TNA PROJECT REPORT

1. Project Information

Proposal reference number	JN_CALL_3_6
Project Acronym (ID)	MultiFluoro
Title of the project	Testing new multi-parameter fluorometer in optically complex environments
Host Research Infrastructure	SYKE MRC-lab SYKE-ALG@LINE FINNMAID
Starting date - End date	01/04/2019 – 31/05/2019
Name of Principal Investigator **Home Laboratory Address** **E-mail address**	01/04/2019 – 31/05/2019 Chelsea Technologies Group 55 Central Avenue, West Molesey, KT8 2QZ, United Kingdom jkirkbride@chelsea.co.uk
User group members	James Kirkbride, Sam Kirby, John Attridge Chelsea Technologies Group, United Kingdom

2. Project objectives

The main objectives of the project were to test the following characteristics of Chelsea Technologies' new VLux multiparameter fluorometer:

- Test the effect of background humic signal on detected algal fluorescence.
- Test the sensitivity and selectivity of different fluorescence bands to different algal classes.
- To compare the selectivity of fluorescence bands targeting phycobilin pigments against other field fluorometers and a bench-top spectrofluorometer.
- Obtain first evidence of operationality of the VLux instrument in a flow-through system.
- Follow the development of the Baltic Sea spring bloom by combining VLux with an operational FerryBox system and demonstrate the added value of having several measurement wavebands.
- Demonstrate the added value of having optical absorbance and turbidity characterisation in correcting the measured fluorescence.

3. Main achievements and difficulties encountered

Difficulties in the development of the multi-parameter fluorometer (VLux) necessitated a change to the initial schedule. Issues were found in the sensor which result in disappointing sensitivity for some applications. A redesign has been carried out to improve on these but was not completed before this work was begun. As such we will not be commenting on the sensitivity of the instrument.

The activity described in this report has received funding from European Commission's H2020 Framework Programme under JERICO-NEXT project, grant agreement No. 654410.

Two representatives from Chelsea took a VLux sensor to the SYKE laboratory on 1st April 2019 and demonstrated the method of mounting the sensor in its flow-through assembly. The VLux was demonstrated to work correctly in a flow-through system and was successfully integrated into the FerryBox system aboard the ferry Finnmaid on 2nd April by SYKE, hoping to catch the end of the spring algal bloom. It was not possible to integrate the sensor with the logging system on the ship, so a separate system was provided, logging to a Raspberry Pi computer. Water samples were collected with FerryBox system, to provide validation data for sensor. A memory issue in the logging program meant that some transects were not logged, these unfortunately corresponding with some transects during which water samples were taken. A second sensor was prepared by Chelsea and taken to SYKE on 16th May 2019 to be used for laboratory testing. A visit was made to the ship, and the logging program updated to fix the memory issue.

4. Dissemination of the results

The results will be utilised as a case study demonstrating the efficacy of the VLux instrument. The data will be used in promotional material for the new instrument, currently scheduled to be launched in September 2019. The data may also be presented at meetings and/or workshops (to be decided upon) to demonstrate and promote the capabilities of the new instrument.

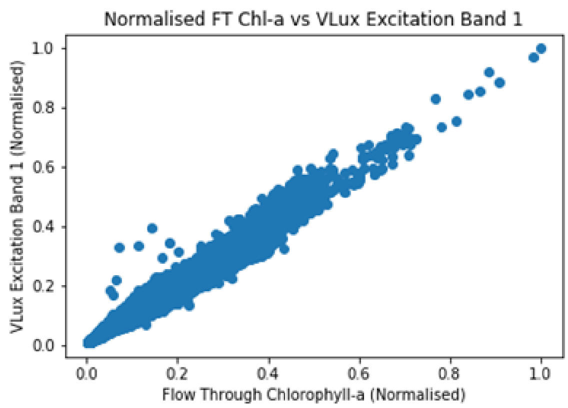
5. Technical and Scientific preliminary Outcomes

The first VLux was installed in the FerryBox system aboard Finnlines' Finnmaid Ro-Ro passenger ferry. The ship travels between Helsinki, Finland and Travemünde, Germany twice per week. The FerryBox system contained the following sensors:

- Chlorophyll & Turbidity (WET Labs, FLNTU)
- Phycocyanin (TriOS, MicroFlu Blue)
- CDOM (TriOS, MicroFlu)
- Phycoerythrin (Chelsea Technologies, UniLux)

A JFA Advantec Multi-Exciter was also installed for comparison.

Preliminary results from the FerryBox system are encouraging, showing good correlation between the VLux and the WET Labs FLNTU chlorophyll sensor installed on the FerryBox system:



In the above plot, both axes have been normalised to their maximum readings. This was done for clarity as differences in calibration and spectral properties between sensors mean the absolute readings (in µg/l of chlorophyll-a) are not equal.

The correlation coefficients between the flow-through chlorophyll measurement and the four VLux excitation bands are presented in the following table:

Excitation Band	Excitation Wavelength	Correlation coefficient
1	450 nm	0.99
2	470 nm	0.99
3	530 nm	0.99
4	625 nm	0.97

While absolute reported concentrations did not match well, which is due to differing calibration methods, the excellent correlation seen between the sensors demonstrates that the VLux is functioning well in measuring chlorophyll. The correlations for phycocyanin and phycoerythrin sensors was poor, however, there was little or no signal present for these sensors in the flow-through system. Essentially, it was just baseline noise being compared on these sensors, which will be uncorrelated. There was also little turbidity or CDOM measured. Consequently, the corrections for absorbance and turbidity made little difference to the reported values over the trial period.

VLux fluorescence also correlates well with the JFA Multi-Exciter data. The table below shows correlation coefficients between the four excitation bands of the VLux and the nearest equivalent bands of the Multi-exciter.

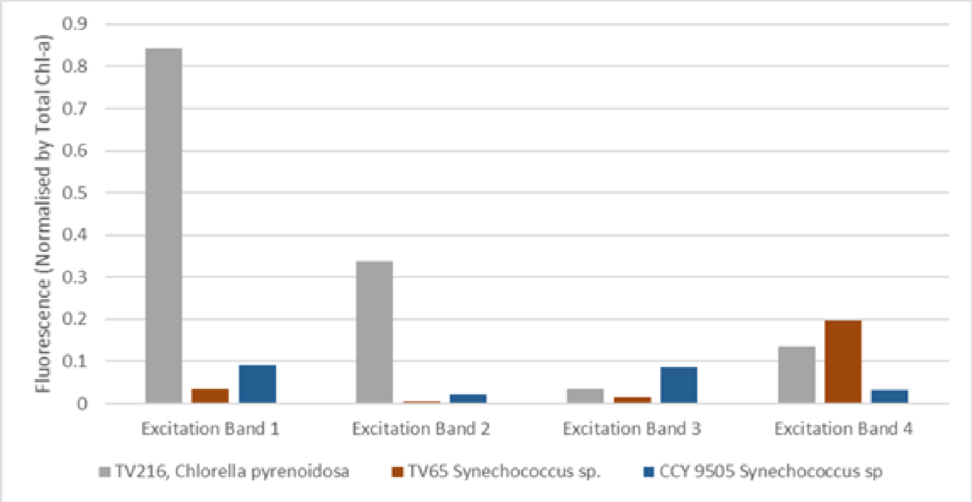
		Multi-Exciter Bands			
		435nm	470nm	525nm	590nm
VLux Excitation Bands	1	0.95	0.92	0.94	0.96
	2	0.88	0.94	0.97	0.95
	3	0.89	0.94	0.97	0.96
	4	0.94	0.87	0.90	0.93

This data shows that the responses from the two sensors are very similar and that both respond in the same way to the conditions in the Baltic Sea.

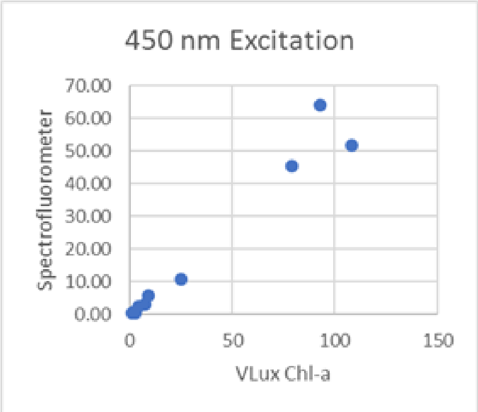
In the laboratory the following sensors were used:

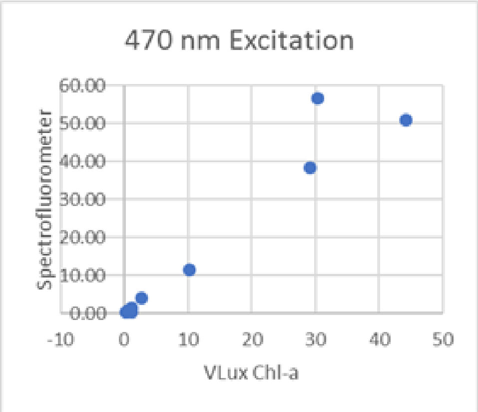
- TriOS, MicroFlu Blue (Phycocyanin)
- Chelsea Technologies, UniLux (Phycoerythrin)
- Chelsea Technologies, TriLux
- JFE Advantec, Multiexciter
- Agilent Varian Eclipse spectrofluorometer

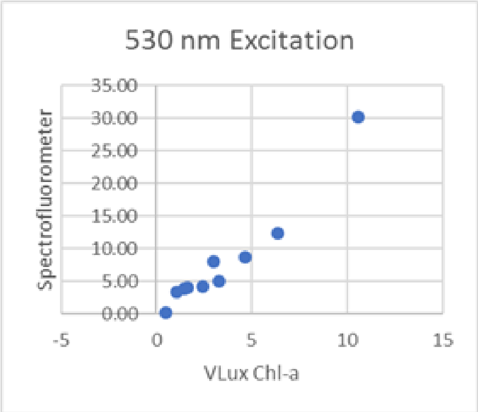
These sensors, along with the VLux, were exposed to a variety of samples containing different pigment groups (green algae, cyanobacteria, diatoms, dinoflagellates, and cryptophytes, totalling 57 samples) as well as CDOM gradient from the Baltic (6 samples). These pigment groups give different fluorescence responses under excitation by different wavebands as demonstrated below.

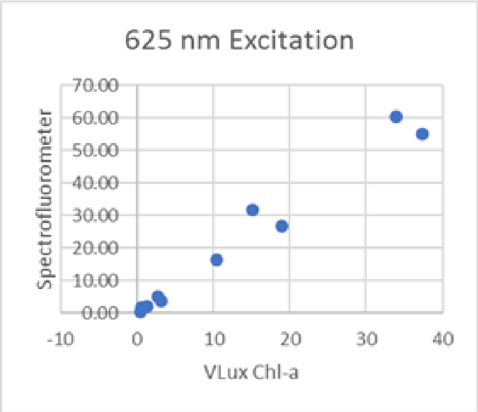


The green alga, chlorella pyrenoidosa, gives its strongest signal under excitation Band 1 with the signals decreasing through Bands 2 and 3 before increasing slightly in Band 4. The cyanobacteria TV65 Synechococcus sp. shows by far the strongest emission with Band 4 excitation, whereas the CCY 9505 Synechococcus sp. shows stronger emission with Bands 1 and 3 than Bands 2 and 4. These VLux responses correlated well with the respective fluorescence induction spectrum measured with the Varian Eclipse spectrofluorometer ($r > 0.96$, n=10 for all excitation bands) as shown below.

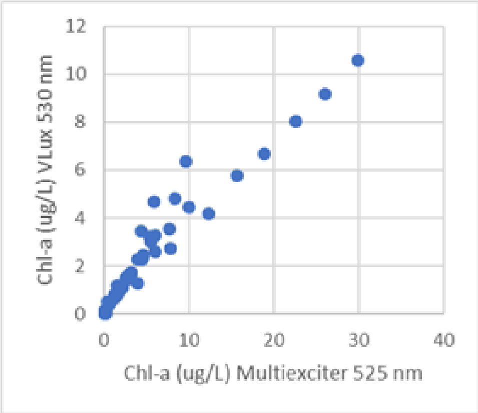


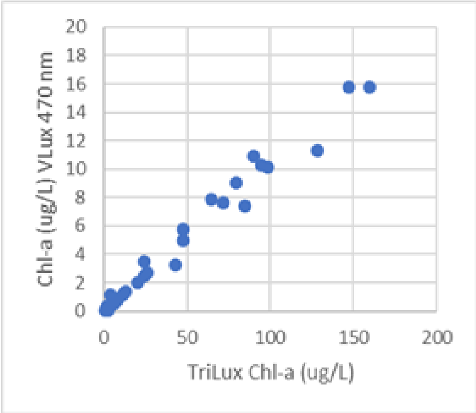






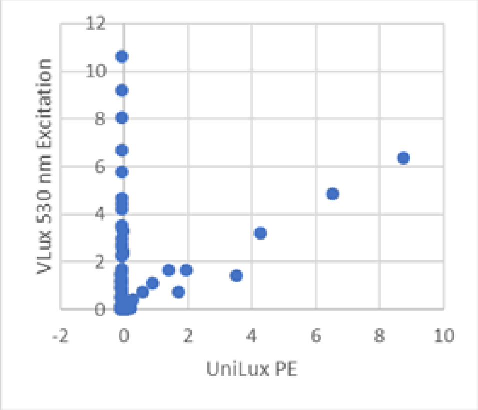
Similarly, high correlation was found between VLUX and other field sensors, when their excitation and emission wavebands (approximately) match as shown in the two example plots and the table below (n=64 for each comparison).

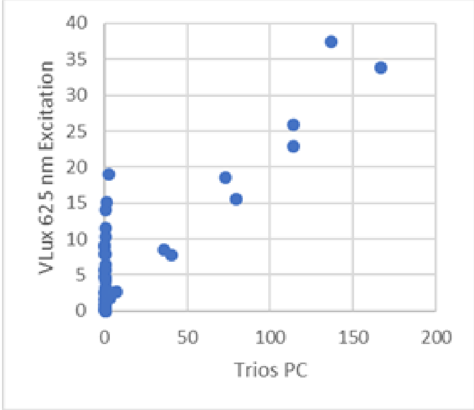




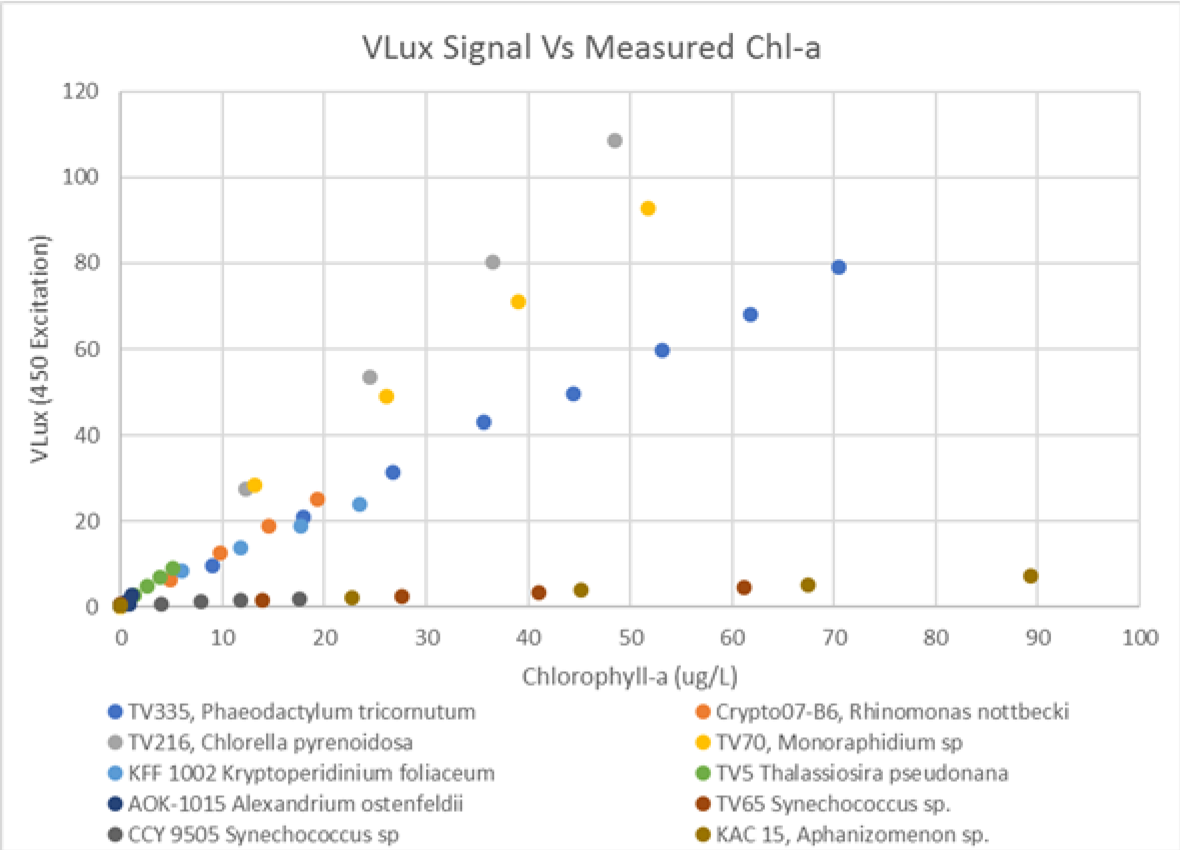
	TRILUX (470/680)	TRILUX (610/680)	JFE (435/680)	JFE (470/680)	JFE (525/680)	JFE (590/680)	UNILUX (530/570)	TRIOS (630/650)
VLUX (450/680)				0.84				
VLUX (470/680)	0.99		0.91					
VLUX (530/680)					0.97		0.251	
VLUX (625/680)		0.99				0.96		0.85

The VLux has excitation wavebands at 530 nm and 625 nm to allow detection of red and blue-green cyanobacteria respectively. However, because the VLux measurement occurs at the peak of the chlorophyll-a emission, the results are not as specific as when measuring phycobilin fluorescence directly. This is demonstrated in the figures below showing VLux response against that of the UniLux PE and Trios PC sensors. The VLux is designed to measure the transfer of energy from the accessory phycobilins to chlorophyll, and hence does not measure the phycobilin fluorescence directly as these sensors do. A linear relationship between VLux and the two phycobilin sensors exists for cyanobacteria whereas the observed high readings in the absence of cynoabacteria, are due to other accessory pigments (Chl's, carotenoids) present in many algae species.

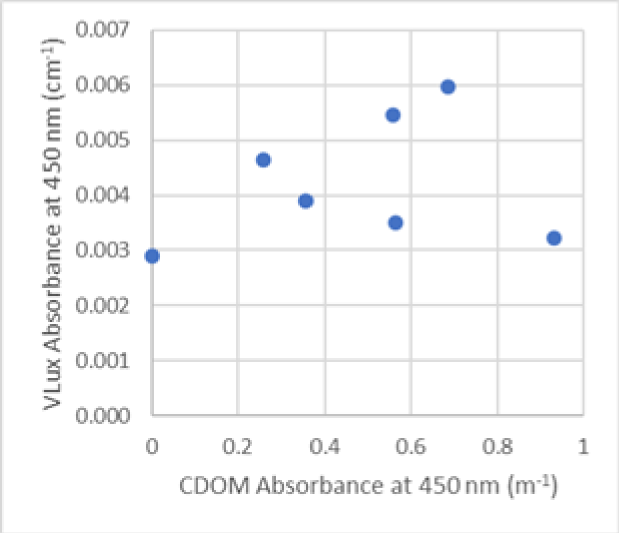




The VLux measurements correlate well with the measured chlorophyll-a concentrations of the samples. There is, however, some scatter in the data due to differences in chlorophyll-a specific fluorescence between the different species. Below, the different species have been plotted in different colours, demonstrating that, while there is considerable scatter in the data as a whole, for each individual species the relationship between VLux signal and the measured chlorophyll-a concentration is linear. This is true for all excitation bands with correlation coefficients being greater than 0.99 for all species.



The correlation between the measured absorbances for the background humic matter and those measured by the VLux was disappointing. The absorbances of real-world water samples was low, meaning that the signals were lost in the excessive noise of the development VLux sensor. A re-design of the internal electronics has since been carried out to reduce the electrical noise and thereby increase the instrument sensitivity.



The effect of CDOM absorbance is to introduce a small error into the measurement of chlorophyll on the order of a few % during normal spring-summer conditions. In coastal waters and fresh waters, the effect of the humic background may be much larger. In these conditions the internal measurement of the absorbance by the VLux will be used to apply a correction algorithm to the measured chlorophyll. In studies using polycyclic aromatic hydrocarbons, the same kind of corrections have been shown to correct for measuremnet errors of 50%, returning the correct concentration to within ±5%. The VLux will make corrections for absorbance and turbidity, returning the corrected concentrations without the need for extra sensors or post-acquistion correction.

Despite a noise issue with the sensor which affected the evaluation of the corrections for humic matter and turbidity, the tests have shown that the VLux respose is in line with other sensors in the market with similar excitation and emission wavebands. Further tests under more demanding conditions and in real deployments are required after the electronics redesign has been completed. This will enable demonstration of the increase in functionality afforded by the absorbance and turbidity corrections, and will be used to evaluate the sensitivity and robustness of the sensor.

SUBMITTED, 27 JUNE 2019; FINAL REVISION, 27 SEPTEMBER 2019