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An alternative method for correcting fluorescence quenching

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Abstract. Under high light intensity, phytoplankton protect their photosystems from bleaching through non-photochemical quenching processes. The consequence of this is suppression of fluorescence emission, which must be corrected when measuring in situ yield with fluorometers. We present data from the Southern Ocean, collected over five austral summers by 19 southern elephant seals tagged with fluorometers. Conventionally, fluorescence data collected during the day (quenched) were corrected using the limit of the mixed layer, assuming that phytoplankton are uniformly mixed from the surface to this depth. However, distinct deep fluorescence maxima were measured in approximately 30 % of the night (unquenched) data. To account for the evidence that chlorophyll is not uniformly mixed in the upper layer, we propose correcting from the limit of the euphotic zone, defined as the depth at which photosynthetically available radiation is ~ 1 % of the surface value. Mixed layer depth exceeded euphotic depth over 80 % of the time. Under these conditions, quenching was corrected from the depth of the remotely derived euphotic zone Z_{eu}, and compared with fluorescence corrected from the depth of the density-derived mixed layer. Deep fluorescence maxima were evident in only 10 % of the day data when correcting from mixed layer depth. This was doubled to 21 % when correcting from Z_{eu}, more closely matching the unquenched (night) data. Furthermore, correcting from Z_{eu} served to conserve non-uniform chlorophyll features found between the 1 % light level and mixed layer depth.

1 Introduction

Monitoring distribution and abundance of primary producers in the marine environment is useful for understanding larger biological and physical processes. Phytoplankton are predominantly single-celled, green microscopic organisms, present at variable concentrations in every ocean (Falkowski and Kolber, 1995; Behrenfeld et al., 2009). They are the first stepping stone in transferring energy into the marine ecosystem. When conditions allow for growth, their collective impact is such that they are able to change the spectral properties of the water (McClain, 2009). This can be exploited to measure abundance and distribution using their photosynthetic pigment, chlorophyll *a* (Chl *a*) as a marker (Morel and Prieur, 1977; Gordon and Morel, 1983; Falkowski et al., 1998; Henson et al., 2010).

Satellite-derived ocean colour is the most comprehensive data set available for monitoring surface Chl *a* concentration. However, the limitations of remote sensing are significant (Dierssen and Smith, 2000; McClain, 2009). Perhaps most significantly, satellite sensors can only see ocean colour from the surface to one optical depth, providing little information on the vertical structure of the water column (Morel and Berthon, 1989). Despite innovative algorithms to improve data collected by satellites, limitations are not likely to be resolved with the next generation of ocean colour sensors. Continued collection of in situ data on phytoplankton distribution and abundance is thus essential (Johnson et al., 2009).

Fluorescence has been widely used as a relatively inexpensive, non-invasive method for quantifying Chl *a* since the 1960s (Lorenzen, 1966; Lorenzen and Jeffrey, 1980; Cullen

and Eppley, 1981; Falkowski and Kolber, 1995; Xing et al., 2011; Lavigne et al., 2012). Chlorophyll pigments packaged inside phytoplankton cells re-radiate $\sim 2\%$ of light energy as fluorescence. Active fluorescence can thus be measured by a fluorometer delivering voltage output equivalent to 460 nm (excitation in the blue) and measuring resultant fluorescence in the 620–715 nm range (detection in the red). Assuming that the measured yield is proportional to the abundance of photosynthesising phytoplankton, relative values of fluorescence can be used to quantify primary biomass. However, yield (and thus proportionality) is affected by several factors, including the intensity of sunlight each cell is exposed to (Behrenfeld and Boss, 2006).

To regulate high sunlight intensity, phytoplankton employ the mechanism of non-photochemical quenching. This process helps cells protect themselves in environments where light energy absorption exceeds the capacity for light utilisation (Müller et al., 2001; Behrenfeld et al., 2009). During periods of high light stress, shallow-mixed phytoplankton in the upper euphotic zone protect their photosystems from bleaching by emitting excess energy as heat (Milligan et al., 2012). In this state of photo-protection, photosynthesis is inhibited and fluorescence yield drops (Müller et al., 2001). However, deep-mixed phytoplankton are shaded by surface biomass and protected by light-attenuating properties of the water itself. Thus, during high sunlight intensity, deeply mixed phytoplankton will fluoresce while shallow-mixed biomass will be invisible to a fluorometer. If uncorrected, quenched fluorescence yields will generate values under-representative of phytoplankton abundance at the surface. Avoiding such under-representation is particularly important for studies comparing satellite-derived surface Chl a with in situ fluorescence-derived Chl a.

On the vertical scale, quenched fluorescence profiles are also problematic in that they mimic the shape of true deep fluorescence maxima (DFM) (Mignot et al., 2011). Sub-surface fluorescence maxima can be indicative of deep chlorophyll maxima (DCM). These features either form due to differences in Chl a packaging, or when the bulk of phytoplankton biomass settles to depths where both nutrients and light are sufficient (Cullen and Eppley, 1981; Holm-Hansen and Hewes, 2004; Mellard et al., 2011). Because DCM are found below one optical depth, abundance and distribution of such features cannot be measured by satellite (McClain, 2009; Charrassin et al., 2010). However, vertical distributions of phytoplankton play important roles in the organisation of pelagic trophic food webs (Mellard et al., 2011). DCM in particular play potentially under-reported roles in net primary production and carbon fixation (Fairbanks et al., 1982; Estrada et al., 1993; Claustre et al., 2008). It is therefore useful to try to accurately identify and map these features.

In previous work on glider data, correcting for quenching involved using backscatter (Sackmann et al., 2008) or surface light intensity (Todd et al., 2009), each measured simultaneously to fluorescence. For autonomous platforms collecting only fluorescence, salinity and temperature data in remote locations, this is not possible. Using the depth of the density-derived mixed layer, Xing et al. (2011) corrected quenched fluorescence data collected by Argo floats in Pacific, Atlantic and Mediterranean offshore zones. This mixed layer depth (MLD) method was then extended to fluorescence data collected by tagged southern elephant seals (*Mirounga leonina*) off Kerguelen Island in the Southern Ocean (Xing et al., 2012; Guinet et al., 2013). Following the method of Xing et al. (2012), the maximum fluorescence yield within the density-derived mixed layer would be representative of the whole, assuming homogeneity. How robust is this assumption for the waters of the Southern Ocean?

The upper layer of the Southern Ocean tends to be deeply mixed, even over austral summers, and DCM are thought to be rare (discussed in Holm-Hansen and Hewes, 2004). However, ship-based studies undertaken in different regions of the Southern Ocean have shown that mixing and settling patterns within and between functional types are dynamic (Holm-Hansen and Hewes, 2004; Mengesha et al., 1998; Quéguiner, 2013; Sangrà et al., 2014). This is especially true for motile (flagellated) and seasonally successive (heavily silicified) species (Mengesha et al., 1998; Quéguiner, 2013). Furthermore, even within homogenously mixed layers of biomass, Chl a packaging has been shown to vary with depth, generating DCM (and DFM) independent of biomass (Behrenfeld and Boss, 2006). Reliance on the assumption of homogeneity is, therefore, possibly problematic.

In this paper, we present an alternative method to account for quenching without relying on homogeneity in the density-derived upper layer. Non-photochemical quenching is corrected using the limit of the euphotic zone (Z_{eu}), defined as the depth at which downward photosynthetic available radiation (PAR) is at $\sim 1\%$ of the surface value (Shang et al., 2011; Soppa et al., 2013). At this level, light should be sufficient for photosynthesis (Ryther and Menzel, 1959; Morel and Berthon, 1989; Saulquin et al., 2013; Palmer et al., 2013) but too weak to cause quenching (Alderkamp et al., 2011; Ross et al., 2011). This method is applied to fluorescence data collected by 19 animal-borne fluorometer, conductivity, temperature and depth satellite-relayed data loggers (FCTD-SRDLs) deployed in the Southern Ocean over several austral summers.

2 Methods

2.1 In situ data

Between 2009 and 2013 18 adult female southern elephant seals from Kerguelen and one from Marion Island were equipped with FCTD-SRDLs (Sea Mammal Research Unit, University of St Andrews, UK, Scotland) before they undertook their post-breeding foraging migration over the austral summer (Fig. 1). Following established tagging protocols
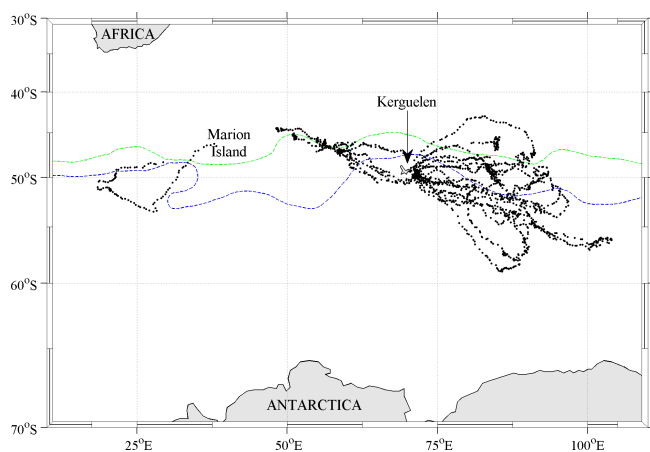
Figure 1. Tracks of 19 southern elephant seals tagged with FCTD-SRDLs over austral summers from 2009 to 2013 in black. The general positions of the subantarctic front and the polar front are shown in green and blue, respectively (Orsi and Harris, 2014). The incomplete track seen off Marion Island is due to the FCTD-SRDL battery failing after 55 days.

(Bester, 1988; McIntyre et al., 2010), the seal on Marion Island was immobilised with ketamine using a remote injection method and the tag was glued to the fur on the head with quick-setting epoxy resin. Seals on Kerguelen were anesthetised with an intravenous injection of tiletamine and zolazepam 1 : 1 and tags were glued to the fur on the head using a two component industrial epoxy (Jaud et al., 2012).

The FCTD-SRDL instrument records behavioural data as well as in situ pressure, temperature, salinity and fluorescence (Charrassin et al., 2010; Xing et al., 2012). At-sea data were relayed via the Argos satellite system (http://www.argos-system.org). Locations were provided by Service Argos based on Doppler shift measurements and data were downloaded from the Sea Mammal Research Unit Instrumentation Group's website (http://www.smru.st-andrews.ac.uk/). Detailed information on the hardware and software of the CTD-SRDL is described by Boehme et al. (2009), and on-board data processing is described comprehensively by Fedak et al. (2002).

Fluorescence is recorded by a Cyclops 7 fluorometer (Turner Designs, CA, USA), which delivers a voltage output proportional to the fluorescence detected in a wavelength between 620 and 715 nm. The Cyclops instrument is programmed to measure fluorescence every 2 s during the ascent (upcast), from 180 m to the surface. Due to the tight limits on data transfer through the Argos satellite system, data have to be compressed (Boehme et al., 2009). Fluorescence yields were therefore binned into 10 m vertical intervals. The reading for 175 m is thus a weighted mean of all fluorescence readings taken between 180 and 170 m. This is the deepest bin available – deep and dark enough to anticipate an absence of live, photosynthesising phytoplankton (Guinet et al.,

2013). However, instruments do not return readings of zero at these depths. This dark count is an offset value added by the manufacturers, and is useful because at very low signal, readings are indistinguishable from noise. The offset value increases the signal to noise ratio, which must be removed during data processing (Lavigne et al., 2012). We therefore calculated the median of readings at 175 m and subtracted this from all measurements collected by the same tag (Xing et al., 2011). This is not only useful for making the fluorescence values more representative, it also serves to reduce variability between tags (Xing et al., 2012). The resulting values are considered proportional to fluorescence, and are termed relative fluorescence units (RFU).

2.2 Satellite data

The depth of Z_{eu} reflects the limit where PAR is 1 % of its surface value. In this study, we use an estimate of the euphotic zone Z_{eu} based on an algorithm by Lee et al. (2007). These estimates are derived from measurements of the water-leaving radiance by the Moderate Imaging Spectrometer (MODIS) as 4 km×4 km monthly composites (http://oceancolor.gsfc.nasa.gov).

To optimise temporal and spatial coverage in a region as cloudy as the Southern Ocean, values of L3 8 day Z_{eu} were used whenever available, and values from monthly composites were then added to fill in the blanks. However, despite the broad coverage in time, gaps were still present in space. For each fluorometer profile the closest Z_{eu} value in space was extracted from the gridded data set. If no value was available, Z_{eu} was interpolated linearly from the nearest seal position with an associated Z_{eu} value.

2.3 Correcting for quenching

Quenching occurs in surface waters during periods of high light stress. Despite daily variability attributed to cloud (changing light intensity), and differences in phytoplankton concentrations between years and regions, suppression of fluorescence is a ubiquitous feature. Fitting a non-linear model (sin function) to surface fluorescence as a function of time of day showed that quenching around midday was significant ($n = 1267$, $R^2 = 0.26$, $P < 0.001$) (Supplement Fig. S1). Data were thus categorised as day: possibly quenched (from sunrise to sunset, with the sun 8 degrees above the horizon) or night: unquenched. To illustrate the quenching effect without interannual and regional variability, day yields were divided by the preceding night yields to generate ratios. Data were then binned by hour and plotted using box plots (Fig. 2).

Before processing, a small proportion of profiles with maximum fluorescence yields below 0.15 RFU were removed from the data set. These low signals tend to fall within the noise, and the resulting vertical profiles cannot be meaningfully interpreted.
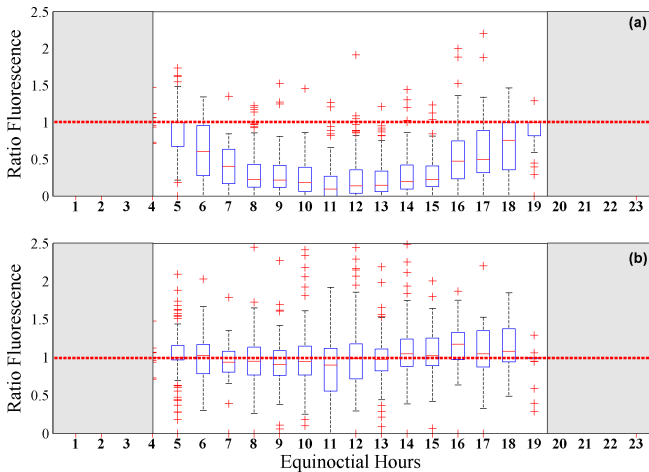
Figure 2. Ratio of day to night surface fluorescence yield (first 15 m) per local hour from data collected by 19 animal-borne fluorometers over five austral summers. Box plots show the distribution of day to night ratios in **(a)**, representing quenched to unquenched yield, respectively. The horizontal red line at 1 indicates the 1 : 1 ratio. Ratios of fluorescence yield decrease from around 06:00 until around 18:00 LT, then recover to the 1 : 1 ratio line. Corrected ratios of fluorescence in **(b)** do not follow the same trend of deviation from the 1 : 1 ratio line at 1. Flanking grey sections represent times of the day where the sun is less than 6 degrees below the horizon.

The night: unquenched data were then normalised and each profile was interrogated for maximum values below the near-surface yield. To ensure a deep maximum value is not within the measurement error for each tag, a 95 % confidence interval for the error was calculated for each instrument using bootstrapping (1000 bootstrap samples). The upper confidence limit was set as a threshold value, subtracted from the deep maximum and added to the near-surface value. Only differences outside of this threshold were flagged as potential DFM, and these profiles were then visually inspected. Vertical profiles conforming to the shape of DFM in this data set were considered true DFM, independent of quenching (Fig. S2).

For the day: quenched data, we compare the results of fluorescence quenching correction from two depths. The first is a density-derived MLD calculated from the concurrent CTD measurements of the FCTD-SRDL. Here, MLD is defined as the depth where the vertical density gradient equals or exceeds a threshold value of $\Delta\rho = 0.03 \, \text{kg m}^{-3}$; as employed by Xing et al. (2011, 2012) (de Boyer Montégut et al., 2004; Kara et al., 2000). The MLD in the region of interest tends to remain deeper than 50 m, even over the austral summer (Dong et al., 2008; Fig. 3). The second depth is the remotely derived Z_{eu} using Lee's algorithm (2007). Separately, maximum fluorescence yield between MLD and the surface, or Z_{eu} and the surface, were then extended to fill in suppressed yield (Fig. 3).

For over 80 % of the day data set, mixed layer depth was deeper than Z_{eu}. However, the small proportion ($\sim$ 20 %) of waters with MLD shallower than Z_{eu} proved problematic for correcting quenching. In these instances, correcting from MLD generates still-quenched values. Conversely, correcting from Z_{eu} would mask shallow but potentially true subsurface features. Where MLD shoaled above Z_{eu}, fluorescence data were thus flagged and removed from the data set.

3   Results

The at-sea phases of the 19 tagged southern elephant seals (Fig. 1) tended to start in November and end in late January of the following year, meaning data were collected over the height of the austral summer. In the top 10–15 m of the upper mixed layer across all years of sampling, suppression of daytime fluorescence yield was significant (Fig. 2a).

To correct for quenching in deeply mixed waters, surface values collected between dawn and dusk were filled in with maximum values from the depth of the mixed layer, or the depth of Z_{eu}. The difference between these methods is illustrated in Fig. 3, where individual normalised fluorescence profiles are treated with the two correction schemes. Corrected surface fluorescence yields are overestimated using the depth of the mixed layer, but perhaps more importantly, vertical complexity is lost. Correcting from Z_{eu} serves to conserve unusual (not homogenous) dynamics on the vertical scale. This is illustrated in Fig. 3b, where phytoplankton may either be settling into different layers (floristic shifts) or where Chl *a* packaging has become measurably different between deep-mixed and shallow-mixed layers (photoacclimation).

The difference between the two methods is also evident looking at fluorescence sections in Fig. 4. Uncorrected vertical profiles in the top row (Fig. 4a, d and g) illustrate how evident suppression of daytime fluorescence yield is in the surface layers (greyscale bar with daylight hours in white and black for night). Quenching is corrected using MLD in the second row (Fig. 4b, e and h) and Z_{eu} in the third (Fig. 4c, f and i). The distinct subsurface fluorescence feature in (a) is within the mixed layer, and is thus masked when corrected from this depth (b). However, correcting from Z_{eu} conserves vertical complexity while still correcting quenching at the surface (c). The deep fluorescence feature in Fig. 4d also has a maximal yield below euphotic depth but within the mixed layer. This deep signal is masked when correcting from MLD (e) but remains distinct when using Z_{eu} (f). Finally, the dynamics in Fig. 4g are lost when correcting from the depth of the mixed layer (h) but conserved when correcting from Z_{eu} (i). The night data from this example in particular corroborates complex Chl *a* dynamics with depth.

Unquenched fluorescence profiles collected at night support the evidence that DCM should be accounted for in this oceanic regime. Of the 19 seals tagged with FCTD-SRDLs
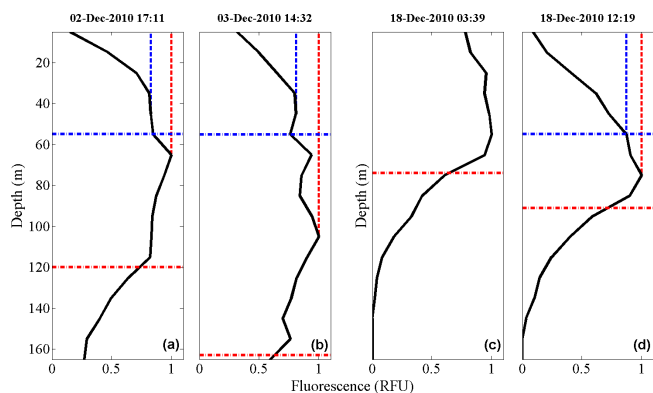
Figure 3. Normalised vertical profiles of fluorescence (solid black line) corrected using either the depth of the mixed layer (dashed red line) or the 1 % light level, Z_{eu} (dashed blue line). The fluorescence in profile **(c)** was measured at night and is unquenched (mixed layer is shown by the dashed red line but Z_{eu} is absent at night). Profiles **(a)**, **(b)** and **(d)** were collected at varying times during the day. For each profile, quenched fluorescence is corrected using either Z_{eu} (depth and corresponding fluorescence yield shown with dashed blue line) or MLD (depth and corresponding fluorescence yield shown with dashed red line).

between 2009 and 2013, 18 instruments measured DFM in the night (unquenched) data. For the combined night data set, approximately 30 % of the 797 profiles showed DFM (mean = 13; standard deviation = 9) (Fig. S3). Correcting with Z_{eu} doubled the number of DFM conserved in the day data (21 %) compared with MLD (10 %), but was still unable to fully match the percentage found in the night data.

4 Discussion

The phenomenon of non-photochemical quenching is well described and appears to be ubiquitous across oceans and seasons (Sackmann et al., 2008; Milligan et al., 2012). The depth where light levels are sufficient for photosynthesis but too weak to generate quenching is key for correcting in situ fluorescence data; ensuring fluorescence yield is representative of vertical Chl *a* concentrations. However, when fluorescence data are collected autonomously, this depth cannot always be measured in space and time.

For this study, removing quenched fluorescence would mean discarding 1352 of the 2149 profiles collected by 19 animal-borne tags over several austral summers in the Southern Ocean. Losing over 60 % of the data collected from such an undersampled region is simply not viable. Thus, two proxy depths are compared for quenching correction. The first is a density-derived MLD, calculated from CTD data measured concurrently by the tag (Xing et al., 2012; Guinet et al., 2013). The second is the depth of the remotely derived euphotic zone, Z_{eu} (Lee et al., 2007).

During daylight hours, a proportion of the euphotic zone (defined as the surface to the 1 % light level) will be light-saturated relative to photosynthetic efficiency (Behrenfeld and Falkowski, 1997). Shallow-mixed phytoplankton are exposed to a range of super-saturating light intensities, while deep-mixed phytoplankton will be exposed to low-levels of light intensity that are suboptimal for photosynthesis in the absence of photoadaptation.

Cells throughout any given euphotic zone adapt to variations in the light field through a range of physiological strategies (Arrigo et al., 2010; Kropuenske et al., 2009, 2010). These include non-photochemical quenching for high light stress, and a range of dark adaptation strategies below a saturating light threshold. Photosynthesis typically saturates at light intensities of ~ 200 mmol photons $m^{-2} s^{-1}$ (van der Poll et al., 2009) and below ~ 100 mmol photons $m^{-2} s^{-1}$ cells must initiate dark adaptation strategies (Garcia and Purdie, 1992). By definition, such dark-adapted phytoplankton at the base of the euphotic zone are not experiencing high light stress. However, light is still sufficient for photosynthesis. Indeed, Hameedi (1978), Platt et al. (1982) and Lee et al. (2010) have shown that shade-acclimated phytoplankton from the 1 % light level are more productive than phytoplankton from the 50 % light level (Alderkamp et al., 2011; Palmer et al., 2013).

At noon on a cloudless summer's day, the Southern Ocean can receive surface irradiance of up to 2000 mmol photons $m^{-2} s^{-1}$ (Ross et al., 2008). Light saturation would be expected at 10 % surface light levels, while dark-adapted phytoplankton would be found at ~ 5 % of the maximal expected surface irradiance. Therefore, at the 1 % light level, phytoplankton are likely dark adapted (i.e. not exhibiting non-photochemical quenching) and actively photosynthesizing.

Composites of MODIS Z_{eu} are provided as evaluative products and these were used to determine euphotic depth, where light intensity would be too low to cause light stress and suppression of fluorescence yield. Lee's algorithm for Z_{eu} provided accurate and reliable estimates of Z_{eu} in open waters of the China Sea (Morel, 1988; Morel et al., 2007; Lee et al., 2007; Shang et al., 2011). We assume that this method can also reliably estimate the limit of the euphotic zone in the open waters of the Southern Ocean. However, validation with in situ light data at the high latitudes would be beneficial for measuring the accuracy of this product.

Combinations of 8 day and monthly Z_{eu} products were used to account for the gaps in coverage. Satellites cannot see through clouds, and the high latitudes are persistently cloudy. Despite the large temporal difference, 8 day and monthly products showed excellent agreement ($R^2 = 0.82$, $p < 0.0001$). This is less likely to be true for coastal or near-coastal regimes, where dynamics change on finer scales. For this reason, we would recommend using daily data combined with 8 day composites in these areas, or any region where cloud cover is less of a persistent problem. As a compro-
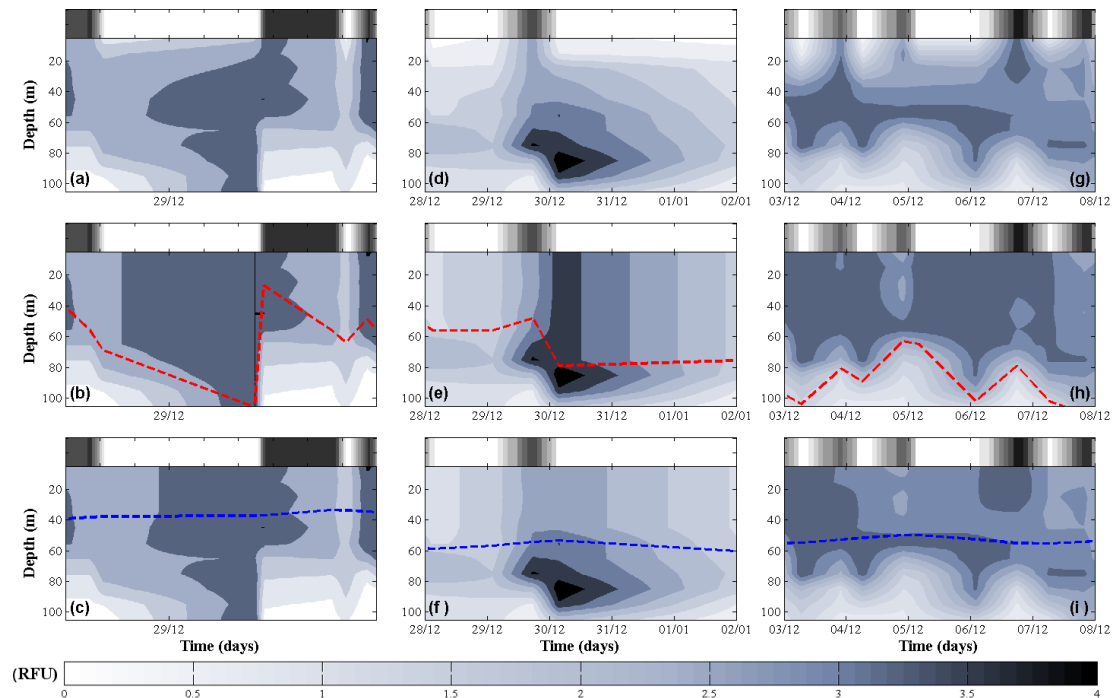
Figure 4. Sections of fluorescence corrected using either the depth of the mixed layer (dashed red line) or the depth of the 1 % light level, Z_{eu} (dashed blue line). The greyscale bar attached to the top of each plot illustrates when fluorescence data were collected relative to the time of day, with black for night and white for day. The top row of vertical profiles **(a)**, **(d)** and **(g)** show sections of uncorrected fluorescence data collected by different instrumented seals over austral summers. The fluorescence data in the second row **(b)**, **(e)** and **(h)** have been corrected using mixed layer depth. The last row of fluorescence data **(c)**, **(f)** and **(i)** have been corrected using euphotic depth. Conditions where MLD shoals above Z_{eu} cannot be corrected and have been filtered from the day data set, but not from the night (unquenched).

mise, generating 16-day composites using SeaDAS 7.0 (Fu et al., 1998) would address the temporal compromise as well as fill in some of the gaps. However, this step requires custom processing, rather than the use of freely available and pre-existing products. This may limit wider application of the method. Our aim is to create a method that is easily applied and universally useful for autonomously collected fluorescence data in regions where waters tend to be deeply mixed.

Surface waters of the Antarctic are deeply mixed and deep chlorophyll maxima were previously thought to be rare (Holm-Hansen and Hewes, 2004). However, while the vertical distribution of phytoplankton is controlled by turbulence and density, it is also driven by nutrient and light gradients (Navarro et al., 2006). Cell motility has been shown to prevail over dispersion by turbulence (Durham et al., 2013). When ideal levels of light and nutrients change with depth over time, so too will the vertical distribution of biomass in the water column (evolutionary stable strategy depth) (Mellard et al., 2011). Indeed, 19 of the 19 seals tagged with FCTD-SRDLs between 2009 and 2013 returned DFM in the night data. These in situ fluorescence data sets add to growing evidence that DCM may be regionally and seasonally persistent in the Southern Ocean (Holm-Hansen et al., 2005; Charrassin et al., 2010; Quéguiner, 2013).

Distinct DCM are only a part of the story, however. Heterogeneous phytoplankton patterns within layers of homogenous density are a common feature of the North Atlantic, resulting from slow mixing rates (relative to growth rates) during periods without deep convective mixing (Taylor and Ferrari, 2011). Although we have no information on mixing or turbulence, finding deep maxima in 30 % of the night data points to moderate or even weak turbulence in the upper layer (Ross et al., 2011; Franks, 2014). Correcting from Z_{eu} serves to conserve unusual (not homogenous) dynamics between the 1 % light level and MLD. From a biological perspective, this vertical information may provide useful insights into mixing and settling patterns of different phytoplankton species (floristic shifts), or differences in chlorophyll packaging in the same species (photoacclimation). However, the fact that DCM and non-uniform phytoplankton patterns persist over the five summers recorded may also be interesting from a purely physical perspective.

The problem of phytoplankton not being uniformly distributed in the mixed layer was commented on by Xing et al. (2011), but not addressed until now. The limitations of our own correction scheme would be improved with testing of the accuracy of the remotely sensed Z_{eu} product in Southern Ocean waters with in situ light data. Furthermore, until we are able to apply our quenching method to fluorescence data

outside of the high latitudes, we are only able to suggest that using Z_{eu} improves the current method in the deeply mixed waters of the Southern Ocean.

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