A proxy of subsurface Chlorophyll-a in shelf waters: use of density profiles and the below The mixed layer depth below the pycnocline (BMLD) as an ecological indicator of subsurface chlorophyll-a

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Abstract

Primary production dynamics are strongly associated with vertical density profiles in shelf waters. Climate change and artificial structures (e.g. windfarms) are likely to modify the strengthvertical structure of stratification and the vertical distribution of nutrient fluxes, especially in shelf seas where the balance between mixing, and stratification defineshence the vertical distribution and production levels of phytoplankton. To understand the effect of physical changes on primary production, identifying the linkage between water column density and chlorophyll-a (Chl-a) profiles is essential. Here, the biological relevance of eight density levels (DLs) characterizing three different portions of the pycnocline (start, centre, endthe top, central aspects, and the bottom) was evaluated to find a valuable proxy for subsurface Chl-a concentrations in stratified conditions. The the best indicator of the vertical distribution of Chl-a in stratified conditions. The association of any DLs with the depth of Chl-a maximum (CMdDCM) was compared to the depth of DLs by hypothesizing their occurrence at the same depthtested using Spearman correlation, linear regression, and a Major Axis analysis. with data covering summertime over 15 years in a shelf-sea region (the northern North Sea) that exhibits stratified water columns. Out of 1237 observations of the water column densities exhibiting a pycnocline, 78% reported CMdDCM above the base ofmixed layer depth below the pycnocline (BMLD) with an average distance equal to 2.74 ± 5.21 m. BMLD appearedacts as a vertical boundary up toat which subsurface Chl-a maxima distribute in shallow waters (SCM) is most frequently found in shelf sea seas (depth ≤ 115 m), suggesting). Overall, BMLD and indicators of the halfway pycnocline highly predicted the location of DCMs than MLD indicators and maximum squared buoyancy frequency (Max N^2). These results confirm a significant contribution of deep mixing processes in supporting subsurface production under specific conditions (stratification and bathymetry). Here, we describe and advisein stratified waters and indicate BMLD as a valuable tool for understanding the spatiotemporal variability of Chl-a in shelf seas, and provide a method, and a function, . An analytical approach as a valuable tool to extrapolate itBMLD and MLD from density profiles. *in situ* vertical samples is also proposed.

Keywords

BMLD, CMd, deep mixing, MLD, pycnocline, SCML, shelf seadeep mixing, depth of Chl-a maxima (DCM), offshore renewables, primary production, subsurface Chl-a maxima (SCM)

1. Introduction

As we begin to manage our oceans and shelf seas for more complex simultaneous uses, such as renewable energy developments, fishing and marine protected areas, it is becoming increasingly important ~~understanding~~to understand the details of primary productivity at fine spatial scales. Besides very shallow waters, the vast majority of phytoplankton ~~in continental shelf waters generally grows under stratified conditions, where the pycnocline acts as a barrier against the mixing of the whole water column and allows cells to buoyance and photosynthesize within the euphotic zone. The balance between stratification and mixing in the water column is determinant for phytoplankton, and, in the North Sea, it fluctuates in time and space by the modulation of daily and biweekly strong tidal cycles (Klymak et al., 2008).~~production in continental shelf waters generally occurs under stratified conditions, where the pycnocline provides a stable habitat for phytoplankton growth in the lower euphotic zone. The balance between stratification and mixing in the water column is the main determinant for phytoplankton production and in the North Sea, the balance between mixing and stratification has been shown to fluctuate in time and space by the modulation of daily, biweekly and seasonally strong tidal cycles (Klymak et al., 2008; Sharples et al., 2006; Zhao et al., 2019b; Müller et al., 2014), which represent the main source of new nutrients' supply to the pycnocline in prolonged stratified conditions. Turbulent mixing of the water column requires energy sources from either the surface (e.g. wind stress, Ekman pump due to wind curl) or deep waters (e.g. upwelling, eddy diffusion, tidal currents), which can be altered by climate change ~~and~~(Holt et al., 2016, 2018) and the introduction of numerous man-made infrastructures (Dorrell et al., 2022). Therefore, ~~changes~~effects are expected in the overall mixing budget of our seas due to both these changes. Anomalies as circulation slow-down, sea-level rise, bottom and surface temperature, wind speed and wave height have largely been described as a consequence of climate change in the last two decades (e.g. Orihuela-Pinto et al., 2022; Taboada and Anadón, 2012; Bonaduce et al., 2019), while the consequences of these changes on the biological processes are still partially understood (Lozier et al., 2011; Somavilla et al., 2017).

1.1 Subsurface chlorophyll-a maxima ~~layers (SCMLs~~(SCM)

Many of the uncertainties regarding the impacts on primary production come from the difficulties in correctly sampling ~~the community composition and the total~~its abundance ~~throughout~~in the whole water column. Contrary to the detection of surface blooms by satellite sensors, subsurface chlorophyll-a maxima ~~layers (SCMLs~~(SCM) are often more difficult to ~~describe and~~ measure. ~~SCMLs represent~~SCM represents significant features in plankton systems (Cullen, 2015), they define where most of the bottom-up processes take place and can exist in separate vertical layers and encompass more than 50% of the entire water column production (Weston et al., 2005; Takahashi and Hori, 1984). In the North Sea, the summertime (May-August) subsurface production contributes to the annual production of up to 20-50% and ~~sustain~~sustains the food chain in continental shelf waters during prolonged stratified conditions (Hickman et al., 2012; Richardson and Pedersen, 1998; Weston et al., 2005). Several studies linked the vertical distribution of maximum chlorophyll-a (Chl-a) to deep mixing processes ~~(e.g. Brown et al., 2015; Richardson and Pedersen, 1998; Sharples et al., 2006; Zhao et al., 2019b)~~(e.g. Brown et al., 2015; Richardson and Pedersen, 1998; Sharples et al., 2006) and identified the occurrence of deep Chl-a assemblages in the proximity of the pycnocline in shelf seas (e.g. Costa et al., 2020; Durán-Campos et al., 2019; Ross and Sharples, 2007; Sharples et al., 2001). ~~The stratification is generally controlled by mixing processes (tidal mixing and surface wind stress) and sources of buoyancy (surface heating and estuarine inputs of low salinity), whose balance allow primary producers to grow in favourable light and nutrient conditions within the pycnocline. In the North Sea, mixing processes are mostly regulated by strong tidal currents (Glorioso and Simpson, 1994; Loder et al., 1992; Sharples et al., 2006, 2001; Simpson et al., 1980; Zhao et al., 2019b), especially in prolonged stratified conditions, when upward fluxes represent the only source of nutrients intake within the pycnocline.~~ Maxima

Chl-a have been identified at the base of the pycnocline in regions of strong tidal mixing at Georges Bank in August (Holligan et al., 1984) and within the western English Channel (Sharples et al., 2001). However, despite the clear linkage between ~~SCMLs~~SCM and ~~tidal mixing~~ deep physical processes in shelf seas, ~~variations on productivity~~surface mixing processes have been ~~mainly conducted at oceanic sites by investigating~~used to investigate the ~~mixing processes above the pycnocline (within the upper mixed layer)~~global variations of primary production (Somavilla et al., 2017; Steinacher et al., 2010)~~, omitting the effects of processes close to the seabed, e.g. variations of mixing processes below the pycnocline. On the other hand, studies on shelf waters suggest variations of the water column due to both surface and deep mixing processes, since the interplay of marine components from surface to seabed are more adjacent than in deep oceanic locations (Durski et al., 2004).~~ making the surface mixed layer depth (MLD) an indicator of variations of Chl-a. The use of MLD is motivated in oceanic sites where the deepest limit of the pycnocline is difficult to draw, while the limits of the pycnocline in shelf waters are more evident due to surface and deep mixings confining the pycnocline in a restricted zone.

1.2 Mixed layer depth (MLD) and pycnocline characteristics

MLD has been largely considered as a central variable for understanding phytoplankton dynamics (Sverdrup, 1953), especially in oceanic sites, where several studies have investigated the ecological relevance of MLD on Chl-a vertical distribution (Behrenfeld, 2010; Carranza et al., 2018; Diehl, 2002; Diehl et al., 2002; Gradone et al., 2020), phytoplankton bloom events (Behrenfeld, 2010; Chiswell, 2011; D'Ortenzio et al., 2014; Prend et al., 2019; Ryan-Keogh and Thomalla, 2020, Sverdrup, 1953), and the effects of climate change (Somavilla et al., 2017). The nutricline's depth exhibits positive correlations with the upper mixed layer depth (Ducklow et al., 2007; Gradone et al., 2020; Holligan et al., 1984; Prézelin et al., 2000, 2004; Ryan-Keogh and Thomalla, 2020; Yentsch, 1974, 1980), and it has been generally associated with surface spring blooms or windstorm events ~~(e.g. Banse, 1987; Carranza et al., 2018; Carvalho et al., 2017; Lande and Wood, 1987; Therriault et al., 1978). However, the effect of climate change on MLD and primary production is~~(Carranza et al., 2018; Carvalho et al., 2017). However, the effects of MLD and climate's variations on primary production are still an unsolved question (Lozier et al., 2011; Somavilla et al., 2017). The need for a much more detailed understanding of the linkage between primary production, pycnocline characteristics and deep turbulent processes (below the pycnocline) is therefore a key area of research, especially in highly productive but spatially heterogeneous areas such as shelf waters and shallow seas.

The methods for identifying MLDs vary among marine environments, hydrodynamic regimes, or the spatial resolution of vertical profiles (Courtois et al., 2017; Lorbacher et al., 2006), because making use of a single method is difficult for spatiotemporally heterogeneous regions. MLDs are typically defined as the depth at which the density exceeds a specific value (threshold) (e.g. Kara et al., 2000), however this method presents issues in specific hydrodynamic conditions, such as over estimating MLD in regions with deep convection (e.g. subpolar oceans) (Courtois et al., 2017), or misidentifying water columns with a newly established shallow MLD over previous periods of stratification (Somavilla et al., 2017). Several sensitivity tests and comparisons have been conducted in oceanic waters ~~(e.g. Carvalho et al., 2017; Courtois et al., 2017; González-Pola *et al.*, 2007; Holte and Talley, 2009)~~(González-Pola et al., 2007; Holte and Talley, 2009; Courtois et al., 2017), however, there are no standard methods for MLD identification neither in shelf nor oceanic waters.

1.3 A new way forward: the ~~base of~~mixed layer depth below the pycnocline (BMLD) as a proxy for Chl-a maximum in shelf waters

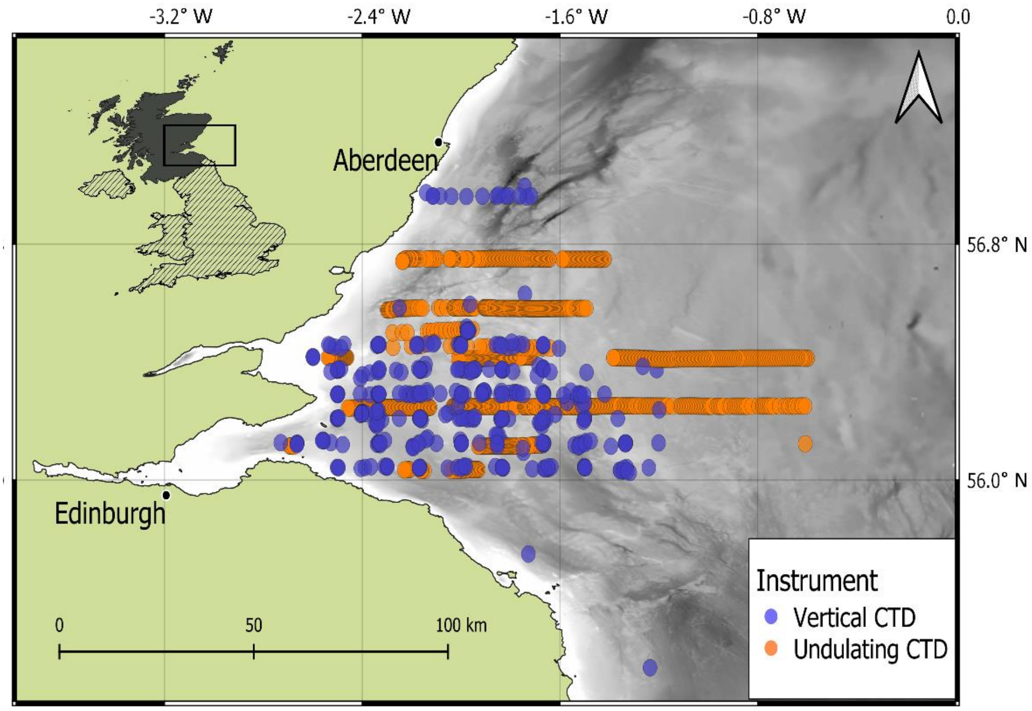
In this study, we proposed the adaptation of existing methods into a new algorithm able to cope with different high-resolution (1 m) vertical distributions of density (~~therefore being able to deal with~~characterized by split pycnoclines and unusual shapes) to ~~characterize the depth between the pycnocline and~~identify i) the surface mixed layer (commonly known as ~~“~~MLD~~”, here renamed as above~~) and ii) the mixed layer depth~~, AMLD) and ii) the~~ below ~~mixed layer depth~~ the pycnocline (BMLD~~).~~) intended as the depth at which the pycnocline ends and deep mixing develops down to the seabed. The method is validated using vertical samples of density and Chl-a to characterize the relationship between stratification features and subsurface Chl-a in waters depths from 20 to 120 m, with ~~14~~15 years of repeated surveys that covers a mosaic of habitats types: seasonal stratified waters, permanently mixed waters, regions of freshwater inputs and strong tidal mixing (Leeuwen et al., 2015). The relationships between the vertical distribution of eight different density levels (DL) and Chl-a profiles are ~~compared~~analysed using three different analytical techniques for comparisons: Spearman’s rank correlation coefficient (ρ_S), a Major Axis (MA) line fitting and ~~the biological relevance of BMLD in investigating subsurface Chl-a is detailed.~~a linear regression model (LM). This approach is being developed in order to help the identification of key linkages between the physical environment and primary production at finer spatial scales ~~(punctual location up to ≤~~(≤ 1 km), which can be ecologically relevant for pressing issues in marine spatial management (e.g. seabed leasing for wind farms, locations of MPAs) and spatially explicit climate change assessments.

2. Methods

~~Vertical samples of density and Chl-a (see Sect. 2.1) were used to characterize the relationship between stratification features (see Sect. 2.2 and 2.3) and subsurface Chl-a (described as abundance and vertical distribution, see Sect. 2.4). The most frequent methods used to identify vertical characteristics of density profiles (density levels – DLs) (see Sect. 2.3) were compared to the proposed algorithm estimating the above and below limits of the pycnocline (AMLD and BMLD, Sect. 2.2). Here, a new method to identify BMLD is proposed, and its potential is evaluated by comparing it with the vertical distribution of subsurface Chl-a during spring and summer (April-August) (see Sect. 2.5).~~

2.1 Physical and biological oceanographic samples

In situ summertime measurements of temperature, salinity, and ~~Chlorophyll-a (~~Chl-a~~)~~ were collected from a towed, undulating, CTD and a vertical CTD in the North Sea off the East coast of Scotland, UK, within the Firth of Forth (FoF) and Tay region for over ~~14~~15 years (from 2000 to 2014) (Fig. 1). A total of 1273 profiles from both types of sampling were extracted from April to August (April=3, May=51, June=1115, July=66, August=38). 426 profiles were gathered using the vertical CTD from 12 oceanographic campaigns carried out by Marine Scotland Science on board of the fisheries research vessels *Scotia* and *Alba na Mara* (www.gov.scot/marine-and-fisheries). The data set comprises temperature, conductivity, and Chl-a measurements from the sea surface to the seabed (vertical resolution equals to 1 decibar) at fixed stations sites. Water samples were collected during each cast for calibration of the *in situ* sensor data. The undulating CTD sampled the water column in June 2003 and July 2014 with a continuous vertical and horizontal oscillation of the instrument throughout the water column from 2-5 m below the sea surface to 5 m from the seabed. The continuous profiles obtained from undulating CTD were converted into 847 single profiles of the water columns. Data were sampled at 1 second intervals, resulting in a vertical resolution comprising between 0.5 and 1 m, in water depths from 25 m to 115 m. ~~More~~Further information about the oceanographic cruise in June 2003 ~~are~~is described ~~in~~by Scott et al. (2010), whose method was ~~used also~~applied in the cruise in July 2014.



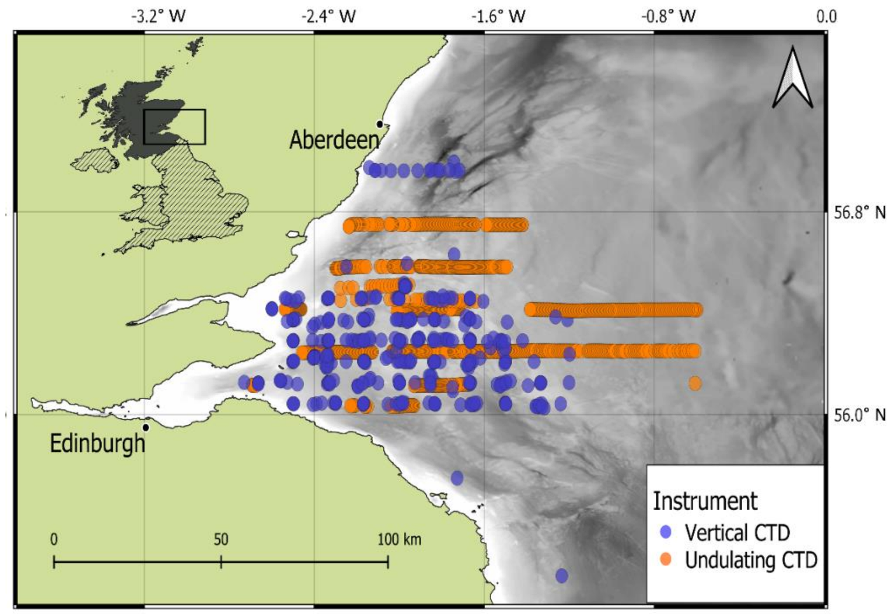


Figure 1: Study area with the in situ surveys measured by a vertical CTD (blue dots) and an undulating CTD (orange dots~~) and a vertical CTD (blue dots~~). Land (green) and bathymetry (grey colour ramp) are pictured (ESRI 2020; EMODnet 2018)

2.1.1 Standardized density profiles

Since the proposed algorithm (described in Sect. 2.2) works with profiles at high vertical resolution (samples' vertical resolution is 1 m), the *in situ* casts must be standardized throughout the water column. Density (ρ) observations taken every 0.5 to 1 m from undulating CTD were converted into measurements over regular depth intervals by smoothing and interpolating. This was achieved by fitting a generalized additive model (GAM) (Hastie and Tibshirani, 1990) using an adaptive spline with ρ as a function of depth. ~~The smoothing basis (knots) were selected in a range from 75% to 90% of~~

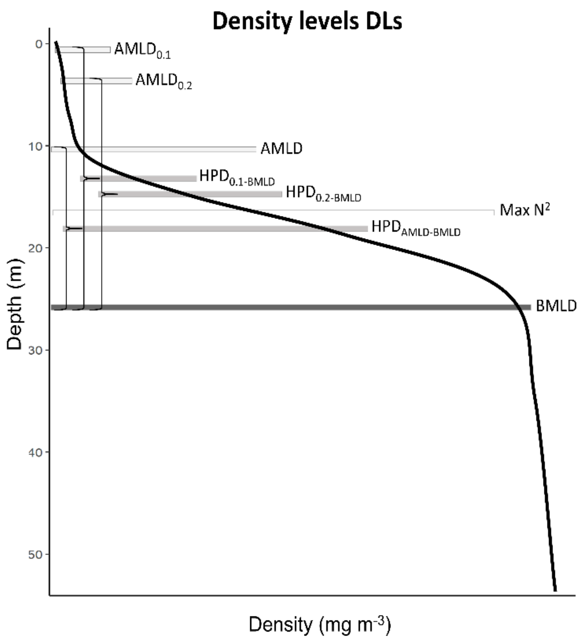
~~the number of observations occurring within each profile.~~ The obtained smooth function for each profile was used to interpolate ρ at regular 1 m depth intervals. In order to maintain the same shape and values in each profile, the fitted curves at 1 m ~~interval~~intervals were visually checked by plotting the estimated and real profiles to ~~visually~~ identify possible errors visually. 4.16% of the shapes (n=53) were manually corrected by changing the number of knots in the GAM~~.~~, which ranged from 75% to 90% of the number of observations occurring within each profile. An example is given ~~at~~in Figure A2 in Appendix A. The analyses were run in R v3.6.3 (R Core Team, 2018) using the *mgcv* v1.8-33 package.

2.2 ~~AMLD~~MLD and BMLD detection

Definition of ~~AMLD~~MLD and BMLD

In stratified waters, the layers above and below the pycnocline are mixed vertical region where the density gradient is significantly different from the pycnocline. The ~~surface~~upper mixed layer depth (~~AMLD~~MLD) and the mixed layer depth below the pycnocline (BMLD) are both ~~transitional layers from a~~the transition regions between mixed ~~to a stratified vertical region occurring at the beginning~~waters and ~~end of~~ the pycnocline~~.~~ (Fig. 2). The most common threshold methods (see Sect 2.3) identify ~~AMLD~~MLD based on the principle that the mixed layer at the surface has a density's variance close to zero, which separates from the pycnocline, exhibiting a larger density gradient. The above assumptions may not always hold, especially when the upper mixed layer is heterogeneous with nested sub-structures such as small re-stratification at the surface, or when the pycnocline can include a small mixed layer (Fig. A1a, e, f in Appendix A) or presents different density gradients (stratified layers) within it (Fig. A1b and c in Appendix A). Such density conditions are difficult to isolate with the available methods.

In the proposed algorithm, the detection of ~~AMLD~~MLD does not assume that the upper mixed layer has a density gradient close to zero up to the top of the pycnocline, and it identifies ~~MLDs~~MLD (and BMLD) regardless any *a priori* threshold~~. It also picks up the shallowest and deepest limits of the pycnocline by excluding middle breaks of the pycnocline, allowing the identification for unconventional density vertical distribution. The definition of AMLD and BMLD are based on common conventions: small and similar $\Delta\rho$ (measured as the difference between two consecutive points, $\Delta\rho_z = |\rho_z - \rho_{z+1}|$) within the mixed layers and within the pycnocline; the pycnocline is enclosed by layers of mixed water above and/or below it exhibiting a different $\Delta\rho$; the mixed layer depth is pinpointed independently from a fixed gradient~~ (Chu and Fan, 2019, 2011; Holte and Talley, 2009). Two approaches, the angle's method from Chu and Fan (2011) and K-mean statistics, are used to analyse the vertical distribution of density (ρ) by comparing the observations to each other in the same profile instead of applying an absolute threshold to all profiles. The ~~AMLD~~algorithm distinguishes in the water column three layers having similar density values (the upper mixed layer, pycnocline and lower mixed layer) (Fig. 2) using K-mean statistics. The MLD represents the ~~last depths~~ shallowest depth up to which the difference of density between adjacent points $\Delta\rho$ is ~~consistently~~ small and similar from the surface ~~to the pycnocline, while the~~. The BMLD is the first depth ~~after~~below the pycnocline from which $\Delta\rho$ is ~~consistently~~ small ~~up~~and similar down to the seabed. This type of detection based on the density shape allows the identification for unconventional density vertical distribution (Fig. A1 in Appendix A). ~~(Fig. 2).~~



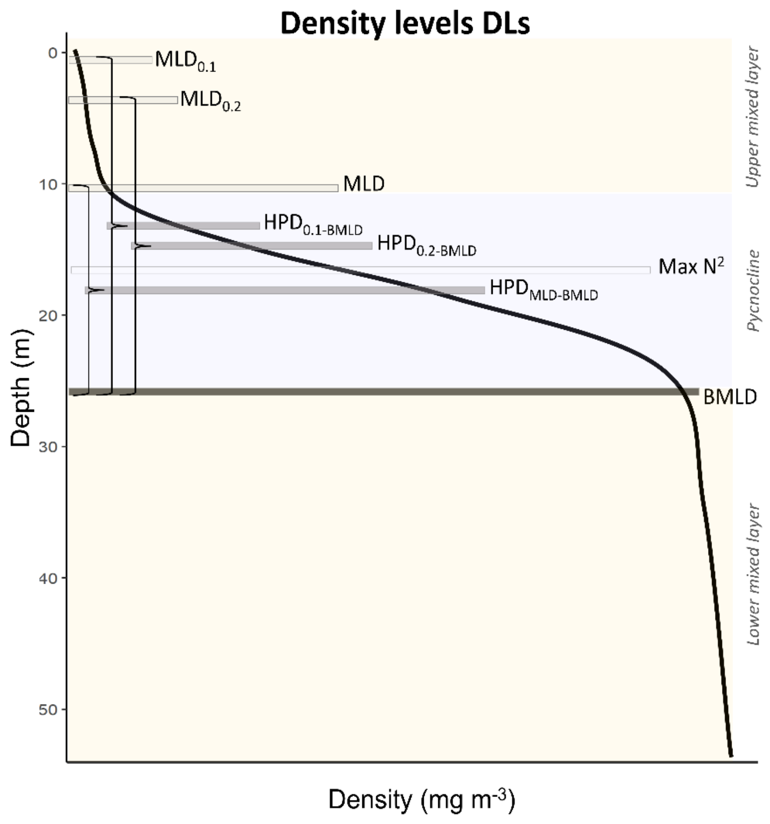


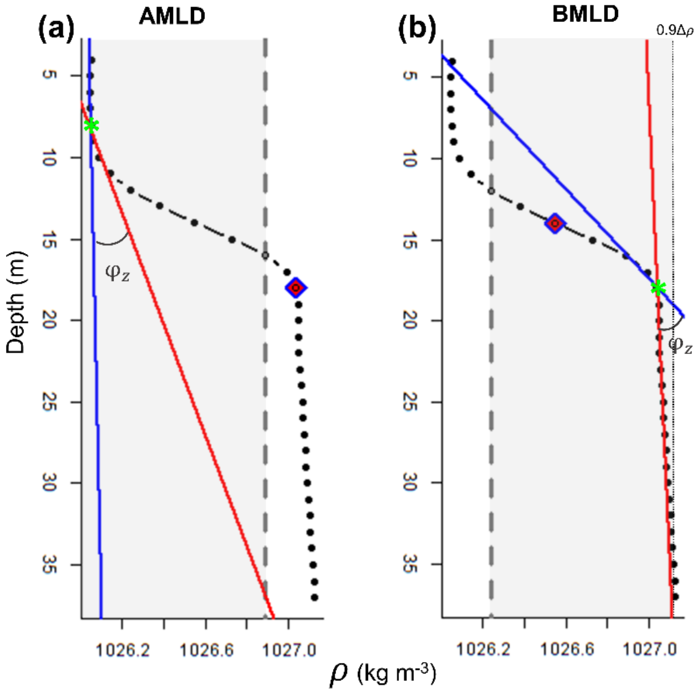
Figure 2: The eight density levels (DLs) are reported for a generic density profile~~.~~ whose upper mixed layer, pycnocline and lower mixed layer are displayed with different coloured rectangles. The curly brackets define the halfway depths (HPDs) between ~~AMLD's~~MLD's indicators (~~AMLD0~~MLD0.1, ~~AMLD0~~MLD0.2, ~~AMLD~~MLD) and BMLD.

Method to extract ~~AMLD~~MLD and BMLD

~~AMLD and BMLD have been identified developing an algorithm based on Chu and Fan (2011) framework to produce a method able to cope with various density profiles exhibiting a pycnocline (examples in Fig.~~ MLD and BMLD were identified by developing an algorithm in R v3.6.3 (R Core Team, 2018) named *abmld.R*, and available at https://github.com/azampollo/BMLD, that analyses the shape of the density profile and distinguishes a mixed from~~A1 in Appendix A). The algorithm's sequence identifies the depth with the largest density difference between a mixed and~~ a stratified layer using i) an adaptation of the maximum angle method (Chu and Fan, 2011) and ii) a cluster analysis on the density difference ~~at each observed depth~~ between two consecutive points ($\Delta\rho_z = |\rho_z - \rho_{z+1}|$). The method is designed to work with equal, high-resolution~~,~~ (1 meter), intervals of density values (z) collected in ~~the profiles. In order to distinguish AMLD from BMLD, their selection is achieved~~stratified shelf waters, with a pycnocline detailed by ~~splitting the number of observations throughout the profile into two distinct groups, *Split1* and *Split2* (Fig. 3), each one respectively used to identify AMLD and BMLD. *Split1* includes the density~~ > 5 values, and BMLD distributed within the first 90% of the observations from the surface ~~(z_1) to two measurement intervals (δ, here 1 m) above BMLD ($z_{BMLD} - 2\delta$); *Split2* extends from 2δ above the halfway depth in ρ range ($0.5\Delta\rho = ((\rho_{max} - \rho_{min})/2) - 2$) to the ninetieth portion of the profile from the surface~~to the deepest point (close to the seabed ~~($z_{0.9\Delta\rho}$ = 90% of $^{n}_{1}z$) (Fig. 3). For all depths between z_1~~). The reason why the method is sensitive to the number of points within the pycnocline, before MLD and after BMLD, is due to the analyses included in the algorithm depending on at least two observations at the beginning of each mixed layer.

The first steps of the algorithm follow the method by Chu and ~~$z_{0.9\Delta\rho}$, the angle φ has been measured at $z(x, y)$ (~~Fan (2011) where ~~x and y are density and~~the depth~~)~~ exhibiting the maximum angle between two vectors ~~(~~, referring to density conditions above and below it, is selected as the mixed layer depth. At each observation (z) of the density profile, the method calculates the angle (φ) from the intersection of two vectors, V1~~,~~ and V2~~) fitting~~, each one fitted using a linear regression ~~(y ~ x)~~.model that accounts for the vertical distribution of the density values above (for V1) or below (for V2) z. At each z of the density profile, a unique V1 (blue line in Fig. 3) is fitted using z and 2 points (2δ) above it, and a unique V2 (red line in Fig. 3) is fitted using z and 2 points below it. The angle (φ) resulting from the intersection of the two lines is measured in degrees using Eg. 1 reported in Supplementary material. A value of φ is hence associated with each point of the density profile. Although Chu and Fan (2011) suggested to ~~measure the tangent of the angle between V1 and V2 (φ), we encountered some issues identifying BMLD in those profiles where density decreases below the pycnocline (Fig. A1d, Appendix A) and φ is bigger than 90 degrees. However,~~identify MLD by measuring the tangent of the angle between V1 and V2, we encountered some issues identifying BMLD in those profiles where φ was bigger than 90 degrees, and where density slightly decreased below the pycnocline (Fig. A1d, Appendix A). At this point, an angle φ is associated with each observation in the density profile. Since the identifications of MLD and BMLD are both based on the ranking of φ, the selection of either one or the other requires splitting the density profile into "surface" (Split1) and "deep" (Split2) observations to avoid any misidentification and interchange between mixed layer depths. *Split1* includes the density values from the surface (z_1) to two measurement intervals (2δ) above BMLD (Fig. 3a), while *Split2* extends from 2δ above the halfway depth in ρ range ($0.5\Delta\rho = ((\rho_{max} - \rho_{min})/2) - 2$) to the ninetieth portion of the profile from the surface to the seabed ($z_{0.9\Delta\rho}$ = 90% of $^{n}_{1}z$) (Fig. 3b). The bottom limit of Split2 was defined at $z_{0.9\Delta\rho}$ following Chu and Fan (2011) to reduce the number of observations close to the seabed. However, the analyses can be extended up to the end of the profile by following the instructions reported at https://github.com/azampollo/BMLD.

After the selection of the largest angles as potential MLD and BMLD, a further K-Mean cluster analysis (Lloyd, 1982) was used to identify the mixed and stratified layers based on the density difference between two consecutive points ($\Delta\rho_z$). The cluster analysis satisfied the assumption that similar observations belong to either the mixed or stratified layers. MLD and BMLD were hence selected above the candidates whether the observations above and below them belonged to the same cluster. More details regarding the decisional tree of the algorithm are reported in the Supplementary materials. Adding the conditions controlling for a similar classification of observations at depths above MLD and below BMLD decisively improved the selection of pycnocline's limits in pycnocline fractured in chunks. Moreover, several trials reported that the exclusive use of the maximum angle method would have biased the selection due to local variation and instability conditions of the water column (Fig. A1b, c, e, f in Appendix A). ~~Therefore, a K-Mean cluster analysis (Lloyd, 1982) was adopted in the algorithm to improve the selection of the pycnocline limits by classifying the density difference at depth ($\Delta\rho_z = |\rho_z - \rho_{z+1}|$) into groups. The use of K-mean meets the assumption that $\Delta\rho_z$ values within a mixed layer would belong to a unique cluster. Adding the conditions controlling for a similar classification of $\Delta\rho_z$ at depths above AMLD and below BMLD resulted in decisive outcomes, correctly identifying the mixed layers within those density profiles having a pycnocline fractured in chunks with different or similar gradients. The algorithm was developed in R v3.6.3 (R Core Team, 2018) and it is available as a function (*abmld.R*) to download from GitHub (https://github.com/azampollo/BMLD). A more detailed description of the method is also reported in Supplementary Materials at the GitHub page.~~



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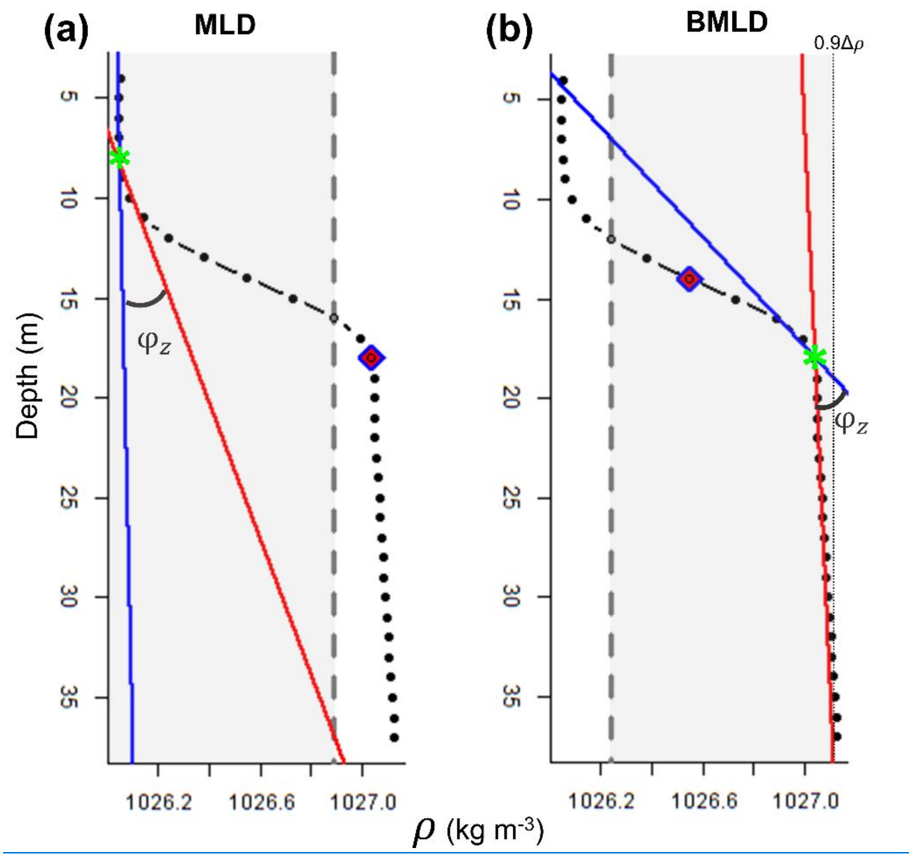


Figure 3: plots of a density profile reporting the attributes calculated by the algorithm: grey region includes the observations (z) (black dots) used to identify ~~AMLD~~MLD and BMLD, which extends in (a) from the surface to 2δ ~~before~~above BMLD (purple rhombus), and in (b) from 2δ ~~before~~above half of the ~~reference point~~density range reported by the profile (0.5Δρ, purple rhombus) to 0.9Δρ. The solid blue and red lines refer to the vectors V1 and V2~~ reporting~~, whose intersection defines the angle φ_z, ~~used to identify AMLD~~selected as MLD and BMLD (green stars).

Performance of the algorithm

~~Following the assumptions described above, the~~ The algorithm ~~failed to correctly identify AMLD~~was validated by manually checking the estimated MLD and BMLD ~~and classified the two limits~~in each profile, which were considered wrongly identified when falling into the pycnocline. Since most of the ~~pycnocline within it (examples in~~ errors located the mixed layer depths clearly at the centre of the pycnocline having thin layers of re-stratification (> 4 observations) (Fig. A1 b, c, e, f, Appendix A~~). The selection was~~), the identifications were considered ~~to have failed~~correct when ~~the AMLD and~~they appeared i) on top of a lower mixed layer (in BMLD~~ were selected ≥ 2 m (2 observations) above or below the mixed layer depth.~~) and ii) on top of a large density gradient (pycnocline) separating surface to deep waters (in MLD). Major errors in identifying ~~AMLD~~MLD (6.76% of the profiles) and BMLD (4.32%) occurred in density profiles with a smooth transition from the mixed layer to the pycnocline, hence reporting a high number of observations ~~in the portion of the water column where mixed layer was transiting into the pycnocline, where φ_z was similar amongst several observations and~~ at the ~~cluster analysis was identifying observations at the end of the pycnocline as part of the mixed layer~~ mixed layer depths (e.g. Fig. A1 a-c, Appendix A). It is important to highlight the sensitivity of this method to ~~Δ~~the difference in density (Δρ) at ~~AMLD~~MLD and BMLD (a large Δρ is preferred), and to the sampling frequency at the transition regions between mixed waters and the pycnocline~~ and the above and below mixed layers~~. The algorithm did not correctly identify ~~AMLD~~MLD in profiles without ~~a surface~~an upper mixed layer~~,~~ and a shallow pycnocline that comprised two different gradients (Fig. A1c, Appendix A). In this case, the cluster analysis split $\Delta\rho$ into two groups, although they belong to the same pycnocline. Other errors were related to profiles having a pycnocline split into two parts by a thin mixed layer ~~with heigth~~having > 4 ~~m (4~~ observations~~)~~ (Fig. A1e, Appendix A). Overall, the identification of BMLD performed better than ~~AMLD's~~MLD's, although it could not deal with profiles having less than 4 observations throughout the pycnocline (~~in this study~~ thickness of the pycnocline < 3 m). This condition occurred due to the location of the *Split2* (which is necessary to distinguish BMLD's from ~~AMLD's~~MLD's selection) i) at depths above ~~AMLD~~MLD (misidentifying ~~AMLD~~MLD as BMLD) or ii) too close to BMLD (missing enough observations to fit V1 properly ~~V1~~). The algorithm always correctly selected BMLD in profiles ~~that have~~with a ~~lower~~ temporary overturn in the density ~~observation below the BMLD~~profile (Fig. A1d, Appendix A).

2.3 Common methods identifying Density Levels (DLs)

~~Among the methods used to detect density levels in coastal and oceanic waters, three approaches were selected to define mixing and buoyancy features in the sampled profiles.~~

~~The AMLDs are~~ The depths detailing the density structure in the water column are defined here as density levels (DLs). Among the multiple indicators of mixed layers that associate with Chl-a vertical distribution, the ecological relevance of the MLD, the halfway pycnocline depth and the maximum buoyance depth were compared to the proposed algorithm's identifications.

The MLD is typically defined ~~as MLD~~ in the literature ~~and representas~~as the depth at which the density exceeds a specific value (threshold method) (e.g. Kara et al., 2000). The threshold is typically selected among a range of values previously tested in the literature (from 0.0025 to 0.125 kg m^{-3}) (summarized in Thomson and Fine, 2003; Montégut et al., 2004; Lorbacher et al., 2006; Holte and Talley, 2009) and measured as the difference ($\Delta\rho_z = |\rho_z - \rho_{ref}|$) between a certain sampling depth (z) and a reference density value (ρ_{ref}), which can be the density at the surface, at 10 m depth, or a consecutive point (e.g. z-1). In this study, two density thresholds (0.01 and 0.02 kg m^{-3}) have been measured as the difference between two consecutive points in the profile ($\Delta\rho_z = |\rho_z - \rho_{z+1}|$) and named as ~~AMLD$_0$~~MLD$_{0.01}$ and ~~AMLD$_0$~~MLD$_{0.02}$.

Since previous studies identified subsurface Chl-a in the proximity of the centre of the pycnocline (~~here~~hereafter called halfway pycnocline depth, HPD, Table 1), we investigated the relationship between ~~CMd~~DCM (depth of maximum Chl-a) and three different HPDs measured as the halfway depth between the base of the pycnocline (BMLD) and ~~AMLD$_0$~~MLD$_{0.01}$, ~~AMLD$_0$~~MLD$_{0.02}$ and ~~adjusted AMLD, and~~MLD from the proposed algorithm. These DLs are named HPD$_{0.01\text{-BMLD}}$, HPD$_{0.02\text{-BMLD}}$, and ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ (Fig. 2).

Moreover, several studies reported positive correlation between the maximum squared buoyancy frequency (Max N^2) and ~~CMd~~DCM at oceanic sites (e.g. Martin et al., 2010; Schofield et al., 2015; Carvalho et al., 2017; Courtois et al., 2017; Baetge et al., 2020) and shelf waters (Lips et al., 2010; Zhang et al., 2016). Therefore, the depth of Max N^2 has been selected from N^2 profiles computed by *gsw_Nsquared* function (*gsw* v1.0-5 package) in R v3.6.3 (R Core Team, 2018), following the most recent version of the Gibbs equation of state for seawater in TEOS-10 systems (Intergovernmental Oceanographic Commission, 2010). The magnitude of N^2 quantifies the stability of the water column and pinpoints the stratified layers where the energy required to exchange water parcels in the vertical direction is maximum (Boehrer and Schultze, 2009).

Table 1: Table of abbreviations used in the paper.

Abbreviation	**Description**

~~SCML~~SCM	*Subsurface Chlorophyll-a maximum ~~Layer~~*
Chl-a	*Chlorophyll-a (mg m^{-3})*
~~CMd~~DCM	*Depth of maximum Chlorophyll-a (m)*
DL	General abbreviation for a *density layer* (e.g. ~~AMLD~~MLD, BMLD, HPD, or Max N^2) *(m)*
~~MLD~~	~~General expression for *Mixed layer depth (m)*~~
~~AMLD~~MLD	*~~Above mixed~~Mixed layer depth*, or ~~starting point~~top of the pycnocline *(m)*
BMLD	*~~Below mixed~~Mixed layer depth below the pycnocline*, or ~~ending point~~base of the pycnocline *(m)*
HPD	*Halfway pycnocline depth*, or centre of the pycnocline *(m)*
Max N^2	maximum squared buoyancy frequency (N^2) *(m)*

2.4 Subsurface Chlorophyll-a parameters

The depth of maximum Chl-a (~~CMd~~DCM) was defined as the deepest maximum inflection point in the Chl-a profile ~~standardized at~~with 1 m sampling frequency (Carvalho et al., 2017; Zhao et al., 2019b)~~, by~~. Here, the inflection point is defined as the depth exhibiting a high concentration of Chl-a and a large change in Chl-a values throughout the profile. The variation of Chl-a throughout the water column was investigated using the adapted Chu and Fan (2011) method identifying for φ that is described in Sect 2.2. ~~The CMd~~The angle (φ) were measured at each depth of the Chl-a profile, and the maximum φ with the largest Chl-a concentration was selected ~~throughout each vertical profile of Chl-a as the depth having the maximum angle (φ) between two vectors (V1 and V2). Details on the number of observations used to fit each vector are reported in Supplementary materials.~~as DCM. The automated identification of ~~CMd~~DCM was checked manually with a visual inspection of each profile. The portion of the method used to measure the angle φ was integrated into a new function *maxChla.R* coded in R v3.6.3 (R Core Team, 2018) and available at https://github.com/azampollo/BMLD.

The total amount of Chl-a were measured using trapezoidal integration (Walsby, 1997) throughout the water column (depth-integrated Chl-a~~) in R v3.6.3 (R Core Team, 2018~~).

2.5 Evaluating the association of density levels with subsurface Chl-a

The ecological relevance of each density level (DL) was evaluated by comparing their coincidence with the depth of maximum Chl-a (~~CMd~~DCM) (e.g. ~~CMd~~DCM = BMLD) and their strength in predicting ~~CMd~~DCM. The coincidence and the prediction of ~~CMds~~DCMs from density profiles return important understanding of the processes driving subsurface concentrations and identify a valuable proxy for modelling analyses and for controlling uncertainty in net primary production estimates.

In this study, we evaluated the coincidence of the ~~CMd~~DCM with eight investigated density levels (~~AMLD$_0$~~MLD$_{0.01}$, ~~AMLD$_0$~~MLD$_{0.02}$, ~~AMLD~~MLD, BMLD, HPD$_{0.01\text{-BMLD}}$, HPD$_{0.02\text{-BMLD}}$, ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$, and Max N^2, Fig. 2) using Spearman's rank correlation coefficient (ρ_S) and a Major Axis (MA) line fitting, and the prediction of ~~CMd~~DCM from DL by performing a linear regression model (LM). All three methods differently assess the level of correlation or prediction. The Spearman's coefficient (Eq. (1) in Table 2) assesses a monotonic linear relationship with values ranging between -1 and +1, which refer to a perfect negative or positive correlation between two variables. Besides the strength of the linear relationship defined by ρ_S, we focused on evaluating the linear relationship between ~~CMd~~DCM and each DL using 3 different linear models $y = \alpha + \beta x$: 1) alpha and beta estimated by linear regression~~,~~; 2) alpha and beta estimated by major axis line fitting; and 3) the one-to-one linear regression with alpha and beta fixed at 0 and 1 respectively. The one-to-one line hypothesizes that ~~CMd~~DCM and DL occur at the same depth. The MA is largely used to investigate how one variable scales against another by accounting for errors from both directions (x and y) and measuring the residuals perpendicular to the line (details in the review Warton et al., 2006). Therefore, the aim of MA is not to predict the y-variable, however evaluating the proximity of the coefficients of the estimated MA line (α and β) to the scenario in which DL equals ~~CMd~~DCM. The coincidence of each DL and ~~CMd~~DCM was summarized by reporting the α and β MA coefficients, which are hypothesized to be intercept ~ 0 and slope ~ 1 when ~~CMd~~DCM occurs at the same depth of the DL in question.

Since the identification of a proxy for subsurface Chl-a represents a useful tool for correctly assessing the abundance and the variations of primary production, we investigated the power of prediction of ~~CMd~~DCM from each DL by measuring the r-squared (R^2) from i) an ordinary least square to estimate parameters from the observations in a linear regression (Eq. (2) in Table 2), and ii) the one-to-one linear regression (which has been forced with the intercept through the origin and a slope equal to 1, Eq. (3) in Table 2). The formulae used to calculate the coefficient of determination R^2 for the one-to-one (R_0^2) and empirical (R_{em}^2) LMs are summarized in Eq. (2) and Eq. (3) in Table 2.

Table 2: Formulae for estimating the bivariate line-fitting. Spearman's rank correlation coefficient (ρ_S), coefficient of determination R^2 for testing the one-to-one linear regression (R_0^2) (e.g. ~~CMd~~DCM ~ BMLD) and the empirical linear regression (R_{em}^2).

	Formula	Purpose
ρ_S	$\frac{\sigma_{xy}}{\sigma_x \sigma_y}$ (1)	Estimate the strength of the relationship between x and y
R_{em}^2	$1 - \frac{SS_{RES}}{SS_{TOT}} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$ (2)	Measure the variation in y that is explained by x in a LM
R_0^2	$1 - \frac{SS_{RES}}{SS_{TOT}} = 1 - \frac{\sum_{i=1}^{n}(y_i - x_i)^2}{\sum_{i=1}^{n}(y_i)^2}$ (3)	Measure the variation in y that is explained by x in a one-to-one LM

Notation: σ_{xy} is the covariance of x and y, σ_x and σ_y are standard deviations, n is the number of observations of x and y, y_i is DMC_i, $\bar{y}$ is the average of ~~CMds~~DCMs, and x_i is the density layers related to ~~CMd~~DCM in each regression (e.g. ~~CMd~~DCM ~ BMLD). SS_{RES} is the residual sum of squares, SS_{TOT} is the total sum of squares.

In the empirical LM, R_{em}^2 was calculated using the typical formula with the residual sum of squares (SS_{RES}) as the square of the difference of y and $\hat{y}$ (estimated y from the model) (Eq. 2)). In the one-to-one LM, the SS_{RES} in R_0^2 was adapted by replacing $\hat{y}$ with x (Eq. (3)), since the values of x and y are assumed to be equal in the one-to-one line regression and the difference between them should be zero. The two R^2 differ also for the denominator SS_{TOT}, which is the sum of squares about the average of the explanatory variable in R_{em}^2 and the sum of squares of the ~~CMd~~DCM values since in R_0^2 the value of ~~CMd~~DCM and DL equals.

Since the SS_{TOT} adopted in the two formulae is different, the proportion of explained ~~CMds'~~DCMs' variance by each DL can be compared only within each linear regression rather than across the one-to-one and empirical regressions. Therefore, the power of prediction among DLs was discussed ~~in~~ within each type of LM.

3. Results

The presented algorithm identifying for ~~AMLD~~MLD and BMLD was applied to 1273 profiles exhibiting a pycnocline. The associations of the density levels (~~AMLD$_{\theta}$~~MLD$_{0.01}$, ~~AMLD$_{\theta}$~~MLD$_{0.02}$, ~~AMLD~~MLD, HPD$_{0.01\text{-BMLD}}$, HPD$_{0.02\text{-BMLD}}$, ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$, BMLD and Max N^2) with ~~CMds~~DCMs and the vertical distribution of Chl-a are described in Sect 3.1 and 3.2.

3.1 Vertical distribution of ~~CMd~~DCM and density levels

The depth of Chl-a maximum (~~CMd~~DCM) was compared to eight different levels of the density profile that are summarized in surface mixed layer depth (~~AMLD$_{\theta}$~~MLD$_{0.01}$, ~~AMLD$_{\theta}$~~MLD$_{0.02}$, ~~AMLD~~MLD), below mixed layer depth (BMLD), the centre of the pycnocline (HPD$_{0.01\text{-BMLD}}$, HPD$_{0.02\text{-BMLD}}$, ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$) and the depth of maximum buoyancy frequency squared (Max N^2) to evaluate i) the strength of a positive linear relationship between each DL and ~~CMd~~DCM, and ii) the prediction of ~~CMd~~DCM from each DL.

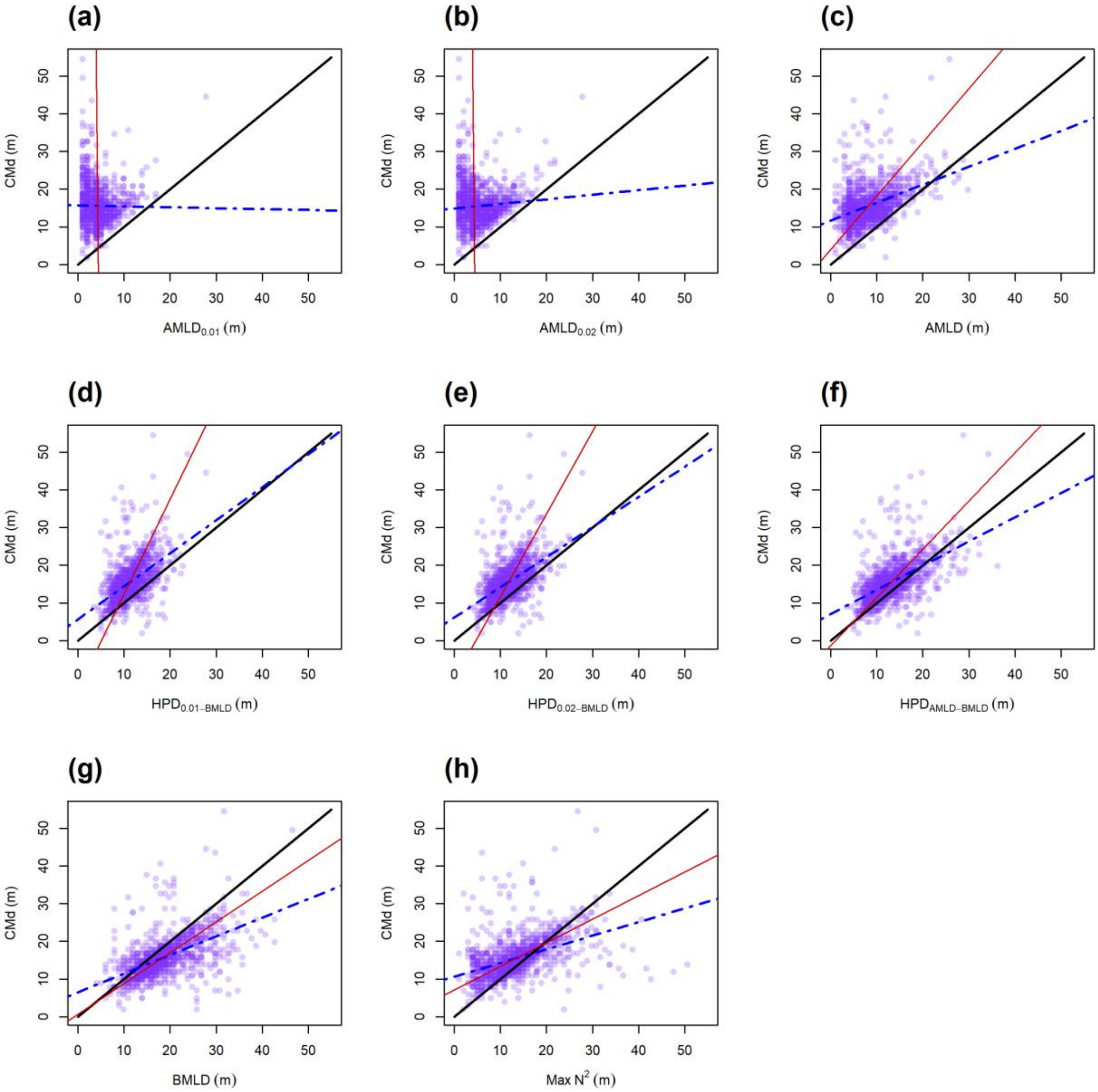
The observations carried out in the FoF and Tay region confirmed the subsurface presence of maxima Chl-a between April and August, with ~~CMds~~DCMs distributing on average (± standard deviation) at 19.29 ± 6.56 m. All the indicator classifying the surface mixed layer (~~AMLD$_{\theta}$~~MLD$_{0.01}$, ~~AMLD$_{\theta}$~~MLD$_{0.02}$ and ~~AMLD~~MLD) distributed generally shallower than ~~CMds~~DCMs (Fig. 4 a-c, Table 3) with a rare coincidence of their vertical distribution (from 0.39% to 1.73% of the profiles, Table 3). In particular, the thresholds' methods used to identify ~~AMLD (0.01 and 0.02 mg m^{-3})~~MLD exhibited the lowest Spearman correlation amongst all DLs, having almost a zero correlation to ~~CMds~~DCMs (ρ_S = -0.01 and 0.08 for ~~AMLD$_{\theta}$~~MLD$_{0.01}$ and ~~AMLD$_{\theta}$~~MLD$_{0.02}$, Table 3) and a limited contribution to define ~~CMd's~~DCM's variability in empirical linear regressions (R^2_{em} = 0.00 and 0.01, Table 3). The Major Axis analysis measured intercepts and slopes in ~~AMLD$_{\theta}$~~MLD$_{0.01}$ and ~~AMLD$_{\theta}$~~MLD$_{0.02}$ almost perpendicular to the y-axis due to the strong presence of ~~CMds~~DCMs in deep waters. Although a clear subsurface aggregation of Chl-a maxima occurs below the surface mixed layer (Fig. 4c), the ~~AMLD~~MLD identified by the algorithm correlated better to ~~CMd~~DCM than ~~AMLD$_{\theta}$~~MLD$_{0.01}$ and ~~AMLD$_{\theta}$~~MLD$_{0.02}$, with a positive linear relationship between the two variables and a greater explained variance of ~~CMd~~DCM by the one-to-one and empirical linear regressions (Table 3). The coefficients measured by MA for ~~AMLD~~MLD (Table 3) reported a positive correlation of ~~CMds~~DCMs, representing a gradual deepening of ~~CMd~~DCM with the top of the pycnocline.

Max N^2 is the density level performing least well after ~~AMLDs~~MLDs in predicting ~~CMds~~DCMs, although it showed the highest percentage of coincidence with ~~CMds~~DCMs (13.51% of the profiles, Table 3). Similar to ~~AMLDs, CMds~~MLDs, DCMs have been recorded in 64.96% of the profiles at layers deeper than Max N^2, indicating that Chl-a maxima area located in waters below surface mixing, at stratified regions within the pycnocline.

Overall, the centre of the pycnocline (HPDs) performed better than ~~AMLD~~MLD and Max N^2, distributing closer to ~~CMds. HPD$_{AMLD}$~~DCMs. HPD$_{MLD\text{-BMLD}}$ reported the highest correlation to ~~CMds~~DCMs (ρ_S = 0.56), and the highest explained ~~CMd's~~DCM's variance from the one-to-one (R^2_0 = 0.90) and empirical (R^2_{em} = 0.31) linear regressions (Table 3). The location of ~~CMds~~DCMs is highly related to ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$, although only 4.63% of the profiles presented ~~CMds~~DCMs and ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ at the same depth (Table 3). Many profiles exhibited ~~CMd~~DCM deeper than ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ (78.69%), of which 81.53% distributed ~~CMds~~DCMs above BMLD (hence, between ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ and BMLD). HPD$_{0.01\text{-BMLD}}$, HPD$_{0.02\text{-BMLD}}$ less related to ~~CMds~~DCMs in Spearman's correlation, MA, one-to-one and empirical linear regressions than the ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ (Table 3).

The below mixed layer depth, BMLD, exhibited a reverse condition compared to the other density levels by encompassing 78.32% of ~~CMds~~DCMs in waters above it (Table 3). BMLDs is the second variable after ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ with the highest correlation to ~~CMds~~DCMs (ρ_S = 0.55). It is distributed at the same depth of ~~CMds~~DCMs in 7.86% of the profiles and linearly predicted the location of maxima Chl-a in both one-to-one and empirical linear regressions (Table 3). BMLD exhibited MA coefficients (α = 0.60 and β = 0.82) close to the hypothesized one-to-one fitting-line (α = 0 and β = 1), indicating a good approximation of ~~CMds~~DCMs at the base of the pycnocline. Moreover, ~~CMds~~DCMs distributed on average at 2.74 ± 5.21 m above BMLD, with a maximum distance above it ~~equals~~equal to 22 m, and 27 m below it.

The overall distribution of ~~CMds~~DCMs is discernible mainly (> 95.84% of profiles) below the surface mixed layers (~~AMLDs'~~MLDs' indicators), within the deepest half of the pycnocline (between ~~HPD_{AMLD}~~$HPD_{MLD\text{-}BMLD}$ and BMLD) and it is bounded for 78.32% of the observations above the BMLD. Although ~~CMds~~DCMs generally reflect the region with the highest concentration of Chl-a throughout the water column, the vertical distribution of Chl-a can vary in the proximity of ~~CMds~~DCMs and accumulate mainly above or below it. Hence, the ecological relevance of the density levels (DLs) has been investigated in comparison with the vertical distribution of Chl-a (Sect. 3.3).



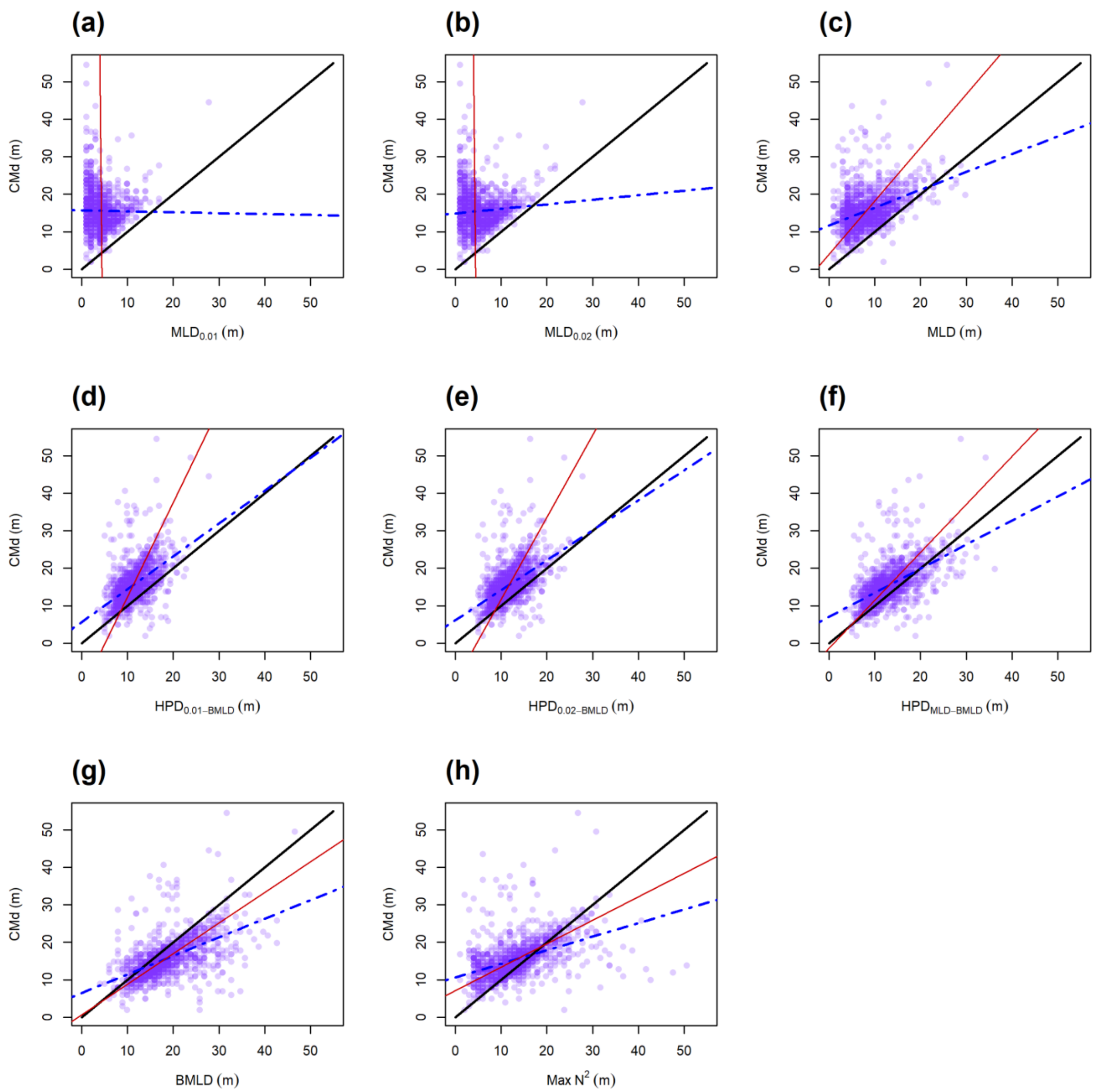


Figure 4: Scatterplots of ~~CMd~~DCM and the eight DLs (a-h). The lines refer to the one-to-one linear regression (LM) (solid black), the Major Axis analysis (MA) (solid red), the empirical LM measured from the observations (~~CMd~~DCM ~ DL) (dot-dashed blue). A good relationship between DL and DMC exhibits similar slope and intercept to the solid black line.

ha fo

Table 3: Statistical parameters and percentage of profiles having ~~CMds~~DCMs above (>), at the same depth (=), or below (<) each DL. A good relationship is described by an $\alpha \sim 0$ and $\beta \sim 1$, high values of ρ_S, R_0^2, and R_{em}^2.

DL	ρ_S	α	β	R_0^2	R_{em}^2	~~CMd~~DCM > DL	~~CMd~~DCM = DL	~~CMd~~DCM < DL
~~AMLD$_0$~~MLD$_{0.01}$	- 0.01	543.35	-124.26	0.40	0.00	99.53	0.39	0.08
~~AMLD$_0$~~MLD$_{0.02}$	0.08	-43.72	11.35	0.47	0.01	99.45	0.31	0.24
~~AMLD~~MLD	0.41	4.01	1.42	0.69	0.17	95.84	1.73	2.44
HPD$_{0.01\text{-BMLD}}$	0.52	-12.81	2.52	0.86	0.27	90.18	1.81	8.01
HPD$_{0.02\text{-BMLD}}$	0.52	-10.20	2.19	0.87	0.27	86.41	3.77	9.82
~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$	0.56	1.31	1.28	0.90	0.31	74.86	4.63	20.50
BMLD	**0.55**	**0.60**	**0.82**	**0.87**	**0.31**	**13.83**	**7.86**	**78.32**
Max N^2	0.45	7.06	0.63	0.84	0.20	64.96	13.51	21.52

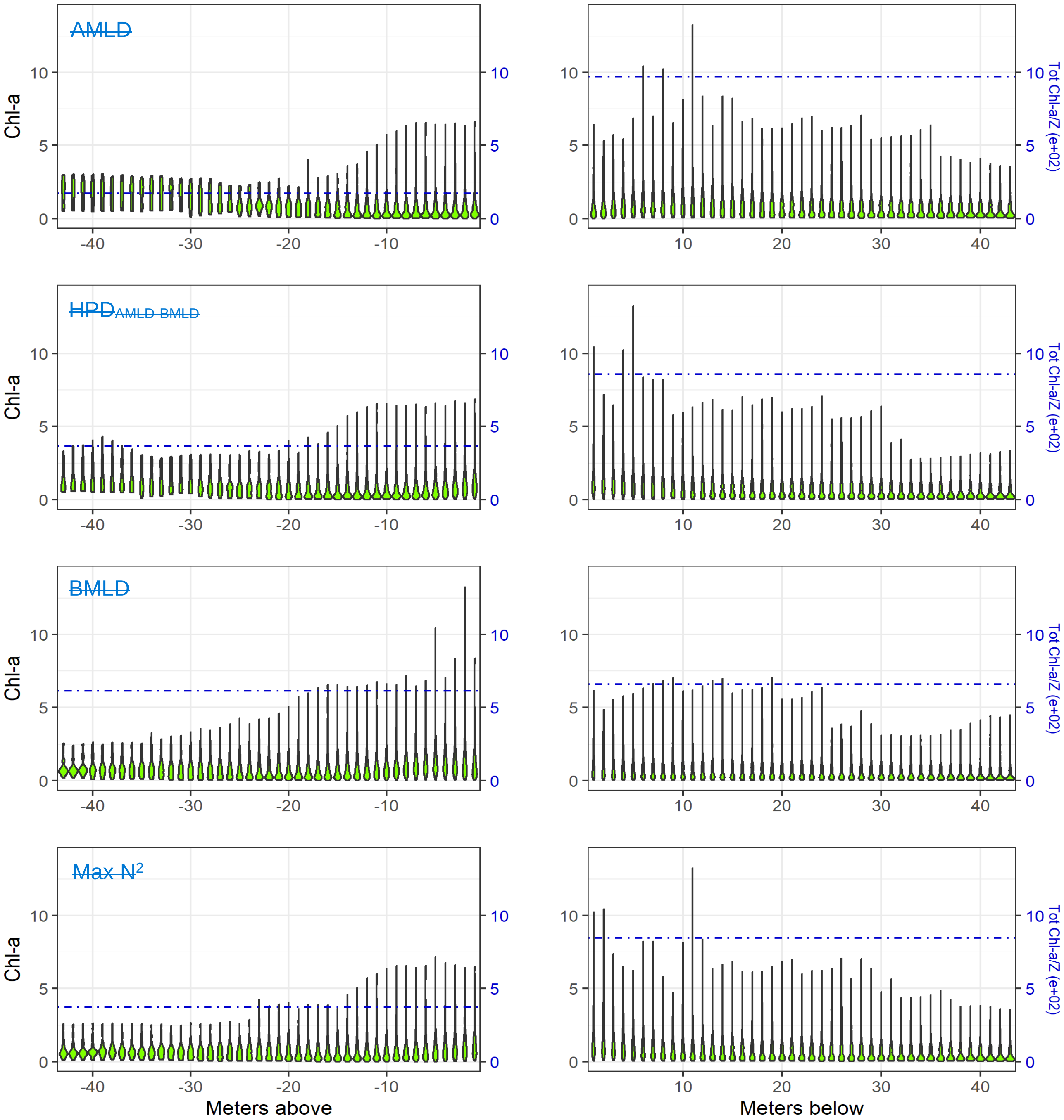
3.2 Chl-a vertical distribution in relation to density levels

~~Since hydrodynamic~~Although DCMs generally reflect the region with the highest concentration of Chl-a throughout the water column, large concentration can still accumulate above or below it. Hydrodynamic and biological conditions generating resuspension, passive drift, and mortality (i.e. zooplankton grazing in stratified waters) can shape Chl-a differently throughout the water column, hence the ~~amount~~ecological relevance of ~~Chl-a was measured above and below each~~ the density levels ~~regardless~~has been investigated in comparison with the vertical distribution of ~~CMd~~Chl-a.

The sum of depth-integrated Chl-a of all profiles was standardized by the number of observations (mg m^{-3}) above and below four DLs (~~AMLD, HPD$_{AMLD}$~~MLD, HPD$_{MLD\text{-BMLD}}$, BMLD and Max N^2~~). AMLD~~), in order to compare the overall Chl-a within each layer of the water column. MLD and ~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$ were selected amongst the density levels to represent the surface mixed layer and the centre of pycnoclines because of their better correlation to ~~CMd~~DCM (see Sect. 3.1). The total amount of Chl-a ~~at each meter depth (mg m^{-1})~~ above and below the four density levels is reported as standardized depth-integrated values in Table 4 and shown at each meter depth in Figure 5.

Table 4: ~~Values~~Sum of all depth-integrated Chl-a (mg m^{-2}) standardized by ~~its range~~the number of ~~vertical distribution (m) (Total Chl-a biomass (mg)/depths (m))~~observations above and below the four density layers. ~~These values are also reported in Figure 5.~~

DL	Standardized depth-integrated (total) Chl-a above DL (mg m$^{-~~1~~3}$)	Standardized depth-integrated (total) Chl-a below DL (mg m$^{-~~1~~3}$)
~~AMLD~~MLD	172.97	971.12
~~HPD$_{AMLD}$~~HPD$_{MLD\text{-BMLD}}$	366.07	859.27
BMLD	615.92	658.72
Max N^2	372.90	848.14



AMLD
HPDAMLD-BMLD
BMLD
Max N²
Chl-a
Tot Chl-a/Z (e+02)
Meters above
Meters below

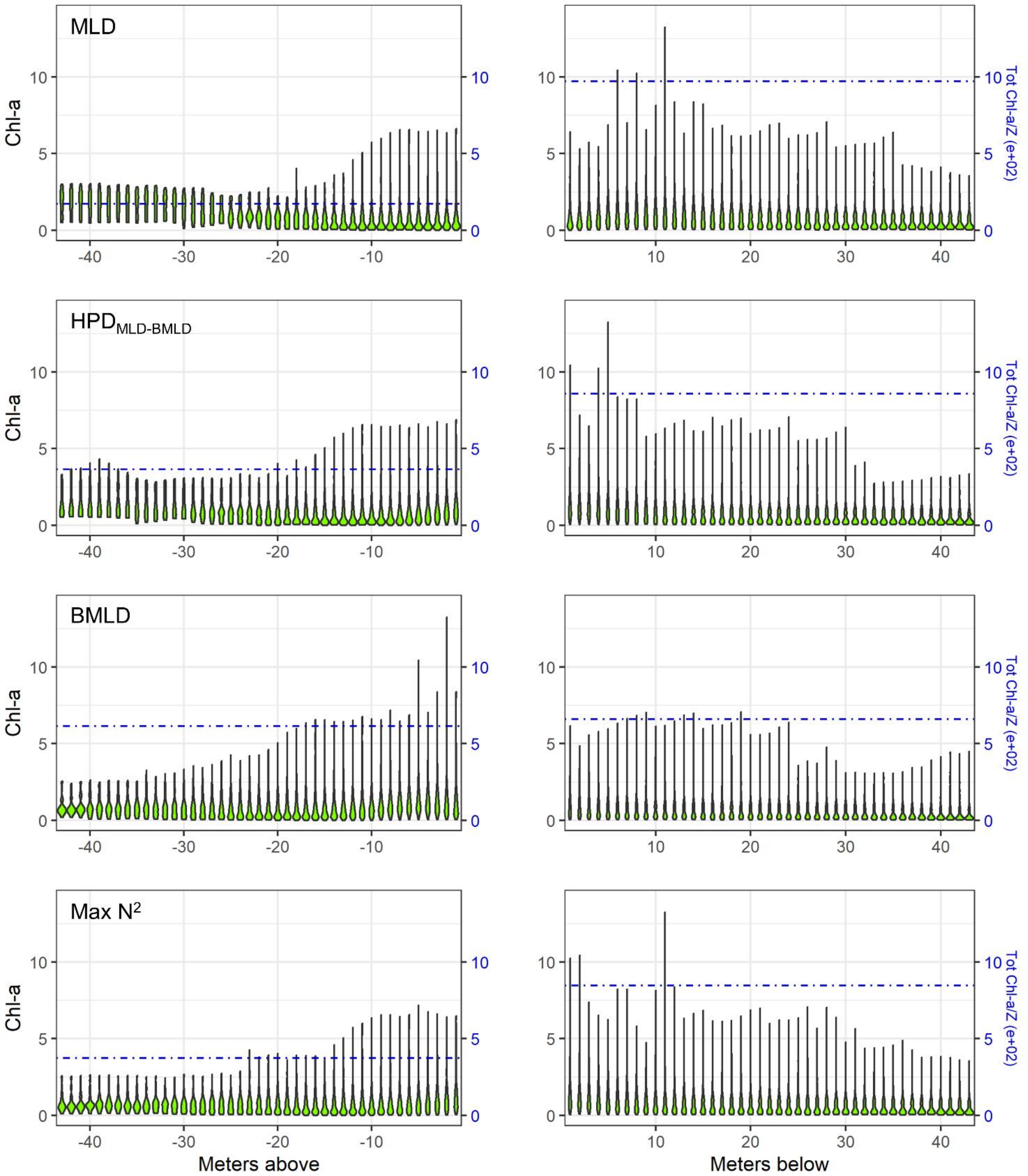


Figure 5: Violin plot of Chl-a (mg) at each meter above and below the four density levels (~~AMLD, HPD_{AMLD}~~MLD, $HPD_{MLD\text{-}BMLD}$, BMLD and Max N^2) for the whole dataset. The dot-dashed blue lines represent the standardized depth-integrated Chl-a measured as the total amount of Chl-a of all profiles (mg m^{-2}) divided by the number of depths (~~z~~m) within each portion of the water column (above and below DLs~~) (values are reported in Table 4~~).

Following the results in Sect. 3.1, a large portion of Chl-a was measured at depths below ~~AMLD, HPD_{AMLD}~~MLD, $HPD_{MLD\text{-}BMLD}$ and Max N^2 (Table 4), where the depth of Chl-a maximum also occurred. From the seabed to ~~HPD_{AMLD}~~$HPD_{MLD\text{-}BMLD}$ and Max N^2, the amount of Chl-a was three times the Chl-a from these DLs to the surface. A reverse condition occurred for Chl-a distributing above and below BMLDs: the standardized depth-integrated Chl-a is higher above BMLDs, although the amount of Chl-a in the deepest layers (below the pycnocline) is still comparable (the difference between Chl-a from the surface to BMLD and from BMLD to seabed is 42.80 mg m^{-1}) (Table 4).

It is therefore sensible to infer the distribution of ~~CMds~~DCMs, and the largest portion of Chl-a, at depths enclosed within the stratified region (~~AMLD~~MLD – BMLD), especially in the second half of the pycnocline (~~HPD_{AMLD}~~$HPD_{MLD\text{-}BMLD}$ – BMLD). At the same time, a noticeable amount of Chl-a still distributes below the pycnocline (BMLD).

4. Discussion

In stratified waters, the vertical distribution of Chl-a is regulated by the balance of stratification and mixing rates across different hydrodynamic regimes over time (Leeuwen et al., 2015). The combination of static, dynamic and biological factors (e.g. grazing, Benoit-Bird *et al.*, 2013) induces phytoplankton communities to adapt their vertical distribution at small scales (< 1 km, Scott et al., 2010; Sharples et al., 2013). Identifying a proxy for subsurface concentrations of Chl-a is essential to investigate the impacts of physical changes due to large scale factors (e.g., stratification strength, sea ~~water increase~~level rise, or turbulence increase downstream wind turbine foundations). To date several studies have identified the mixed layer between the sea surface and the pycnocline as a valuable tool to assess changes in phytoplankton abundance and phenology over time; here we propose a tool to identify the vertical limits of the pycnocline and indicate the ~~base of~~mixed layer depth below the pycnocline (BMLD) as a variable ~~tightly~~ influencing the vertical distribution~~ of Chl-a and likely to affect~~, abundance and phenology of Chl-a.

4.1 Ecological relevance of ~~AMLD~~MLD and Max N^2 in defining ~~CMds~~DCMs

Oceanic sites ~~exhibit~~often exhibited phytoplankton blooms within the upper mixed layer (e.g. Behrenfeld, 2010; Costa et al., 2020; Somavilla et al., 2017) to coincide with ~~AMLDs'~~MLDs' vertical fluctuations due to e.g. windstorm events deepening the pycnocline into nutrient-enriched waters (Detoni et al., 2015; Carranza et al., 2018; Höfer et al., 2019; Montes-Hugo et al., 2009)~~. In this study~~Although MLD are linked to the physical processes setting the vertical distribution of DMCs in deep oceanic environments, all the investigated surface mixed layers' indicators (~~$AMLD_0$~~$MLD_{0.01}$, ~~$AMLD_0$~~$MLD_{0.02}$ and ~~AMLD~~MLD) weakly predicted ~~CMd. The algorithm used in this study has identified AMLD to have an overall higher performance~~DCM in ~~predicting the location of CMds than the thresholds' methods and maximum squared buoyancy frequency (Max N^2). Since AMLD~~the shelf waters investigated in this study. MLD has been largely considered as a central variable for understanding phytoplankton dynamics (Sverdrup, 1953), ~~it has been investigated in~~and its relation ~~to~~with climate change has been investigated to infer possible significant changes in the amount, spatial distribution and phenology of ~~oceanic~~future primary production (Boyd et al., 2015; Montes-Hugo et al., 2009; Somavilla et al., 2017; Prend et al., 2019; Richardson and Bendtsen, 2019; Schmidt et al., 2020). ~~However, the effect~~The effects of climate change on ~~AMLD~~MLD and primary production is still ~~an unsolved question~~unclear (Lozier et al., 2011; Somavilla et al., 2017)~~. The unclear effects of climate change on AMLD~~ and ~~primary production~~ might be related to i) the ~~type~~large amount of Chl-a data ~~used~~required to ~~measure~~investigate variations in ~~Chl-a,~~ primary production that have to rely on e.g. satellites' observations at the sea surface ~~and their uncertainty related to~~that leave out subsurface Chl-a (Baldry et al., 2020; Erickson et al., 2016; Lee et al., 2015), and ii) the exclusive investigation of the ~~effects of~~ surface ~~mixing processes~~physics on primary production (e.g. temperature, wind-induced mixing) by neglecting deep processes that are responsible for the pycnocline's stability (Dave and Lozier, 2015, 2013; Lozier et al., 2011; Somavilla et al., 2017). The ~~AMLD~~MLD is informative for surface concentrations, but it may not be biologically relevant for subsurface Chl-a that are maintained at the pycnocline by deep turbulent mixing. The need for a much more detailed understanding of the linkage between subsurface Chl-a, pycnocline characteristics and deep turbulent processes is therefore a key subject, especially in highly productive but spatially heterogeneous areas such as shelf waters and shallow seas.

In the FoF and Tay region, Max N^2 exhibited higher percentages of coincidence with ~~CMds~~DCMs (13.51% of 1273 profiles) than other DLs (Table 3). The depth of Max N^2 is a less turbulent region where the energy to exchange parcels in the vertical is maximum (Boehrer and Schultze, 2009), and it is frequently used to identify the upper mixed layer depth (e.g. Carvalho et al., 2017). The location of ~~CMds~~DCMs at Max N^2 might reflect the distribution of phytoplankton within a less turbulent region where nutrient particles, which have been resuspended by mixing, can persist for longer time periods. The ~~mild turbulent layer at~~ Max N^2 would therefore represent a ~~hot spot of nutrients reached by~~mild turbulent layer where resuspended phytoplankton cells accumulate, while ~~strong~~ mixing processes ~~still undergoing~~ above and/or below ~~it, or diluted gradients of phytoplankton and nutrients~~Max N^2 redistribute phytoplanktonic organisms throughout the water column~~, would avoid the creation of highly productive subsurface patches~~. However, the amount of standardized depth-integrated Chl-a below Max N^2 is almost three times higher than above it (Table 4 and Fig. 5) suggesting that Max N^2 is a layer of suitable conditions for phytoplankton to grow, but it lacks informing where most of the Chl-a vertically distribute. Although the depth of Max N^2 appeared to better inform ~~better~~ the exact location of ~~CMds, BMLD exhibited a clear pattern by distributing below CMd in 78.32%~~DCMs, the percentage of ~~the profiles~~its coincidence with DCMs is still low and ~~representing the deepest limit up~~might relate to ~~which CMds distributed~~specific conditions. Overall, the linear correlation (ρ_S), the MA coefficients and the one-to-one linear regression R_0^2 described a ~~low~~poor association of ~~CMds~~DCMs with Max N^2 compared to ~~HPDs'~~HPD indicators and BMLD, and hence the use of Max N^2 to locate subsurface Chl-a patches in summertime shelf waters may lead to underestimate the amount of Chl-a in the whole water column.

4.2 Vertical distribution of Chl-a and BMLD

The observations carried out in the FoF and Tay region confirmed the subsurface presence of maxima Chl-a between April and August. A recent study in the German Bight described ~~CMds~~DCMs located mainly at the centre of the pycnocline and the overall amount of Chl-a at depths distinctly lower than the surface mixed layers (Zhao et al., 2019a). The location of ~~CMd~~DCM at the pycnocline is regulated over time by upward nutrient-enriched fluxes entering the pycnocline from deep waters (Pingree et al., 1982; Rosenberg et al., 1990). In the Skagerrak strait between Denmark and Norway, deep ~~SCMLs~~SCM were recorded at a nutricline (rate of change in nitrate and phosphate) located below the base of a shallow pycnocline (< 15 m) (Bjørnsen et al., 1993). The low concentration of ~~CMds~~DCMs below BMLD might reflect a limited erosion of Chl-a by mixing (Zhao et al., 2019a) and, possibly, grazing (Benoit-Bird et al., 2013). The physical factors developing subsurface Chl-a are defined by mixing processes below the pycnocline that provides an indispensable upward flux of nutrients in the euphotic zone, where e.g. dinoflagellates are able to compete successfully in slightly turbulent conditions (< 0.1 mm s^{-1}) (Ross and Sharples, 2007). Therefore, the erosion as well as the resuspension of sinking phytoplankton cells and nutrients can maintain the proximity of ~~CMds~~DCMs at BMLDs setting the location of the nutricline at the base of the pycnocline. It is also noticeable that a large amount of diluted Chl-a in deep waters (51.67% of depth-integrated Chl-a below BMLD) might be crucial in maintaining primary production ~~at~~in the subsurface over the summer, since deep mixing processes eroding and sustaining Chl-a at BMLD would also contribute ~~also~~ to reducing the overlap between ~~SCMLs~~SCM and predators (Behrenfeld, 2010). However, it should be noted that the high concentrations of Chl-a at DCM may reflect the photoacclimation of phytoplankton rather than an actual increase in carbon biomass (Marañón et al., 2021). Hence, the location of DCM close to the base of the pycnocline informs on a large concentration in pigments rather than in carbon production, which should be considered when DCM is used as a proxy of food patches. Overall, DCMs, and most of the depth-integrated Chl-a, distribute in the proximity of the centre and the base of the pycnocline suggesting the maintenance of subsurface Chl-a by deep turbulent fluxes which supply nutrients into the deepest portion of the pycnocline.

Overall, the deep distribution of CMds, and most of the depth-integrated Chl-a, in the proximity of the centre and the base of the pycnocline suggests the maintenance of subsurface Chl-a within shelf waters through the regulation of nutrient supply by waters below the pycnocline and makes this linkage responsive to variations in deep physical processes.

4.3 Using BMLD to investigate impacts on primary production

The marine photosynthetic activity represents an essential biological pump of carbon sequestration (Boyd et al., 2015), whose extent is often invalidated by the exclusion of subsurface Chl-a of up to 10%-40% (Sharples et al., 2001). The correct measurement of primary production throughout the whole water column is essential to address which factors affect absorbing atmospheric carbon dioxide in the marine environment. Recent studies reported a decrease of Chl-a biomass (Capuzzo et al., 2018; Schmidt et al., 2020) and a temporal shift of phytoplankton bloom (Silva et al., 2021) due to significant changes in the surface MLD. The Northeast Atlantic shelves experienced a summertime reduction of Chl-a in the last 60 years leading to significant impacts on the food web, caused by an intensified stratification of the water column that maintains nutrient fluxes in deep watersIn this section are introduced some of the potential contexts in which BMLD's use would be advantageous. The linkage between the mixed layer depth below the pycnocline and subsurface Chl-a advocates BMLD as a key variable to address the effects of climate changes and man-made structures (e.g. offshore wind farm foundations) on the food resources, and defines BMLD as a potential proxy of subsurface food patches to investigate the vertical and spatial distribution of grazing and predator species.

Climate change

Since primary production is sustained by deep turbulent processes in prolonged stratified conditions, investigating the effect of future climate change, where stratification has been reported to increase over time (Capuzzo et al., 2018), using BMLD as a proxy of the vertical distribution of deep physical processes might be more informative than MLD in shelf waters. The Northeast Atlantic shelves experienced a summertime increase of stratification and a consequential reduction of Chl-a in the last 60 years in waters where nutrient fluxes had been confined in deep layers (Capuzzo et al., 2018; Schmidt et al., 2020). Prolonged stratified conditions were reported to define deeper concentratedare known to promote subsurface patches of Chl-a (Somavilla et al., 2017; Scott et al., 2010), where phytoplankton stabilize at deep low-turbulence layers (Bopp et al., 2013) having still sufficient light to photosynthesize and set the nitracline position. The starvation of nutrients at surface force phytoplankton to re-distribute in the water column(Ross and Sharples 2007; Somavilla et al., 2017) due to the depletion of nutrients at shallow layers after surface blooms. The starvation of nutrients at the surface forces phytoplankton to re-distribute (e.g. Bindoff et al., 2019; Boyd et al., 2015; Schmidt et al., 2020) in deeper nutrient-enriched waters, within the euphotic zone. Hence, the location of CMdsDCMs in the proximity of the deepest portion of the pycnocline, between HPD$_{AMLD}$HPD$_{MLD}$-BMLD and BMLD, (78.32% of the profiles) is not surprising during summer in the Firth of Forth and Tay regions. Although a consistent portion of depth-integrated Chl-a is reported below pycnocline, the vertical distribution of BMLD resulted in setting the position of subsurface productive patches in stratified waters, representing an important indicator of the vertical distribution of phytoplankton in shelf waters.

, where the production of Chl-a is sustained by weak upward fluxes of nutrients from deep layers. The effects of an intensified stratification on primary production in the continental shelf waters are still entangled and suggest an overall deepening of subsurface Chl-a, which is likely to delineate a knock-on effect on redistributing most of the higher trophic levels (e.g., zooplankton, fish) and affect the foraging success of highly adapted species~~.~~ such as surface feeding seabirds (OSPAR, 2017). Hence, the role of climate change in increasing stratification is likely to affect the distribution of BMLD and the upward fluxes, which may either redistribute food patches and causing a variation in abundance and community composition of primary production (Holt et al., 2016, 2018; Capuzzo et al., 2018). The potential deepening of BMLD within or even below the euphotic zone may lead Chl-a to decrease across shelf seas since phytoplanktonic cells would increase buoyance at deeper and darker depths.

However, the deepening of productive patches is difficult to examine over large spatial scales, and remote sensing methods often lack reliability for subsurface data. ~~The role of climate change in increasing stratification are likely to affect the distribution of BMLD and the upward fluxes, which may either redistribute food patches at major depths together with the deepening of BMLD and causing an overall reduction of primary production or shifts of community compositions.~~The global estimates of carbon sequestration have often failed to include 10% to 40% of subsurface Chl-a (Sharples et al., 2001). Since the correct measurement of primary production throughout the whole water column is essential, key drivers of subsurface production are demanded to correctly predict, measure, and estimate DMCs from widely-used remote sensing data. Although data on the nutricline position were unavailable in this study, the vertical distribution of BMLD informed adequately on the position of subsurface productive patches in stratified waters, making this variable an important indicator of the vertical distribution of phytoplankton in shelf regions.

Offshore renewable infrastructures

It is ~~hence~~ reasonable to ~~notice~~stress that ~~the~~ potential effects on primary production ~~involves~~involve both surface and deep (below the pycnocline) processes, especially where multiple local changes (i.e. wind turbine foundations changing levels of mixing) repeated over large spatial areas (i.e. the North Sea) can have an effect at different scales (van der Molen et al., 2014; De Dominicis et al., 2018; Carpenter et al., 2016). The upcoming interest of the offshore renewable sector in building offshore wind farms ~~(OWFs) in the FoF and Tay region (www.marine.gov.scot)~~in stratified regions rises the need of drafting reliable environmental impact assessments able to identify key variables for estimating the effects in a holistic way~~.~~ (Dorrell et al., 2022). The consequences of offshore wind farms are likely to be related to bathymetry and mixing budgets, by affecting the stratification rate differently across ~~several bathymetries. The~~ depths. In this study area with spring tidal speeds < 1 ms^{-1} , the vertical distribution of ~~CMds~~DCMs at BMLDs appeared to be correlated to the bathymetry by exhibiting ~~CMds~~DCMs closer to BMLDs at water depths comprised from, approximately, 40 to 70 m, ~~CMds~~DCMs deeper than BMLD mainly in shallow waters < 60 m, and ~~CMds~~DCMs above BMLD towards deeper waters up to 100 m (Fig. A3 in Appendix A). Previous studies identified a similar pattern in shallow waters (25-85 m) where ~~CMds~~DCMs were mainly recorded at or below the base of the pycnocline (Barth et al., 1998; Durán-Campos et al., 2019; Holligan et al., 1984; Zhao et al., 2019a). Although stratification is reported to intensify in shelf waters with climate change, the increase in turbulence downstream of wind farms may counteract the local stratification (Carpenter et al., 2016; Schulien et al., 2017; Schultze et al., 2020) and affect the temporal and spatial distribution of Chl-a. Since the variation in stratification is a useful tool to address possible impacts on primary production, ~~using~~ understanding the potential impacts on the vertical distribution of BMLD is likely to ~~be more efficient in predicting~~efficiently predict changes in the vertical distribution of Chl-a and its possible ~~consequences. The deepening of BMLD within or even below the euphotic zone may lead Chl-a to decrease across shelf seas since phytoplanktonic cells would buoyance at deeper and darker depths. Hence, the use of AMLD to investigate physical alteration of climate change and man-made structures should be integrated with the use of BMLD and the understanding of physical processes at depth, together with changes in seabed temperature, and the slow down or increase of upward fluxes~~predators.

5. Conclusion

Chl-a vertical distribution ~~gives~~provides important information about the state of development of the phytoplankton community~~,~~ which is associated with mixed and stratified layers. The mixing processes above and below the pycnocline can have very different influences on Chl-a vertical distribution, dictating the concentration at subsurface patches that can distribute close to, above, or below the pycnocline.

Although the association of phytoplankton with ~~AMLD~~MLD has been largely described at large spatial scales within oceanic habitats, the presented study shows a weak linkage between ~~AMLD~~MLD and ~~CMd~~DCM in shelf waters, at a very high vertical resolution (1 m~~), compared to HPDs'~~). The results indicate that HPD indicators ~~or~~and BMLD, ~~which has led to hypothesize~~have a ~~stricter~~stronger association ~~of~~with summertime subsurface Chl-a ~~with~~ maxima within the bottom-half of the pycnocline. Therefore deep mixing processes, such as tidal currents~~ in the North Sea~~, play a role in regulating summertime subsurface primary production and ~~may~~ regulate their distribution at BMLDs in the stratified conditions found in the North Sea. Considering the described associations of subsurface Chl-a with BMLD, it is evident how this variable can play a role in the assessment of productivity, since the deep mixing processes are equally (or more) relevant than the surface process in determining a shift of primary production at local ~~or~~and large scales. This association therefore advocates the~~-~~ investigation of the effect of anomaly-inducing processes occurring at and below the pycnocline (e.g. ~~deep sea~~bottom temperature~~, deep~~ and salinity, turbulence and physical processes at the BMLD), which are likely to influence primary production and on to the whole ecosystem dynamics within shelf seas (Trifonova et al., 2021). Understanding mechanisms affecting primary production at fine scales is very important to investigate as we are moving rapidly towards the deployment of thousands of structures and hundreds of GW in ~~the~~ wind energy ~~sector~~extraction from worldwide shallow seas (Gielen et al., 2019). This work proposes BMLD~~ is proposed~~ as an ecological relevant variable for further oceanographic investigations in shelf waters, ~~and~~along with the proposed analytical approach ~~is~~as a valuable tool to extrapolate this variable from *in situ* vertical samples.

Appendix A

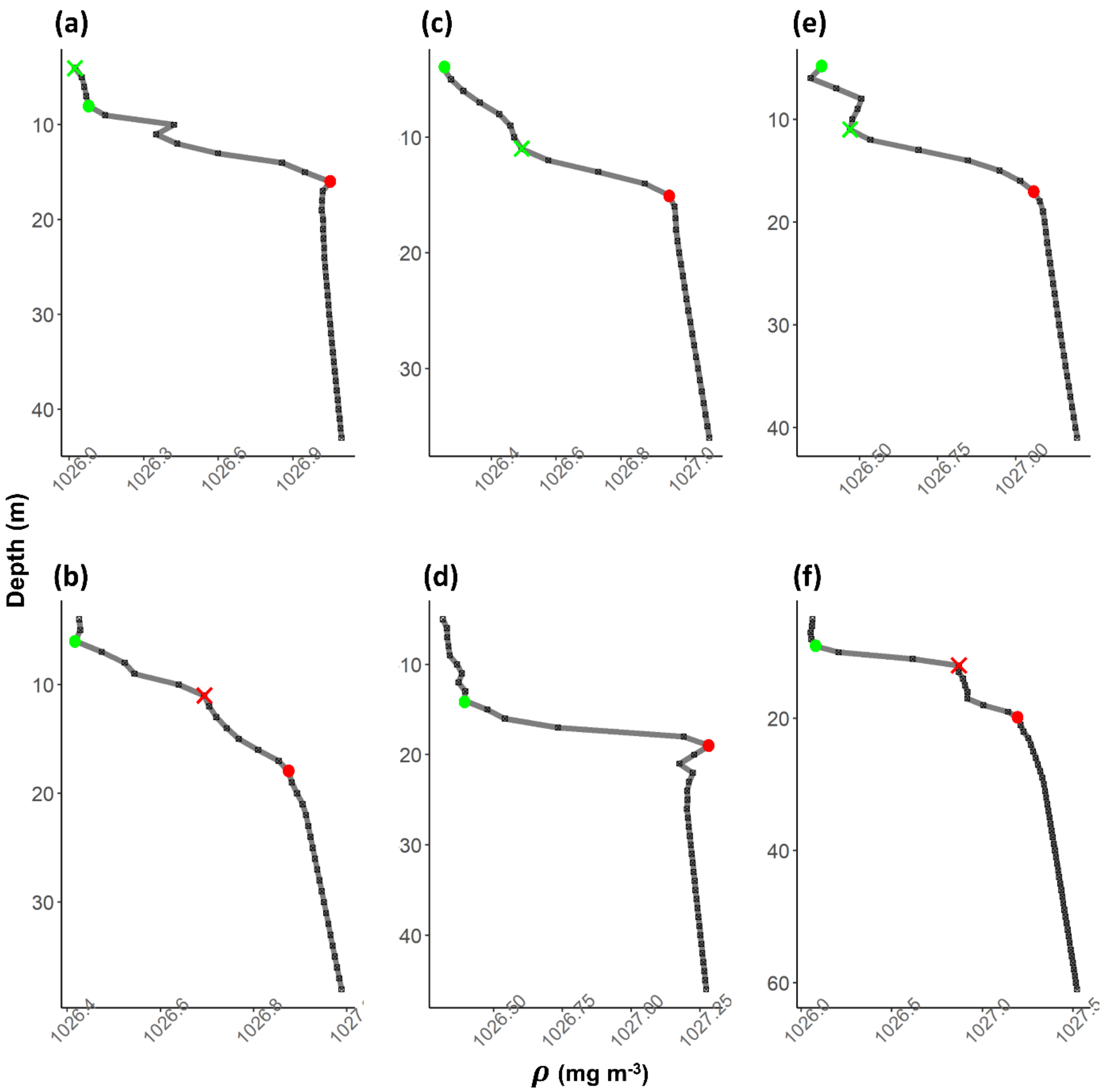


Figure A1: examples of density profiles (grey line) (a-f). The black squares are ~~the values~~observations at 1 m resolution. Red dots refer to BMLD, green dots to ~~AMLD~~MLD. Crosses refer to misidentified ~~AMLD~~MLD (in green) and BMLD (in red) that ~~needed to be~~were manually corrected.

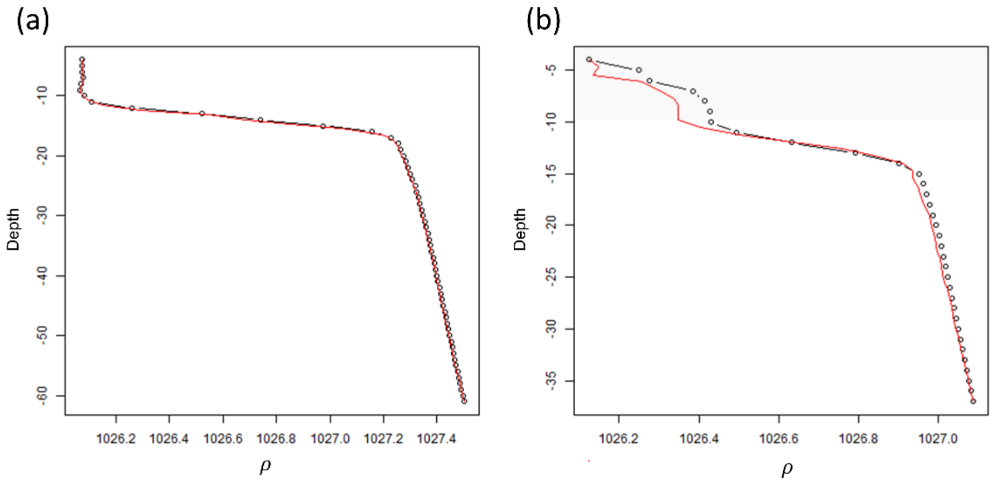
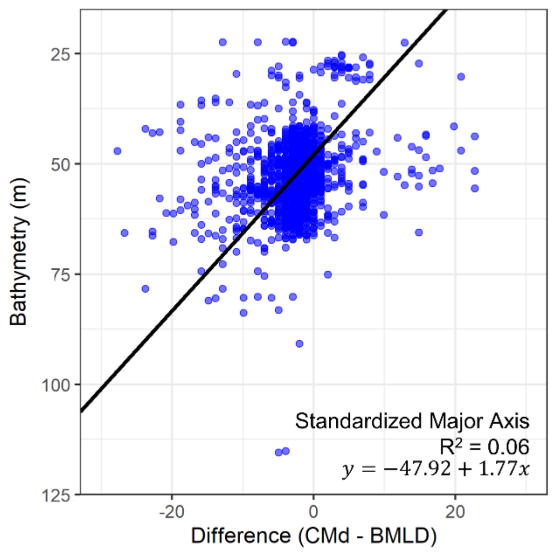


Figure A2: two density profiles whose observations were standardized at equals 1 m ~~depths~~intervals using generalized additive model (GAM). (a) ~~reports a~~ density profile (black dotted line) where the GAM correctly fitted (red solid line) the vertical distribution. (b) ~~reports a~~ density profile where the GAM wrongly fitted the upper portion of the profile (grey polygon area) and, hence, required a manual correction of the values.



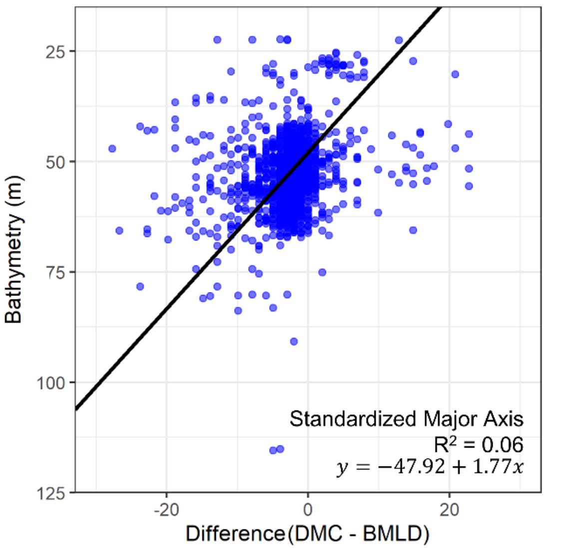


Figure A3: scatterplot of the difference between ~~CMd~~DCM and BMLD against the bathymetry at which each profile was sampled. The solid black line reports a Standardized Major Axis analysis, whose equation and R squared values are reported.

Author contribution

Arianna Zampollo contributed to the conceptualization of the study, formal analyses, methodology on ~~AMLD~~MLD and BMLD, writing of the original draft, and software use; Thomas Cornulier contributed to the conceptualization and supervision of the statistical method, writing of the original draft, methodology and visualization of the results; Rory O'Hara Murray contributed on the data curation, writing of the original draft, supervision, visualization and validation; Jacqueline F. Tweddle contributed to the conceptualization and the supervision of the study; James Dunning contributed to the methodology of the ~~AMLD~~MLD and BMLD algorithm; Beth Scott contributed to the conceptualization of the analyses, writing of the original draft, revision of the two versions, contextualization and discussion of the results, supervision, funding acquisition, resources and data curation.

Code availability

The ~~code~~codes for the ~~AMLD~~identification of DCM, MLD and BMLD ~~algorithm~~ are available at https://github.com/azampollo/BMLD.

Data availability

Data are available upon request and agreement with the co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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