Using next-generation multi-spectral FRRf to improve current estimates of marine primary production (MPP) within Australian waters

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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David J. Hughes

12/04/2018

Dedication

This thesis is dedicated to my parents, Peter and Rose, who shared their appreciation for the natural world with me from an early age, and sparked a lifelong interest in marine science.

Many times over the years they have encouraged me to follow the path that makes me happy and have supported me every step of the way. It is with their support that I embarked on this journey, and this thesis stands as a testament to the belief that they have shown in me.

Now I have reached the conclusion of this journey I look back with full appreciation for their love and support.

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List of Abbreviations

AEF	Alternative electron flow
ANACC	Australian national algal culture collection
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Bac	Bacillariophyceae
C	Carbon
CCM	Carbon concentrating mechanism
CDOM	Coloured dissolved organic matter
CEF-PSI(II)	Cyclic electron flow around PSI(II)
CO_2	Carbon dioxide
Chl	Chlorophyceae
Chl-*a*	Chlorophyll *a*
Cry	Crptophyceae
CTD	Conductivity, temperature and depth
Cya	Cyanobacterium/Cyanophyceae
dbRDA	Distance-based redundancy analysis
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Dia	Diatom
DIC	Dissolved inorganic carbon
Din	Dinophyceae
DistLM	Distance-based linear modeling
DOC	Dissolved organic carbon
DPM	Disintegrations per minute
EAC	East Australian Current
Eus	Eustigmatophyceae
FDOM	Fluorescent dissolved organic matter
FDP	Flavodiiron protein
FIRe	Flash Induction and Relaxation fluorometry
Fla	Flagellate
Flv	Flavoproteins
FRRf	Fast Repetition Rate fluorometry
FY	Fluorescence Yield
GAP	Glyceraldehyde 3-phosphate
GC	Gas chromatography
GPP	Gross primary production
Hap	Haptophyte
HCl	Hydrochloric acid
$HgCl_2$	Mercuric chloride
HPLC	High-performance liquid chromatography
IMOS	Integrated Marine Observing System
LED	Light emitting diode

LEF	Linear electron flow
MDS	Multidimensional scaling
MIMS	Membrane inlet mass spectrometry
MLD	Mixed layer depth
MPP	Marine primary production
N	Nitrogen
N_2	Atmospheric Nitrogen
$NADP^+(H)$	Nicotinamide adenine dinucleotide phosphate
$NaH^{14}CO_3$	^{14}C-labelled Sodium bicarbonate
NaH_2PO_4	Sodium phosphate dibasic
NE	North east
NH_4^+	Ammonium
NH_4NO_3	Ammonium nitrate
NO_2^-	Nitrite
NO_3^-	Nitrate
NPP	Net primary production
NPQ	Non-photochemical quenching
NPQ_{NSV}	NPQ(normalised Stern-Volmer coefficient)
NSW	New South Wales
O_2	Oxygen
OCP	Orange carotenoid protein
P	Phosphorus
Pabs	Particulate absorption spectra
PAM	Pulse amplitude modulated fluorometry
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PE	Photosynthetic-irradiance
Pel	Pelagophyceae
PH	Port Hacking
Pmf	Proton motive force
PO_4^{3-}	Phosphate
POC	Particulate organic carbon
PON	Particulate organic nitrogen
POP	Particulate organic phosphorus
PP	Primary production
PQ	Plastoquinol
PQ	Photosynthetic quotient
Pry	Prymnesiophyceae
PSI	Photosystem I
PSII	Photosystem II
PSU	Practical salinity unit
PSU	Photosynthetic unit
PTOX	Plastiquinol terminal oxidase
Q_A	Quinone-A electron acceptor

q_E	Energy-dependent Quenching
ROS	reactive oxygen species
rpm	Revolutions per minute
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
S	Sulphur
Scf	Spectral correction factor
Si	Silicate
SiO_4	Silicate
SST	Sea surface temperature
ST	Single turnover
SVP	Surface velocity program
TOC	Total organic carbon
V	Cell volume

List of Symbols

a^{Chl}	Spectral light absorption
a_{LHII}	PSII absorption coefficient of the light-harvesting pigments
$A(\lambda)$	Wavelength-dependent absorbance
β	Pathlength amplification factor
C	Fraction of [RCII] in the closed state
d^{-1}	Daily division rate
ΔpH	Proton gradient
E	Irradiance
E_K	Light saturation parameter
E_{LED}	Intensity of the fluorometers 450 nm measuring beam
$\mathrm{ETR_{PSII}}$	Electron Transport Rate through PSII
Φ_{PSII}'	quantum yield of photochemistry under actinic light
F'	Fluorescence yield under actinic light at time t
F_0	Minimum PSII fluorescence yield (dark-acclimated state) where all PSII reaction centres are open
F_0'	Minimum PSII fluorescence yield (light-acclimated state) where all PSII reaction centres are open
F_m	Maximum PSII fluorescence yield (dark-acclimated state) where all PSII reaction centres are closed
F_m'	Maximum PSII fluorescence yield (light-acclimated state) where all PSII reaction centres are closed
F_t	Steady-state fluorescence
F_v	Maximum variable PSII fluorescence yield (dark-acclimated state)
F_v'	Variable fluorescence yield under actinic light
$\mathrm{F_v/F_m}$	Maximum photochemical efficiency (dark-acclimated state)
$\mathrm{F_v'/F_m'}$	Maximum photochemical efficiency (light-acclimated state)
$\mathrm{F_q'/F_m'}$	Effective photochemical efficiency under actinic light
$\mathrm{F_q'/F_v'}$	PSII efficiency factor (under actinic light
K_C	Electron requirement for carbon fixation
K_R	Instrument-specific constant
L	Optical pathlength of filter particulates
λ	Wavelength
μ	Growth rate
n_{PSII}	Assumed ratio of PSII reaction centres per unit chlorophyll-*a*
qE	Energy-dependent quenching
qJ	FRRf connectivity model (assumes partial connectivity between RCIIs)
qP	Photochemical quenching parameter
[RCII]	Concentration of functional PSII reaction centres
$[\mathrm{RCII}]^{(\mathrm{FRRf})}$	Concentration of [RCII] as estimated by FRRf
ρ	PSII Connectivity Factor

ρ'	PSII Connectivity Factor under actinic light
ROS	Reactive Oxygen Species
σ_{PSII}	Functional absorption cross-section of PSII
$\sigma_{PSII}(')$	Functional absorption cross-section of PSII (under actinic light)
τ_{PSII}	Turnover time of PSII
Y	Fractional yield
YF	Fluorescence yield
YPSII	Photochemical yield of PSII

Summary

Bio-optical tools remain key technologies to address a long-standing goal in oceanography: to improve understanding of how marine primary productivity (MPP) varies over space and time. A major goal for one particular technique, Fast Repetition Rate fluorometry (FRRf), is to retrieve highly resolute patterns of carbon (C) uptake *in situ* to improve satellite retrieved predictions of MPP. However, this goal hinges upon the application of a highly-variable, yet poorly-understood conversion factor to scale FRRf-derived electron transport rates (ETRs) to rates of C-uptake. Understanding of the conversion factor, termed the "electron requirement for carbon fixation" (K_C) is limited, in particularly for Australian waters where K_C has rarely been measured.

This thesis focuses on coupled ETR – C-uptake measurements, to examine how key factors drive variability in K_C, utilising both laboratory and field studies to isolate the respective influences of growth environment and phytoplankton taxonomy. I performed nutrient addition bioassays upon natural phytoplankton assemblages to demonstrate for the first time how macronutrient availability (N, P and Si) regulates K_C at an Australian coastal reference station when nutrient concentrations are low during summer. To examine taxonomic variability of K_C together with metrics influencing phytoplankton growth and physiology (cell size and non-photochemical quenching, NPQ), I grew phytoplankton covering a broad range of taxonomic and size classes within a controlled laboratory setting where environmental variability could be excluded. Finally, to examine how well K_C could be predicted in a highly-dynamic system with multiple environmental stressors and phytoplankton assemblages, I performed a novel high-throughput assessment of K_C (n = 80) along the eastern Australian coast spanning multiple water masses including the Tasman Sea and the East Australian Current

(EAC). Prevailing environmental variables, physiological (non-photochemical heat dissipation, NPQ_{NSV}) and phytoplankton community structure (size-fractionated Chl-*a*) were also measured for each sample to allow evaluation of their respective performance in empirically modelling K_C variance.

This thesis highlights the importance in characterising both environmental and taxonomic factors to most robustly retrieve K_C, but also demonstrates that a single FRRf parameter (NPQ_{NSV}) may reliably explain ~50% of variability in eastern Australian waters. These new findings potentially provide new and unprecedented capacity to retrieve C-fixation rate from FRRf-based productivity assessments, but ultimately require further validation that may be possible through re-visiting past FRRf data sets. These findings are then considered to propose a roadmap to enable broader implementation and uptake of FRRf for widespread assessments of marine (and freshwater) primary productivity into the future.

Declaration of the Contribution to Each Chapter

Chapter 2

This chapter has been submitted for publication in Limnology and Oceanography as:

Hughes DJ, Varkey D, Doblin M.A, Ingleton T, McInnes A, Ralph P.J, Van Dongen-Vogels V, Suggett D.J (2017) Impact of nitrogen availability upon the electron requirement for carbon fixation in Australian coastal phytoplankton communities. Currently under review. Experimental design was by DJH with help from DJS. Fieldwork was conducted by DJH, VVDV and TI. Laboratory sample analysis was performed by DH with help from DS. Data analysis and interpretation was done by DJH with help from DV and DJS. Writing of the manuscript was completed by DJH with help from DJS, DV, MAD and PJR.

Chapter 3

The experiments of this study were designed by me, with help from Assoc. Prof. David Suggett (UTS). I was responsible for the majority of laboratory work, data analysis and interpretation, and the writing-up of the manuscript. Dr. Maria Giannini (UTS) and Arjun Verma (UTS) grew and provided several phytoplankton strains used in this experiment. Dr. Joseph Crosswell (CSIRO) provided assistance with analysis of dissolved inorganic carbon. Dr. Deepa Varkey (Macquarie University) provided support with R-software. Assoc. Professor David Suggett, Prof. Peter Ralph (UTS), Assoc. Professor Martina Doblin (UTS) and Dr. Deepa Varkey provided detailed feedback on the manuscript at various stages.

Chapter 4

The data presented in this chapter reflects a joint laboratory effort. I was responsible for the experimental design, methodological development, data interpretation, and write-up of the manuscript with help from Assoc. Professor David Suggett (UTS). I performed the coupled ^{14}C/FRRf incubations, and jointly conducted size-fractionated Chl-a analysis with Assoc. Professor David Suggett (UTS). Assoc. Professor David Suggett collected multispectral FRRf measurements. Dr. Joseph Crosswell (CSIRO) performed analysis of dissolved inorganic carbon. Assoc. Professor Martina Doblin (UTS), Prof. Peter Ralph (UTS) and Assoc. Professor David Suggett were instrumental in securing ship-time.

Chapter 6

This opinion paper reflects a joint effort. I was responsible for writing the majority of the original manuscript which was then significantly improved by contributions from Assoc. Professor David Suggett (UTS), Dr Doug Campbell (Mount Allison University), Professor Mark Moore (Univeristy of Southampton). Dr Evelyn Lawrenz and Prof. Ondrej Prasil (Czech Academy of Sciences) performed time-resolved measurements of ^{14}C-incubations and provided significant intellectual input into the manuscript. Assoc. Prof. Martina Doblin and Marco Alvarez (UTS) performed short vs long-term ^{14}C incubations and provided comprehensive feedback on the manuscript.