

Photoprotection in Southern Ocean phytoplankton: missing link for low primary productivity

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The Southern Ocean is considered a zone of high nutrients and low chlorophyll (HNLC). Despite the year round availability of nutrients, phytoplankton biomass remains low. Key modes of control are now thought to be light, grazing, iron and supply of silicic acid¹. Physiological photoprotective mechanisms used by phytoplankton to regulate photosynthesis versus rapid light fluctuations have not been considered important. In diatoms and haptophytes, which are the major component of phytoplankton biodiversity in the Southern Ocean, the one step de-epoxidation of diadinoxanthin (Dd) into diatoxanthin (Dt) of the Dd-cycle constitutes the main photoprotective mechanism^{2,3}. Here we show that Southern Ocean diatoms and haptophytes have higher concentrations of cellular Dd-cycle pigments than other oceanic regions. The Dd-cycle is activated under low irradiance conditions and the pool of photoprotective pigments increases under intermittent light conditions. The high cellular concentration of Dd-cycle

pigments and our observations of an increase of the pool size of Dd-cycle pigments towards the surface implies that the Dd-cycle pigments bind to light harvesting proteins other than with fucoxanthin and chlorophyll *c*. Within photosystem II, enrichment of Dd and the physiological acclimation to light changes constrains photosynthetic activity, efficiency and potentially growth rates³⁻⁷. These results raise important questions about the role of photoprotective mechanisms in limiting Southern Ocean primary productivity and how these mechanisms will respond to predicted climate change impacts⁸.

The nonradiative dissipation of excess light energy is considered to be an important photoprotective mechanism in phytoplankton. The dissipation of excess light and thermal energy is attributed to rapid modifications within the light harvesting complexes (LHC) of photosystem II that results in nonphotochemical fluorescence quenching (NPQ)^{3,7}. NPQ occurs with the formation of a proton gradient across the thylakoid membrane and by the reversible conversion of specific carotenoid pigments. In diatoms, dinoflagellates and haptophytes which are largely responsible for oceanic primary production, dissipation of excess thermal energy occurs via the Dd-cycle in which the mono-epoxide carotenoid Dd is converted to the de-epoxide form Dt under high irradiance with the reverse conversion under low light or darkness.

We sampled the concentration of Dd-cycle pigments and chlorophyll *a* (Chl *a*) in surface mixed layer at twenty-six stations in subantarctic waters south-east of New Zealand during austral autumn (Fig. 1). The concentrations of Dd, Dt and Chl *a* in the water column were analysed in 156 samples with High Performance Liquid Chromatography (HPLC) using a specific carotenoid protocol¹⁰. Our results show that the phytoplankton population in our study area have a higher concentration of Dd than other oceanic regions. Dd was well distributed throughout the study area in

concentrations varying between 0.2 and 0.3 mg m⁻³ (Fig. 2). The concentrations of Dd are significantly higher than levels of Dd observed in adjacent subtropical waters¹¹, and in Northern hemisphere temperate waters¹². The concentration of Dt was highly variable (Fig. 2). The concentration of Chl *a*, which is generally used as a proxy for phytoplankton biomass was low (< 0.4 mg m⁻³), consistent with previous satellite and ship observation in this area¹³. Nearby Subtropical waters have Chl *a* biomass up to four times greater than subantarctic waters.

The total Dd-cycle pigment pool (Dd+Dt) can be normalised to Chl *a*, to provide an indication of the amount of photoprotective pigments relative to the phytoplankton biomass. The ratio of Dt to Dd both in diatoms and haptophytes was higher in surface waters and decreases with depth. Recently, another analysis of phytoplankton collected in the Southern Ocean along 110°E during austral summer and along the edge of the seasonal ice of the Antarctic continent during austral autumn support our observations of high concentrations of Dd in the Southern Ocean and of a (Dd+Dt)/Chl *a* ratio increasing towards the surface¹⁴.

The ratio Dt/(Dd+Dt) is an indicator of the de-epoxidation state (DES) of the phytoplankton population and a measure of the photoprotective state of the population. In our study, while the ratio was highly variable, at a number of locations the DES ratio was surprisingly high up to 0.8 (Fig. 3). As Dt is formed on very short time scales of minutes to hours, the DES index represents a clear indicator of fast activation of photoprotection even under the generally low irradiance conditions of austral autumn. Results from a series of on-board incubation experiments we conducted under natural light using phytoplankton collected from the surface at two hours before dawn at three different locations in the study area show that the Dd-cycle is activated at low irradiances (< 50 μmol photons m⁻² s⁻¹) with rapid interconversion

of Dd to Dt. This was associated with the induction of non-photochemical quenching (NPQ) indicating a photoprotective response. Under constant artificial light incubations over 8 hours ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), the pool size (Dd+Dt) showed no change while under intermittent light the pool size increased. The high DES index found in some locations show that a large portion of Dd is converted to Dt under low irradiance conditions.

The subantarctic waters south-east of New Zealand and other areas of the Southern Ocean are considered a region of high nitrate and low chlorophyll (HNLC) with low but persistent phytoplankton production. The reasons for this low production despite high nutrients are currently considered to be a mosaic of environmental factors of light, grazing, iron and supply of silicic acid. Our field observations and *in situ* incubations when considered with other field observations and laboratory experiments suggest that the Dd-cycle is an additional and important factor in limiting primary productivity in the Southern Ocean.

We suggest that the presence of high amounts of cellular Dd-cycle pigments reduce the ability of Southern Ocean diatoms and haptophytes to harvest light and that the physiological cost of maintaining high DD-cycle activity reduces growth rates.

Laboratory experiments show that at a cellular level, Dd competes with light harvesting pigments mainly fucoxanthin for the absorption of light diminishing the light energy, which is transferred into photosystem II^{5,9}. Additional Dd binds to the ‘major’ light-harvesting complex, which while enhancing the dissipation of excess energy reduces the ability of the cell to harvest light. Within natural phytoplankton communities a high concentration of Dd is consistent with observations of reduced photosynthetic efficiency^{15,16}. Cellular pigment concentrations of Dd and Dt of 40% represent 30% of the total absorption of incident photosynthetically available

radiation (PAR), making this portion of the absorbed energy unavailable for photosynthesis¹⁵.

From our observations, the relationship between the vertical distribution of the Dd-cycle pigments and the hydrodynamics in the study area, show that higher Dd occurs when phytoplankton are subject to a rapid fluctuating light environment due to hydrodynamic conditions. Laboratory experiments on diatoms also show that an intermittent light regime results in a high Dd content with additional Dd molecules bound to the light-harvesting fraction¹⁶. A high concentration of Dd and the fast kinetics of de-epoxidation and epoxidation of the Dd-cycle confer an adaptive advantage in the highly variable underwater light environment. *In situ* data supports this idea with observations of an increased Dd content in diatoms exposed to fluctuating high light conditions¹⁷. This may be a consequence of replacement of fucoxanthin molecules by Dd molecules or the relative enrichment of subunits rich in Dd at the cost of fucoxanthin/Chl *c* rich subunits^{9,16}. Our and other observations of the pool size of Dd-cycle pigments increasing towards the surface implies that the Dd-cycle pigments bind to light harvesting proteins other than with fucoxanthin and Chl *c*¹⁴. The physiological response to rapid light changes is a costly process. It has been suggested that the energy cost of enhanced photoregulation due to rapid light fluctuation may be responsible for the decrease in growth rate in some species of phytoplankton, which possess the Dd-cycle¹⁸.

Surface mixed waters within the study area have been shown to have subnanomolar concentrations of dissolved iron over the same period as this cruise (April – May)¹⁹. Algae growing in very low iron concentrations under low light/high light conditions show an increase by several times of the Dd cellular content comparable to cells exposed to higher irradiance conditions²⁰. The high Dd concentration observed in this

study may be a consequence of low dissolved iron concentrations with the algae responding as if they had been exposed to higher irradiance conditions. We suggest that low iron results in higher photoprotective pigments but a lower photosynthetic capacity and efficiency.

A number of global circulation models predict that climate change will have a marked effect on the structure of the surface mixed layer of the Southern Ocean with predicted warming, increased stratification and a shallowing of the mixed layer⁸. If the upper ocean stratifies, phytoplankton will be on average closer to the surface and be exposed to higher levels of solar radiation, significantly altering the current light environment. How the Dd-cycle in shade-adapted Southern Ocean phytoplankton responds to these changes will be a key factor in understanding future changes in primary productivity.

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Acknowledgements We thank the National Institute of Water and Atmospheric Science (NIWA) and M. Williams for support and participation in subantarctic cruise (SAA5). K. Bonney for assistance with HPLC pigment analyses. G.P.G was supported by the Division of Science, University of Otago

Author Contributions G.P.G and R.V contributed equally to the work. G.P.G collected and analysed the pigment data. Both authors discussed the results and commented on the manuscript.

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Figure 1 Sampling stations in the subantarctic region, South-east of New Zealand.

Figure 2 Distribution of diadinoxanthin and diatoxanthin.

Figure 3 Daytime vertical profiles of the DES index ($Dt/Dd+Dt$).





