APPENDIX 3

TA PROJECT REPORT (TEMPLATE)

(see following pages)

TA PROJECT REPORT PACKAGE

- The completed and signed forms included in this package should be sent by email to jerico.ta@marine.ie and **jerico**-s3**@ifremer.fr** within **one month after the completion of the TA project** by the User Group Leader.
- **Refunding of the TA reimbursement to the user group will be processed as soon as these forms will be submitted**.
- The TA project report will be published in the JERICO-S3 website. The report, as well as other information collected with the attached forms, will be used to report to the European Commission.
- **Please note that any publication resulting from work carried out under the JERICO-S3 TA activity must acknowledge the support of the European Commission – H2020 Framework Programme, JERICO-S3 under grant agreement No.871153.**

1. Project Information

Proposal reference number[1]	21/1001637
Project Acronym (ID)[2]	OligoSTAF
Title of the project[3]	Field testing and validation of a new STAF sensor in oligotrophic Mediterranean waters
Host Research Infrastructure[4]	HCMR
Starting date - End date[5]	17th to 28th October 2021
Name of Principal Investigator[6] **Home Laboratory** **Address** **E-mail address** **Telephone**	Kevin Oxborough Chelsea Technologies Ltd. 55 Central Avenue, KT8 2QZ, UK koxborough@chelsea.co.uk +44 2084819002

2. Project objectives[7] (250 words max.)

Photosynthetron-based measurements of ^{14}C assimilation can provide accurate estimates of net primary productivity over a long time period. However, this method cannot provide information on dynamic processes or downregulation during the incubation, which is typically two hours or more. Furthermore, long incubation times, expensive reagents, and a complex experimental protocol mean that only a few ^{14}C data sets can be acquired per day. In contrast, Fluorescence light curves (FLCs) provide detailed dynamic data over a 20 minutes measurement cycle (typical). The main issue with comparing ^{14}C and STAF data is that the STAF measurements quantify PSII photochemical (electron) flux, rather than carbon uptake. This study was conducted to investigate the quantitative relationship between ^{14}C fixation and STAF-derived PSII electron flux in oligotrophic waters.

The overall goal of the work was to determine whether LabSTAF could be used to monitor primary productivity in oligotrophic waters. While the methodology for STAF-based measurements of PSII electron flux on a volume basis was published ten years ago (Oxborough et al. 2012), previous generations of fluorometers have lacked the required level of sensitivity.

[1] Reference number assigned to the proposal by the TA-Office.

[2] User-project identifier used in the proposal.

[3] Title of the approved proposal. The length cannot exceed 255 characters

[4] Name of the installation/infrastructure accessed with this project. If more than one installations/infrastructures are used by the same project, please list them in the box.

[5] Specify starting and end date of the project (including eventual preparatory phase before the access).

[6] Fill in with the full contact of the Principal Investigator (user group leader).

[7] Write the short-term, medium and long-term objectives of the project. Use no more than 250 words.

There were three additional aims of the experiments: 1) to assess the level of variability in primary productivity estimates between the ^{14}C and STAF-based methods; 2) to determine how primary productivity changes during the long incubation times used in a photosynthetron, particularly at the high light levels; 3) to determine the electron per carbon ratio ($\Phi_{e,C}$) for carbon assimilation by comparing the primary productivity estimations from ^{14}C uptake and LabSTAF.

3. Main achievements and difficulties encountered (250 words max.)[8]

LabSTAF was able to acquire high-quality data from 2 m, 20 m and 60 m seawater samples. Continuous FLC analysis showed that LabSTAF had the sensitivity to identify diurnal trends in oligotrophic water, at much higher temporal resolution than ^{14}C-uptake experiments.

Detailed experiments were conducted on the 20 m samples, to compare FLC-derived measurements of PSII electron flux (JV_{PII}) with ^{14}C-based estimates of primary productivity. The range of electron per carbon ratios ($\Phi_{e,C}$) derived from these measurements were within the expected range, at approximately 8 to 40. A strong positive correlation was observed with the photosynthetron actinic light level. In all samples, the JV_{PII} and $\Phi_{e,C}$ values were significantly lower after 120 minutes of incubation relative to 20 minutes of incubation.

One practical consideration with these measurements was the need to apply spectral matching between the photosynthetron-derived ^{14}C-fixation data and LabSTAF-derived FLC data. This was complicated by a significant spectral change in the photosynthetron actinic light experienced by the samples going from front to back (highest to lowest actinic light levels). The correction method applied within this study is detailed within the report. Since this analysis was carried out, a number of new functions have been added to the RunSTAF software used to control the LabSTAF system which make it much easier to apply spectral matching between the light source used to drive photosynthetron-based ^{14}C-fixation and the integrated LabSTAF light source used to generate FLC data.

1. Dissemination of the results[9]

The data presented within this report are extracted from a more extensive document. The more extensive document will be made publicly available.

2. Technical and Scientific preliminary Outcomes (2 pages max.)[10]

[8] Describe briefly the main achievements obtained and possible impacts, as well as possible difficulties encountered during the execution of the project. Use no more than 250 words.

[9] Describe any plan you have to disseminate and publish the results resulting from work carried out under the Transnational Access activity in JERICO -S3: scientific articles, books - or part of them -, patents, as well as reports and communication to scientific conferences, meetings and workshops. Highlight peer-reviewed publications. **Note that any publications resulting from work carried out under the JERICO -S3 TA activity must acknowledge the support of the European Commission – H2020 Framework Programme, JERICO -S3 under grant agreement No. 871153.**

[10] Describe in detail results and main findings of your experiment at the present stage.

Sample collection

Carboys of seawater were collected on 18/10/2021 from depths of 20 m and on 22/10/2021 from depths of 2, 10, 20, and 60m. The sample from 18/10/2021 was only used for experiment prototyping and initial tests (not included within this report). The sample from 22/10/2021 at 20 m was used to compare LabSTAF-based and photosynthetron-based measurements.

Experimental setup

Samples were incubated in a photosynthetron incorporating a uniform radiance PAR lamp (Photon Systems Instruments SL3500-W-C white lamp running at 70-90% full intensity). The light levels in the incubation bottles were controlled by distance from the lamp and strategically placed 30% neutral density (ND) filters. The entire photosynthetron area was surrounded by black sheeting to exclude external light sources, and the temperature of the photosynthetron was maintained at 23°C using an air conditioning unit connected through the black sheeting.

Two parallel rows of incubation bottles were run with matched light intensities and temperatures to provide replicate datapoints for each condition (Figure 1). The two rows were labelled as A and B, and the bottles were labelled 1 to 7. The temperature was monitored in bottle 3B and 7B. Incubations were run for between 2 h 5 mins and 2 h 25 mins.

Two Chelsea Technologies LabSTAF systems (004 and 008) were installed in the lab next to the photosynthetron (Figure 1). While ^{14}C incubations were ongoing, seawater from the same Carboy was analysed in the LabSTAF units using the 'Repeat FLC' protocol to acquire parallel estimates of PSII electron flux (JV_{PII}). Table 1 summarises all measurements made during this study.

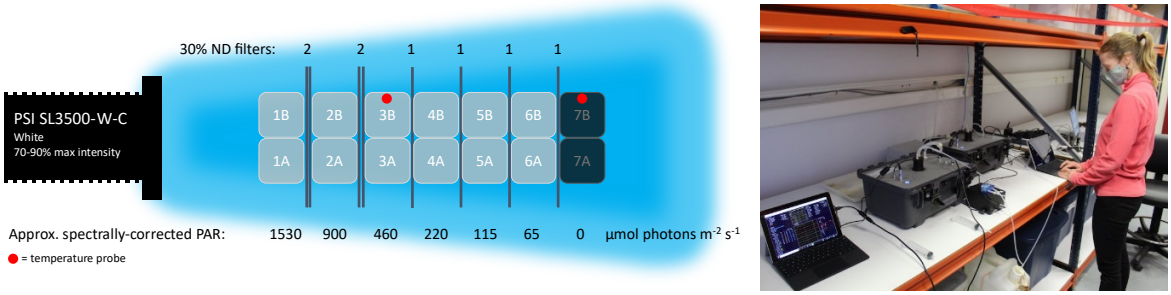

Figure 1: The setup for the photosynthetron (left) and the LabSTAF units (right).

Expt.	Date	14C experiments	004 FLCs	008 FLCs
LS0	19/10/2021	Trial light curve, 125-minute incubation	No data	No data
LS1	21/10/2021	Trial light curve, 132-minute incubation	Continuous from Carboy	Discrete from photosynthetron
LS2	22/10/2021	Trial light curve, 127-minute photosynthetron incubation	Discrete from photosynthetron	No data
LS3	25/10/2021	Light curve, 130-minute photosynthetron incubation	Discrete from photosynthetron	Discrete from photosynthetron
LS4	26/10/2021	Light curve, 145-minute photosynthetron incubation	Discrete from photosynthetron	Discrete from photosynthetron

Table 1: Overview of experiments. Where 'Discrete from photosynthetron' is used to describe the protocol used, measurements were at 20 and 120 minutes.

LS0 and LS1 were used to optimise the experimental protocol and timings for the ^{14}C assimilation and LabSTAF procedures.

LS2 was used to trial the final experimental procedure. Only one FLC measurement was acquired for each light level at each timepoint with this series.

LS3 and LS4 followed the same experimental protocol as LS2, except two complete FLC measurements were acquired for each light level at each time point. Timing issues experienced in LS2 were improved for LS3 and resolved for LS4.

Summary of results

The photosynthetron-based measurements provided ^{14}C-fixation rates within units of µmol C m^{-3} s^{-1}. The STAF Fluorescence Light Curves (FLC) measurements provided PSII electron flux with units of µmol electrons m^{-3} s^{-1} from. Points along the FLCs were matched to the light intensity within each of the photosynthetron sample bottles. These primary data allowed for calculation of electron per carbon ratios ($\Phi_{e,C}$). The $\Phi_{e,C}$ values within Table 2 are within the expected range for oligotrophic conditions (Lawrenz et al. 2013).

	LS2 (minutes)		LS3 (minutes)		LS4 (minutes)	
	20	**120**	**20**	**120**	**20**	**120**
Matched $\Phi_{e,C}$	18.1±4.5	13.2±4.2	14.1±2.2	12.2±1.8	22.8±8.6	19.2±4.6
Maximum $\Phi_{e,C}$	23.2±5.8	15.6±5.8	16.1±2.9	14.5±2.0	24.6±8.6	20.4±4.0

Table 2: The 'matched' $\Phi_{e,C}$ values shows the average with each ^{14}C-fixation rate coupled to the FLC step at the same actinic light intensity. The 'maximum' $\Phi_{e,C}$ values from each experiment is also shown. All values are corrected for differences in the spectral output of the actinic light sources used.

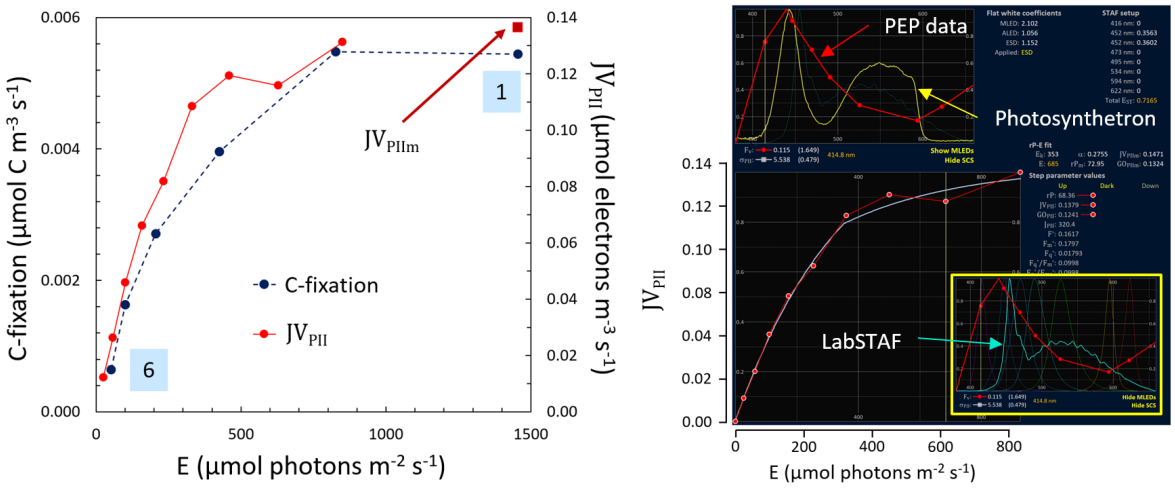

Figure 2: Sample data comparing photosynthetron-derived ^{14}C-fixation and LabSTAF-derived PSII electron flux. The blue labels **1** and **6** in the left plot indicate the bottle locations within Figure 1. The right plot is a crop from the RunSTAF screen and includes the Photochemical Excitation Profile (**PEP data**). The **Photosynthetron** line is the spectral output from the actinic light measured within the sample vessel. The **LabSTAF** line within the inset is the spectral output from the LabSTAF actinic light. Automated spectral correction between the two is now possible within RunSTAF.

Additional technical information plus cited and other relevant references can be found within the LabSTAF and RunSTAF Handbook: http://dx.doi.org/10.25607/OBP-1029.4

UK, 08/02/2022

Kevin Oxborough

Location and date

Signature of principal investigator