necessary if we are to effectively assess the impacts of climate warming in the coastal and shelf environment. This will require further investigation of the physical forcing factors, and the physiology and ecology of key taxa associated with SCMTL.

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HIGHLIGHTS

Biological & physical survey of summer stratified shelf sea: Western English Channel (84)

Phytoplankton community structure of subsurface chlorophyll maxima (SCM) assessed (81)

SCM thin layers (< 5 m thick) occur with greatest stratification (64)

SCM thin layers have distinct phytoplankton community structure from broader SCM (80)

SCM thin layers may expand in importance with predicted increase in stratification (82)

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