TNA PROJECT REPORT

1. Project Information

Proposal reference number	JN_CALL_2_11
Project Acronym (ID)	FluorMed-1
Title of the project	Phytoplankton fluorescence studies in Mediterranean. Part 1. Feasibility and comparability of different methods in oligotrophic seas
Host Research Infrastructure	Heraklion Coastal Buoy (HCB) Poseidon Calibration Lab (PCL)
Starting date - End date	09/04/2018 – 20/05/2019
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2. Project objectives

The FluorMed-1 project aims in providing information on the applicability and comparability of various fluorescence detection methods for phytoplankton community structure at a high frequency in the oligotrophic conditions of the Mediterranean waters. Those methods are used in contemporary online phytoplankton diversity and physiology research on various platforms (buoys, bench with pumped water, ships of opportunity, scientific vessels) and are mostly tested in eutrophied sea areas, where diversity and biomass are important. Since bulk or specific fluorescence sensors are not well defined in terms of detection limits but that they are depicted as required when selecting technology for monitoring the biological state of marine areas, it is important to define the possible applications in the oligotrophic conditions, and when developing the methodology further.

The validation and combination between bulk and physiology analysis in oligotrophic areas will be coupled with single cell analysis using high frequency pulse shape recording flow cytometry. This project will enable to get insight on the picoplankton community functioning, dominating oligotrophic areas.

3. Main achievements and difficulties encountered

Project started with a laboratory experiment. Water was collected from the oligotrophic coastal site (10.4. & 16.4. 2018). 200 L of water was incubated in a tank for 6 and 3 days respectively. Water was fertilized with nutrients and development of phytoplankton community was followed using a suite of sensors (3 different LED fluorometers, 1 spectral fluorometer, 1 integrating cavity spectrophotometer, 2

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FRRF fluorometers, flow cytometer, membrane inlet mass spectrometer, spectroradiometer, probes for oxygen, salinity and temperature). Some instruments were placed directly in the tank while some were used in flow-through mode. All sensors were functional throughout the experiments and their dynamic responses in varying oligotrophic conditions were obtained. Discrete sampling for analytical laboratory measurements was carried out to validate sensor data.

The field deployment of fluorometers required more interfacing work than anticipated. New model of Trios NanoFlu Chlorophyll fluorometer was deployed at HCB at 25.6. 2018 and data was received in real time. After approx. 2 months of deployment, the sensor readings became unstable due to biofouling. Sensor was cleaned manually couple of times during the deployment until finally delivered back to user mid-May and finally re-inspected 21 May by user. The initial two month period with HQ data will be studied along with light data to analyse effect of non-photosynthetic quenching on fluorescence. Trios Unilux-PE sensor deployment was planned along with NanoFlu. The analysis of fluorescence profiles along the water sampling (for experiment) indicated that phycoerythrin at the surface waters is not measurable and other sampling strategy was decided. PE sensor was attached to CTD and several casts were performed during spring 2019, to assess phycoerythrin in deeper layers.

4. Dissemination of the results

Results have been presented (poster) at Jerico-Next general assembly (Galway, IR, 24.-27.9 2018).

Results will be presented (poster) at Jerico-Next general assembly (Brest, FR, 2.-5.7. 2019).

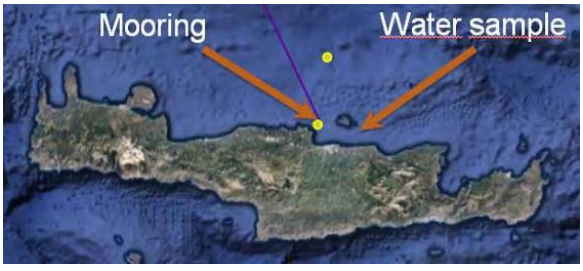
Results will be published in scientific peer-reviewed journal.

Results will be communicated towards sensor manufacturers.

5. Technical and Scientific preliminary Outcomes

Experiment: POSEIDON PCL

Water was collected from the oligotrophic coastal site (10.4. & 16.4. 2018, Figure 1) and incubated in a 200L-tank for 6 (EXP1) and 3 (EXP2) days respectively. Water was fertilized with nutrients and development of phytoplankton community was followed using a suite of sensors (Figure 2) and standard laboratory sampling. Some instruments were placed directly in the tank while some were used in flow-through mode (Figure 1).



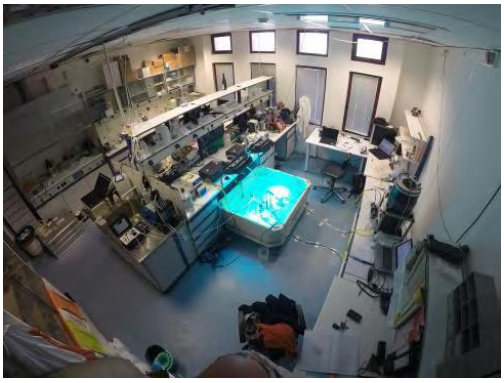


Figure 1. Location of water sampling and mooring (up) and experimental setup at calibration lab (right).

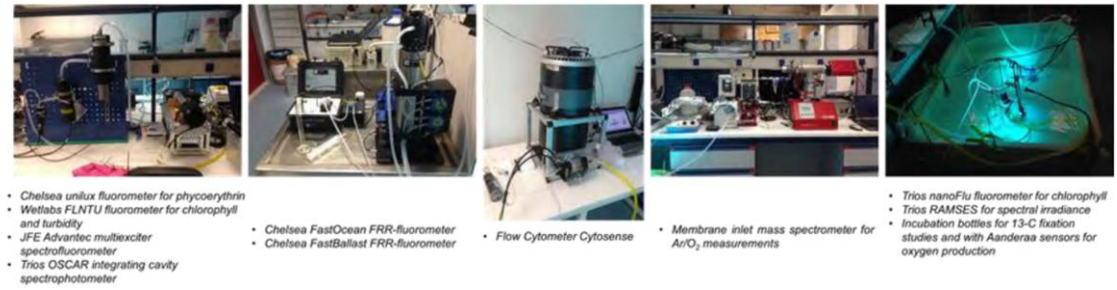


Figure 2. Sensors used in the experimental period.

The key findings of the experimental results are highlighted for EXP2, as follows:

- Phytoplankton subpopulations were identified by cytometer, and *Synechococcus* sp. picocyanobacteria being important component of community (Figure 3A).
- Cell numbers of pico- and nanoeucaryotes decreased during experiment (Figure 3C), which was also reflected in Chlorophyll a fluorescence (Figure 3B). Chlorophyll a concentration from discrete samples decreased similarly from the start value 0.35 down to 0.08 µg Chla L^{-1}.
- Higher decrease of chlorophyll a fluorescence during light periods was observed, due to nonphotochemical quenching (Figure 3B), but similar response is not seen for phycoerythrin fluorescence (Figure 3F).
- *Synechococcus* sp., with two subpopulations (high FLO and high FLR), maintained it's abundance, with a switch between subpopulations during the experiment (Figure 3E).
- Phycoerythrin fluorescence increased during the first night (Figure 3F), as well as the number of *Synechococcus* sp. cells.
- Photosynthetic efficiency, measured with Chelsea Fast Ocean FRRF, declined during the experiment. During the latter part of the experiment, the signal-to-noise ratio increased, indicating that for this instrument the range of phytoplankton concentration was too low.

Results will be studied more in detail and instrument intercomparison will be carried out, especially evaluating their capacity to provide reliable measurements in low phytoplankton concentrations.

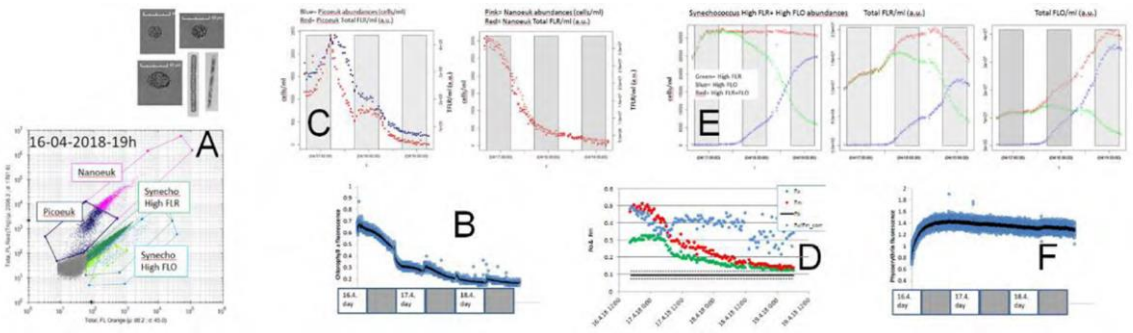


Figure 3. Highlights of the results in EXP2

Deployment: POSEIDON HCB

During the collection of water samples for the experiments, the vertical profiles of spectral irradiance and fluorescence were measured (Figure 4). These measurements highlight the light climate of the study area, with blue light penetrating the deepest and vertical profile of phytoplankton with deep layer chlorophyll maxima. Spectral adaptation/acclimation of prevailing deep layer phytoplankton populations to harvest blue light is seen by both spectra peaking around 470 nm. Based on this information and the experimental results, Chlorophyll a fluorometer (Trios Chlorophyll NanoFlu) was selected as the best option (connections, size, power consumption, reliability) to be deployed in the Poseidon HCB mooring, to follow phytoplankton dynamics. The phycoerythrin fluorometer (Chelsea Unilux PE) was considered as a second option, but as the mooring provides measurements at the surface only, it was considered that the phycoerythrin signal will be very low. Instead it was decided to install the phycoerythrin fluorometer on a profiling CTD.

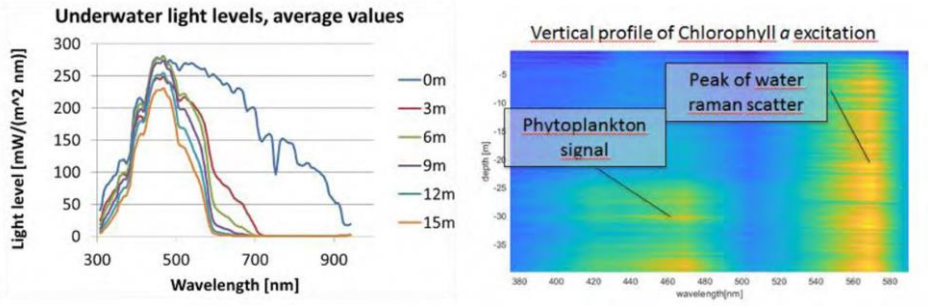


Figure 4. Spectral irradiance profile (left) and spectral fluorescence

Fluorometer data was with good quality for approximately 3 months, thereafter affected strongly by biofouling (Figure 5). Removal of biofouling material was tested couple of times, but it was likely that the signal was no more reliable. The initial records however provide insight of the inherent problem in fluorescence detection at high irradiance and oligotrophic conditions. The diel rhythm of fluorescence follows the irradiance levels, with low fluorescence at time of high irradiance, due to non-photochemical quenching. The observed differences in day-night values were 1.5-10 fold. Most obviously the night time values reflect better the real concentrations. With the available light data we aim to estimate the rate constants for non-photochemical quenching and its relaxation.

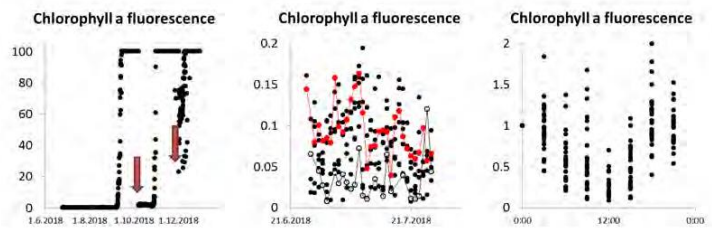


Figure 5. (Left) Fluorescence readings at the mooring for period from 25.6.-31.12.2018 (moments of cleaning noted by red arrows) especially showing the biofouling events. (Middle) Fluorescence readings (black circles) for the first month of deployment, showing the high night-time values (red circles) and low daytime values (white circles). (Right) Fluorescence values for the first month normalised to values observed at midnight, showing the consistent diel pattern with minima during midday.

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