An alternative method for correcting fluorescence quenching

L. Biermann¹, C. Guinet², M. Bester³, A. Brierley⁴, and L. Boehme¹

¹Sea Mammal Research Unit, Scottish Oceans Institute, St. Andrews, UK
²Centre National de la Recherche Scientifique, Centre d’Etudes Biologiques de Chizé, Villiers en Bois, France
³Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa
⁴Pelagic Ecology Research Group, Scottish Oceans Institute, St. Andrews, UK

Correspondence to: L. Biermann (lb66@st-andrews.ac.uk)

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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 Zeu, 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 Zeu, more closely matching the unquenched (night) data. Furthermore, correcting from Zeu 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., 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 ∼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 homogeneously 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 eup), defined as the depth at which downward photosynthetic available radiation (PAR) is at ∼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 (Alderdkamp 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
sence of live, photosynthesising phytoplankton (Guinet et al., 2011). Readings taken between 180 and 170 m. This is the deepest reading for 175 m is thus a weighted mean of all fluorescence values were therefore binned into 10 m vertical intervals. The 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 (Supplement Fig. S1).

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.
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...
The second is the depth of the remotely derived MLD, calculated from CTD data measured concurrently by the tag (Xing et al., 2012; Guinet al., 2013). The first is a density-derived MLD, calculated from CTD data. Such an undersampled region is simply not viable. Thus, two proxy depths are compared for quenching correction. 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 \( \sim 200 \text{ mmol photons m}^{-2} \text{ s}^{-1} \) (van der Poll et al., 2009) and below \( \sim 100 \text{ mmol photons m}^{-2} \text{ s}^{-1} \) 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, Hameed (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 \( \sim 5 \% \) 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-
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 $Z_{zu}$ improves the current method in the deeply mixed waters of the Southern Ocean.

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