Mapping Antarctic phytoplankton physiology using autonomous gliders

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I. INTRODUCTION

Environmental factors, such as nutrient availability and irradiance, play the key role in phytoplankton physiology. Phytoplankton growth and marine primary production depend on physiological performance of phytoplankton that in turn respond to varying environmental conditions. The use of variable fluorescence kinetics has increasingly become a vital method in oceanographic studies; however, its use within the community is limited by the complexity of physiological data interpretation and the cost of available instruments. Physiological responses also span across different scales that are hard to record with the discrete manual sampling methods available.

The Quantum Yield of Photosynthesis (ϕ) is defined as the ratio of oxygen evolved in photosynthesis (or carbon assimilated) to the number of photons absorbed in the process [1]. Evaluating the Quantum Yield of Photosynthesis *in vivo* provides an alternate approach to C^{14} uptake, for example, for investigating how photosynthetic processes are affected by environmental factors, as it is highly sensitive to environmental stresses. The variable fluorescence [2] is the most sensitive signal detected from the ocean, which provides considerable insight into the photophysiology of phytoplankton, in particular the structure and function of Photosystem II (PSII). In the field, the maximum yield of photochemistry in PSII (Fv/Fm) has been widely used as a diagnostic tool to rapidly assess the health of phytoplankton and infer potential stresses or primary production controls [3].

Traditional sampling strategies such as the pump-and-probe, Fast Repetition Rate Fluorometers (FRRF) and more recently the Fluorescence Induction and Relaxation (FIRe) sensor [4] have been employed to characterize and understand the factors controlling phytoplankton physiology and primary production in the ocean. While continuous automated acquisition of physiological parameters is commonly used for surface waters using different fluorometers, repeated depth measurements over diel cycles are less often reported. When available, depth profiles have been built from discrete samples collected in bottles and ran on the benchtop instrument. Besides being a destructive sampling method, it is also constrained by the fact that the water collection and instrument run need to be done manually. Another benefit of measuring variable fluorescence *in situ* lies in the fact that the assessment of the photo-physiological properties of phytoplankton happens in the actual light fields in which these organisms are growing [5]. An important point since these physiological properties are highly sensitive to the ambient light fields.

Phytoplankton developed photoadaptation mechanisms to overcome light-induced stresses [6] i.e. to optimize light absorption under low light conditions or reducing total photon absorption under supra-optimal irradiances. These mechanisms, reflecting changes in the functional absorption cross-section of PSII (σ_{PSII}), are short-term light adaptation mechanisms such as Non-Photochemical Quenching (state transition, energy dependent and photoinhibition) and long term modifications in the light harvesting complex of phytoplankton (i.e. photoacclimation) [1]. The integration of an instrument, such as the FIRe sensor, that is capable of evaluating depth-dependent phytoplankton physiology in situ and in high resolution is an important step to fully understand phytoplankton dynamics and marine primary production.

II. MATERIALS AND METHODS

A. Slocum gliders

Slocum electric gliders are a robust tool to map, with high spatial and temporal resolution, the upper water column properties in different environments [7], including polar regions. These 1.5 m torpedo-shaped buoyancy driven autonomous underwater vehicles provide high-resolution surveys of the physical and bio-optical properties of the water column [7].

Glider used for these deployments (ru24) was equipped with a Seabird Conductivity-Temperature-Depth (CTD) sensor. Glider based conductivity, temperature and depth measurements were compared with a calibrated ship CTD sensor on deployment and recovery to ensure data quality, as well as with a calibrated laboratory CTD prior to deployment.

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Glider profiles were binned into 1-meter bins and assigned a mid-point latitude and longitude. For each profile, Mixed Layer Depth (MLD) was determined by finding the depth of the maximum water column buoyancy frequency, max (N^2). An upward facing Photosynthetic Active Radiation (PAR) sensor was also integrated in the glider to record PAR in the range of 400-700 nm.

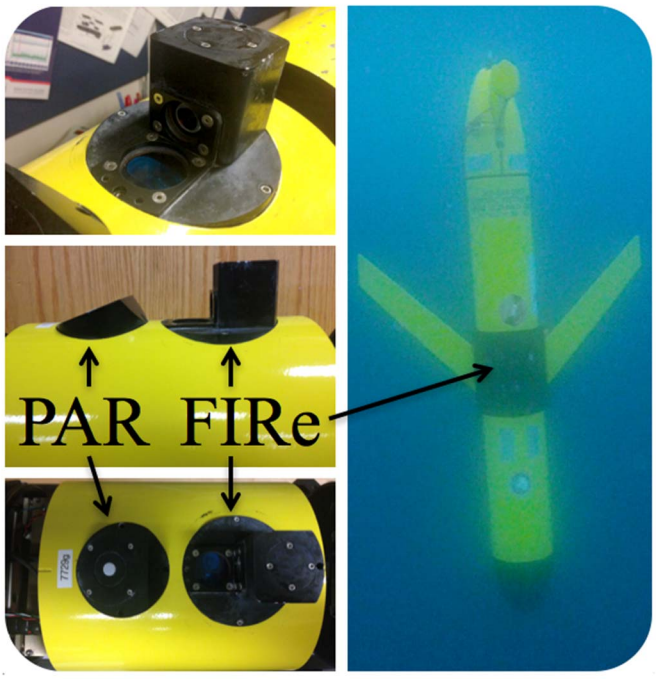

Figure 1: Fluorescence Induction and Relaxation (FIRe) and PAR sensors integrated into a Slocum glider.

B. FIRe sensor

In order to evaluate physiological responses of phytoplankton to physical forcing, ru24 was equipped with a FIRe sensor [8], the first sensor of its kind to be integrated in a glider. This allowed a high-resolution continuous mapping of the phytoplankton physiological responses to variable light regimes in the water column.

The FIRe sensor [4] provides a comprehensive suite of photosynthetic characteristics of the organisms, such as the minimum (F_o) and maximum (F_m) fluorescence yields corresponding to open and closed reaction centers of PSII, respectively, variable fluorescence component (F_v) and the functional absorption cross section of PSII (σ_{PSII}). This is accomplished by employing a sequence of excitation flashes of light with controlled intensity, duration and interval between flashes. The maximum quantum yield (efficiency) of photochemistry in PSII, denoted by ϕ_{PSII}, is given by the ratio F_v/F_m, i.e. $[F_m-F_o]/F_m$. Dark-adapted F_v/F_m has been widely used as an algal "health" parameter, which is responsive to the short-term (hours) light and nutrient history of the cells [9]. By definition, the actual quantum yield of photochemistry in PSII at a given PAR level is denoted Fv'/F_m'. A cap was used to cover the FIRe sample chamber so the signal was measured in the dark (the prime (') symbol after the variable denotes any light acclimated sample measured in darkness [10]). At low irradiance (under 100 $\mu E/m^2/s$, which comprises over 98% of the data points), Fv'/Fm' approaches Fv/Fm, and so for simplicity, Fv'/Fm' will be referred to as photosynthetic efficiency for the remaining of the manuscript.

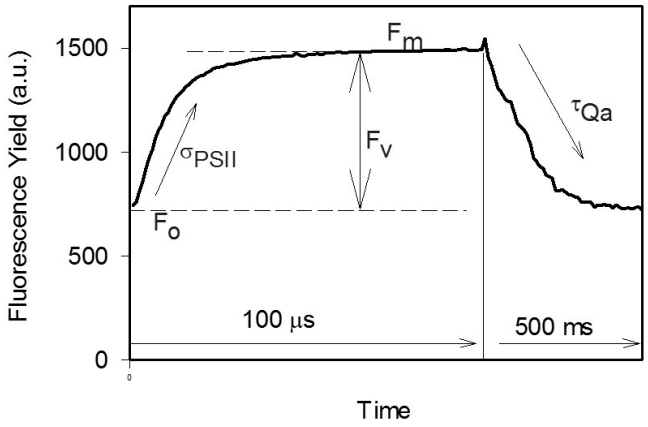

Figure 2: FIRe parameters from the Single Turnover Flash (STF) protocol. Minimum (Fo) and maximum (Fm) fluorescence yields corresponding to open and closed reaction centers of PSII, respectively, variable fluorescence component (Fv, where Fv=Fm-Fo), the quantum yield of photochemistry in PSII (Fv/Fm) and the functional absorption cross-section of PSII (σ_{PSII}).

C. FIRe glider data post-deployment processing

Several steps comprise the FIRe glider post-deployment processing. After the glider is recovered, raw data are downloaded from the onboard FIRe memory. Binary raw data is run through the Satlantic software and converted to ascii format. Data was then corrected for gain of the detector before applying any other corrections:

a) Blanks

"Blank" is the background signal recorded from the sample without phytoplankton in it. The blank signal includes a small amount of fluorescence from dissolved organic matter (DOM) and phytoplankton degradation products dissolved in water. As in any fluorometer, blanks must be removed from fluorescence signals (only Fm and Fo, as other FIRe parameters are blank-independent) to get accurate, blank-corrected values of chlorophyll fluorescence. Although blanks in the FIRe sensors are usually relatively small and may be neglected in many cases, the blank correction procedure will be critical in waters with high amount of DOM and/or small amount of phytoplankton (e.g. oligotrophic regions or deep layers below the euphotic zone).

As it is not possible to collect blanks while the glider is deployed, discrete *in situ* water samples were collected from the surface and at a depth well below the deep chlorophyll maximum (DCM), several times before and after the glider deployments. Surface and deep water samples were filtered and measured using the FIRe glider. Average surface and deep values were calculated and subtracted from the FIRe glider

fluorescence signals during the deployment from the surface up to the DCM and below the DCM, respectively.

b) Functional absorption cross-section of PSII (σ_{PSII})

In order to convert the measured σ_{PSII} (in relative units) into absolute units (Å^2 quantum^{-1}), a correction coefficient was determined by cross-calibrating the FIRe glider against a "standard" calibrated benchtop FIRe instrument. A correction factor of 1650 was determined for the FIRe sensor RU24.

c) Converting FIRe Fm to chlorophyll-a concentrations

Discrete water samples at 8 different depths within the euphotic zone were collected during each glider deployment and recovery to further convert the measured Fm (maximum fluorescence intensities, in relative units) into absolute chlorophyll concentration (µg L^{-1}), a variable measured by other sensors, facilitating further comparisons with other studies. Chlorophyll-*a* (chl-*a*) is a proxy of phytoplankton biomass. Water samples were filtered onto 25 mm Whatman GF/F filters and extracted using 90% acetone.

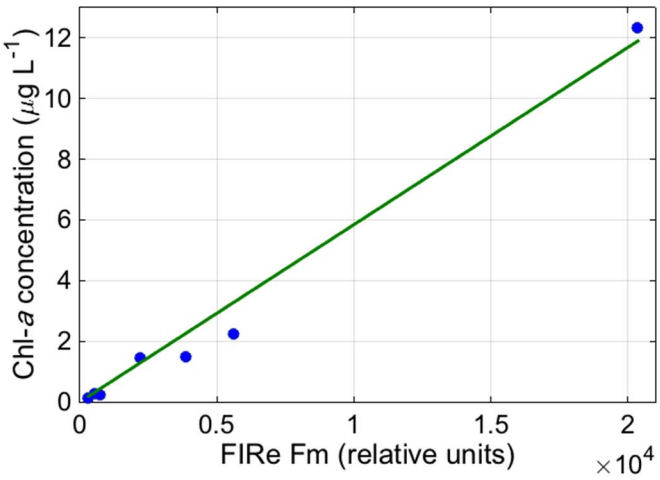

Figure 3: Scatter plot and respective linear trend from discrete water samples measured by the FIRe sensor (FIRe Fm) and chlorophyll-*a* concentrations obtained by the fluorometric method.

As a linear correlation is expected between FIRe Fm and chl-*a* concentration, the same sample was also run through the FIRe glider and its Fm measurement recorded. FIRe Fm throughout the deployment was then converted into chl-*a* concentration (Equation 1) using the high linear correlation (r^2=0.98) found between the 2 variables:

$$Chl = Fm \times 5.84 \times 10^{-4} \quad (1)$$

where *Chl* is the derived chlorophyll concentration and *Fm* is the maximum fluorescence measured by the FIRe sensor, after gain and blank corrections. QA/QC methods were applied to the data to ensure data quality.

D. Sampling overview

Palmer Deep (PD) is one of the cross-shelf canyons located in the West Antarctic Peninsula (WAP) where there is evidence of increased primary production [11, 12] and localized penguin foraging [13]. The National Science Foundation (NSF) funded Palmer Long Term Ecological Research (PAL-LTER) project [14] has been monitoring this ecosystem since 1991. The dependence of higher trophic levels on primary producers has led to increased efforts to try to understand the link between some of the physical drivers (such as stratification and mixed layer depth, MLD) and phytoplankton dynamics [12, 15, 16].

Part of this project has been focusing on understanding the phytoplankton physiological responses due to physical forcing. Until recently, sampling methods were restricted to discrete samples taken by a rosette or go-flo bottles collected from the ship or zodiac, respectively. This method provides insight into the depth dependent response of phytoplankton physiology, however it requires manual water collection at certain depths and times. A second shipboard method measures physiological parameters continuously using the onboard flow-through system at 5 m depth. This provides higher resolution horizontal maps, but lacks the depth component. The integration of a FIRe system into a glider allowed us to overcome these constrains and to sample *in situ* phytoplankton physiological responses in high-resolution both vertically and horizontally.

By measuring changes in maximal (F_m') and minimal (F_o') fluorescence in the same water mass over a diel cycle, one can get fluorescence values representative of a darkened adapted and relaxed state [17]. Evaluating diel cyles will also allow us to better understand the light effect on phytoplankton physiology by isolating the effect of supra-irradiance during peak daytime hours.

Here we present the results of two FIRe glider missions that have been designed to evaluate physiological responses at different temporal and spatial scales:

a) Temporal evolution – "the drift mission"
b) Spatial variability – "the station keeping mission"

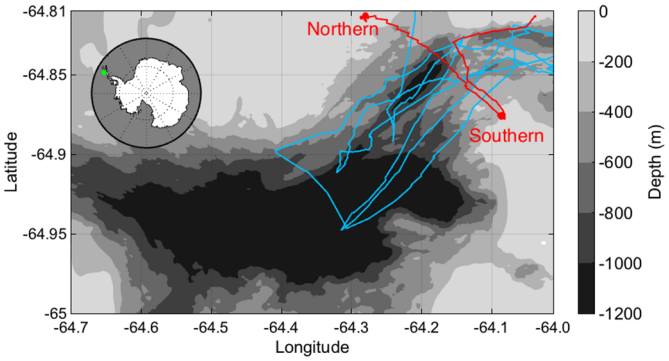

Figure 4: Bathymetry map of Palmer Deep canyon (green dot) in the WAP, Antarctica with the 2 sampling strategies used in the study: 1) drift mission (blue) to evaluate temporal changes in phytoplankton physiology and 2) station keeping mission (red) to evaluate spatial variability in phytoplankton physiology due to physical forcing.

III. Evaluating Temporal Changes

Previous studies have shown that the community structure, such as phytoplankton cell size and taxonomy, has influence on the photosynthetic rates, and therefore on the variable fluorescence signal [18]. In order to better isolate the temporal signal in phytoplankton photosynthetic efficiency, a mission was designed (Figure 4, blue) where the same water mass would be followed. This would allow a better characterization of physiological changes of the same community over time. The principle behind this mission was to conduct drift missions starting southwest of the canyon head as the dominant currents would push the glider towards the head of the canon. Every hour, the glider would perform a corkscrew dive and climb (fin set all the way to starboard side), while drifting at the surface in between dives. This way the same water mass was being followed and, by default, the same phytoplankton community would be evaluated during that drift. Four drift transects were conducted that lasted around 2 diel cycles each.

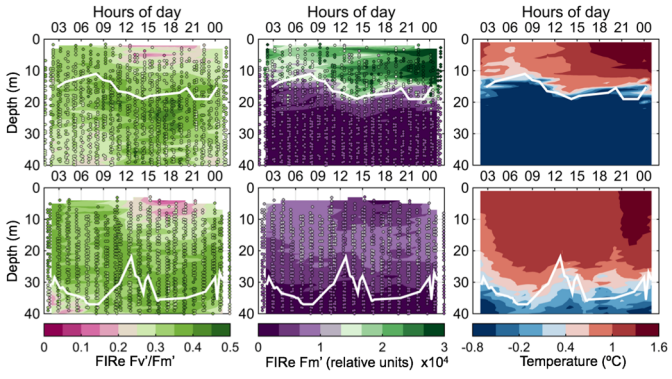

Figure 5: Averaged diel cycles of Fv'/Fm' (Photosynthetic efficiency), Fm' (proxy for phytoplankton biomass) and temperature for 2 different MLD (white line) regimes. Dots represent actual in FIRe glider measurements.

Phytoplankton acclimate to light levels averaged over the MLD. As stratification increases during spring/summer time, cells start to acclimate to the light intensity at each depth. Shallow MLDs provide a relatively stable light environment that allows phytoplankton to photoacclimate on the timescale of 1-2 days [19]. Intense mixing can bring dim-light adapted phytoplankton to the upper MLD where phytoplankton get exposed to supra-optimal irradiances and a decrease in Fm and Fv/Fm is recorded. Non-Photochemical Quenching (NPQ) is evident from reduction in both Fv'/Fm' and Fm' during daytime hours. Under higher irradiance (11:00-22:00 GMT), both Fv/Fm and Fm decrease, with the lowest values and deeper light penetration during peak irradiance hours (15:00-18:00 GMT). NPQ signal is more marked (Fv'/Fm'<0.1) when MLD is deeper as phytoplankton cells are acclimated to a mid-MLD light level, and therefore show higher light stress under supra-optimal irradiances.

IV. Evaluating Spatial Variability

Spatial variability in the water column structure has been recorded across the head of the canyon [12]. In order to evaluate whether phytoplankton physiological responses are related to physical forcing, a mission was designed (Figure 4, red) where diel cycles of FIRe parameters were recorded at each location together with the standard CTD measurements. The station keeping glider recorded 2 consecutive diel cycles at each location (Figure 6), where at least one out of three dives/climbs would record FIRe data (approximately one FIRe dive an hour). In the remaining dives, the glider would only capture water column physical parameters.

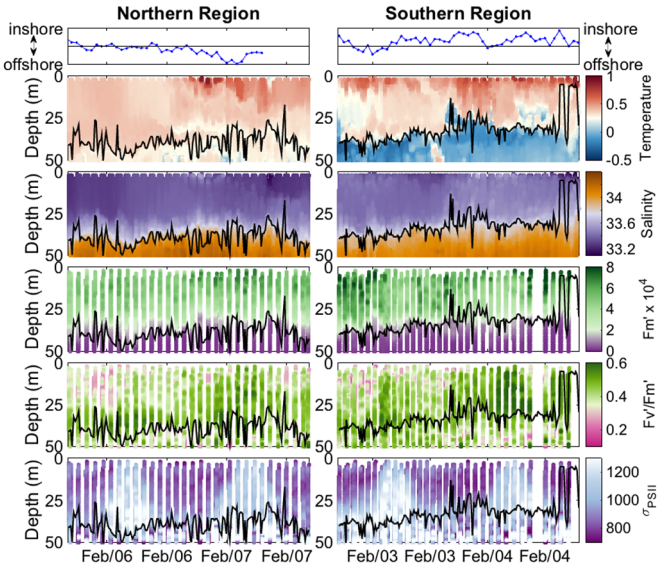

Figure 6: Comparison of 2 diel cycles at each region. Top row indicates dominant surface currents: inshore (oceanic "warm" influence) and offshore (coastal "cold" current). Temperature (°C), Salinity and FIRe parameters Fm' (relative units), Fv'/Fm' and σ_{PSII} (functional absorption cross-section of PSII, Å^2 quantum^{-1}) are presented for both regions. Black line denotes MLD.

V. Evaluating Photoacclimation Mechanisms

Phytoplankton developed photo-adaptation mechanisms to overcome light-induced stresses, i.e. to optimize light absorption under low light conditions or to reduce total photon utilization under supra-optimal irradiances. Our preliminary analyses have shown different photoacclimation responses resulting from different MLD dynamics due to different solar radiation exposure conditions (both time and intensity).

An exponential relationship was found between Fv'/F_m' and PAR (Figure 7, left). Under low light (nighttime periods and deeper depths) Fv'/F_m' was maximum and equal to F_v/F_m, while under high light (high PAR) Fv'/F_m' decreased, an evidence of light-induced down regulation of PSII. A power fit curve (Equation 2) was applied to the FIRe data to evaluate differences in photoacclimation regimes under two different MLD conditions:

$$\frac{Fv'}{Fm'} = a\, e^{-\frac{PAR}{E_k}} \qquad (2)$$

where Fv'/F_m' is photosynthetic efficiency and PAR is Photosynthetic Available Radiance, both measured by the glider, E_k is light saturation parameter and a is a constant.

By comparing E_k values calculated for each depth/PAR bin, we can begin to distinguish between similar photo-physiologic communities, i.e. communities that have similar photoacclimation regimes and different photo-physiological communities and relate that to the depth of the ML.

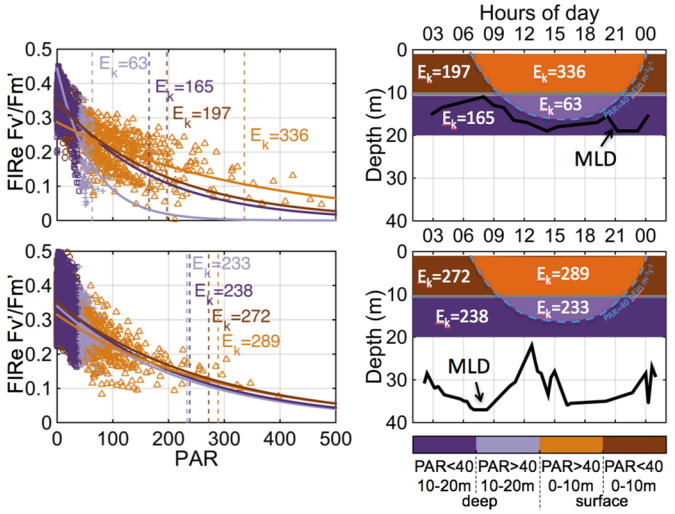

Figure 7: (left) Scatter plots of Fv'/F_m' and PAR with power curve fits for each PAR and depth bins highlighted in the legend. E_k values for each fitting are also presented; (right) schematics on difference in photoacclimation regimes presented in the plots on the left, evaluating the light saturation parameter in relation to the MLD (black line).

When MLD is shallow (Figure 7, top), the two layers show different E_k values. The much higher E_k, seen at the surface, gives an indication of phytoplankton acclimated to high irradiances while the lower E_k seen below the MLD shows low light acclimation. On the other hand, when MLD is deeper, and the 2 layers (0-20 m) fall within the ML, E_k values are much more similar, indicating that phytoplankton have similar photoacclimation regimes in the 2 layers. Note that the E_k of both layers when ML is deeper also fall between the first 2 E_k, indicative of a mid-light level acclimation.

Table 1: Summary on depth-dependent photoacclimation regimes in a nutrient replete environment.

MLD (m)	Stratification max[N^2], s^{-2}	Biomass (FIRe Fm')	Layer 0-10m	Layer 10-20m
Shallow ($\overline{MLD} \approx 16$)	High ($\overline{N^2} \approx 2\text{x}10^{-3}$)	High	High-light acclimated (higher E_k)	Low-light acclimated (lower E_k)
Deeper ($\overline{MLD} \approx 32$)	Low ($\overline{N^2} \approx 6.7\text{x}10^{-4}$)	Low	Mid-light (MLD) acclimated E_k(0-10m) ≈ E_k(10-20m)	

VI. Conclusions

The integration of a FIRe sensor into a glider allows us to map, with high temporal and spatial resolution, phytoplankton physiological responses to physical forcing. Different missions were designed to evaluate the temporal and spatial variability of phytoplankton physiology by using a drift and a station keeping mission, respectively. Diel cycles collected show a clear diurnal variations driven by incident radiation, with both maximal fluorescence and photosynthetic efficiency (in any light adapted phytoplankton) showing reduced values only in the upper 10-15 meters of the water column at the highest irradiances. Further analyses comparing different MLD regimes have shown different photoacclimation responses (light saturation parameter, E_k) resulting from differences in solar radiation exposure conditions (both time and intensity), reflected in the depth of the ML. Further analyses include determining a method to correct the FIRe glider fluorescence profiles in the upper ocean during daytime by comparing the maximum fluorescence during the highest irradiance (daytime) with the lowest irradiance (nighttime).

Acknowledgments

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