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.**

TA PROJECT REPORT

1. Project Information

Proposal reference number[1]	JS3_CALL_2_4037_APHYMOSO
Project Acronym (ID)[2]	APHYMOSO
Title of the project[3]	Automated phytoplankton monitoring at ship of opportunity
Host Research Infrastructure[4]	Ms Fantasy
Starting date - End date[5]	02/05/2022 – 10/05/2022
Name of Principal Investigator[6] **Home Laboratory** **Address** **E-mail address** **Telephone**	Machteld Rijkeboer Rijkswaterstaat-CIV-Laboratory for Hydrobiological Analyses Zuiderwagenplein 2 8224AD Lelystad, the Netherlands machteld.rijkeboer@rws.nl +31 620407667

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

Explore the feasibility of unattended and continuous monitoring of phytoplankton species composition and primary production with underway flow cytometric and fast repetition rate fluorometer on board a ship with FerryBox system

Questions to answer:

- Can these delicate instruments (FCM, FRRf) work in an unattended, noisy and warm environment like the engine room of cruise ferry or a bulk container
- Is the temperature control in the engine room stable enough to enable reliable observations
- Can the instruments perform the measurements fully automatically
- Can we automatically upload our data to the internet, allowing the operator and stakeholders to follow online measures and results online and near-real time
- Can additional observations (with FRRf) help to correct for non-photochemical quenching

[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).

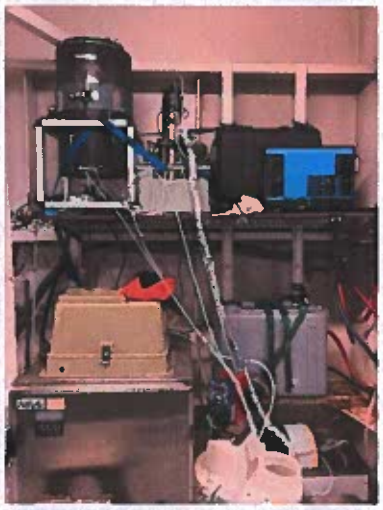
[7] Write the short-term, medium and long-term objectives of the project. Use no more than 250 words.

effects in fluorescence observations that are commonly made from Ferrybox systems

The obtained results will be helpful to define the conditions needed for installation on Ships of Opportunity (SOP) like the cs. Connector, which has a Ferrybox system run by NIVA and RWS since 2021.
Long-term these results and technologies will be used for the optimalization of the monitoring of Rijkswaterstaat (and other partners) on the North Sea. This will change the common monthly, discrete sampling strategy with a RWS vessel into a fully automatic monitoring using High Frequency sampling and integrating diverse instruments. In a similar way, automated monitoring platforms can be developed near windfarms to generate near-real time results, which are available for third parties and can act as early warning systems.

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

During the first round trip (Oslo-Kiel-Oslo) installation of FCM and FRRf in the engine room was achieved smoothly. FCM (every 30 min) and FRRf (every 20 min) automatically ran their measurement protocols and data of FCM were near-real time available on a website by using EasyClusLIVE software. A IT infrastructure with internet is required. The FRRf data can also be sent to the website, but needs some extra environmental input to calculate primary production from the raw data. This input (i.e. total irradiance) was not available on the ms Fantasy. The FCM is an optical instrument with lenses and light sensitive detectors, which are influenced by temperature to a certain degree. The focusing optics showed a stable alignment although environmental temperature conditions were not optimal. The FCM was placed in an airflow with a slightly lower temperature. The special temperature box, we intended to test could not be brought on board due to a communication mistake. Also the images made by the FCM are temperature sensitive, the focal quality could have been much better. The opening of the instrument in the engine room to adjust the system to the local temperature was not performed due to the risk of getting dirt inside the system. Recent FCM instruments have automated adjustable translators (of the injector) to solve realign the system without opening it. The FRRf has its own temperature control unit and had no temperature issues.



Water inflow towards the instruments was regulated via a sampling chamber, but the flow-through rate had to be set much lower than preferred, because of a reduced waste water remove opportunity. Impact was not observed in the results, but this can be optimized in future.
The overall conclusion is that the instruments worked stable and fully automated in this environment. No serious problems were encountered, which opens the way for further implementation on other non-manned platforms or SOP's.

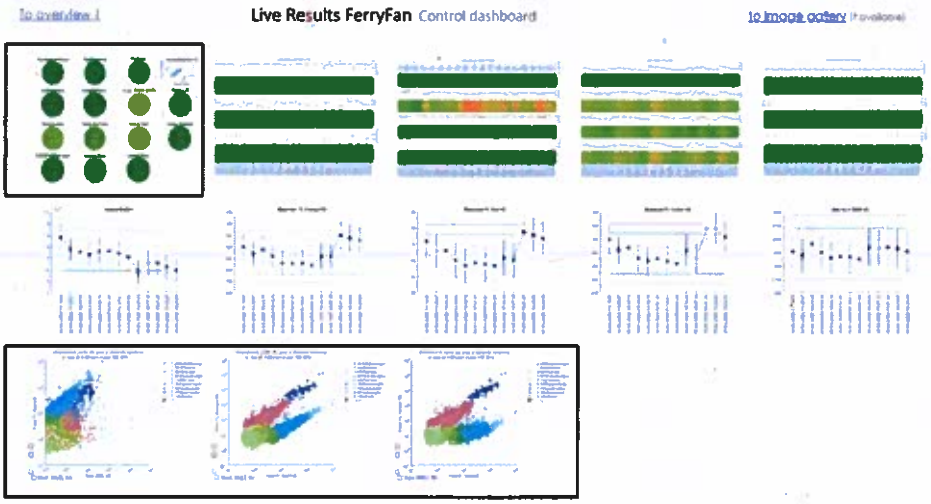
[8] Describe briefly the main achievements obtained and possible impacts, as well as possible difficulties encountered during the execution of the project. Use no more than 250 words.

4. Dissemination of the results[9]

The prime and overall results will be published in a working report which will be shared with RWS and the Jerico-community and can be used as a guideline for installing High Frequency instruments (like the FCM and the FRRF) at SOP's. The raw data are kept at RWS server (not public at this moment). The results of analyzed flow cytometer data are available via www.phytoplanktonlive.com A PowerPoint presentation was held for the own organization department CIV-Laboratory for Hydrobiological Analyses (in Dutch). A short new item was written for the newsletter of IGA-CIV (in Dutch).

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

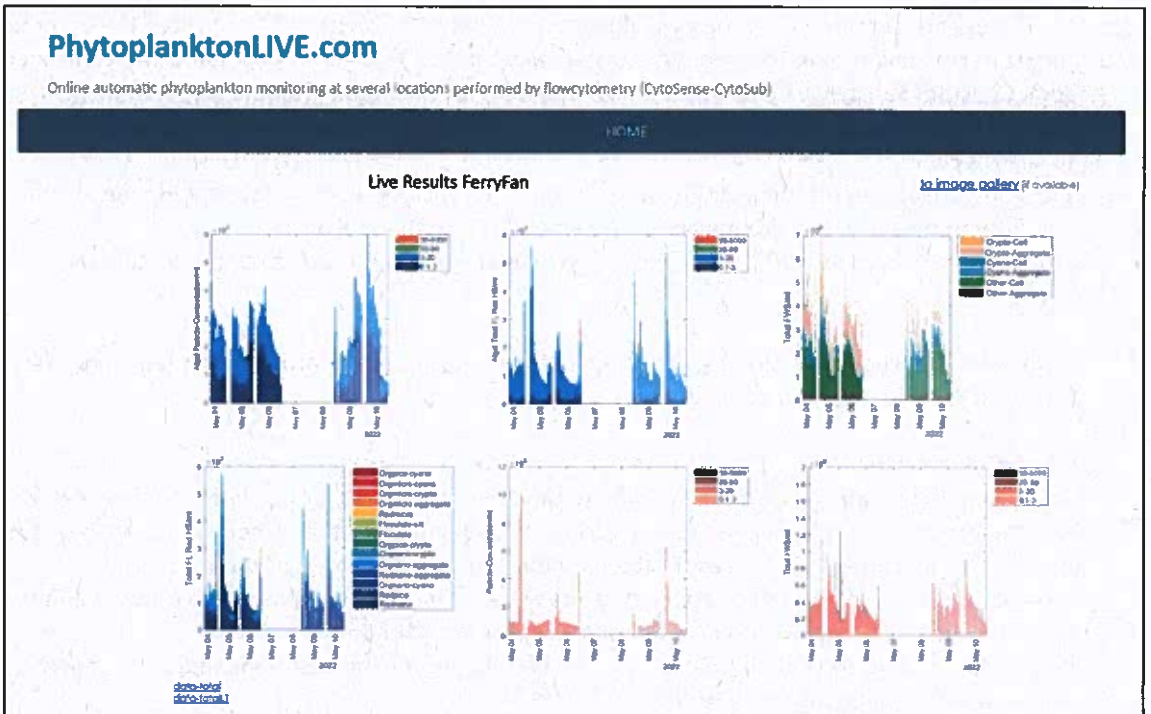
In principle both CytoSense flow cytometer and FRRF worked fully automated in the unmanned environment. The measurements started and stopped automatically. The data for the flow cytometer were automatically analyzed with EasyClusLIVE software and uploaded to the internet.



The above figure is automatically generated information for the operator, which can be accessed remotely. The overview in time of the most important parameters, like number of particles and used analysis volume, alignment of the FCM, fluidics pressure, temperature in the FCM and some scatter plots of the clustered data. The encircled data are referring to the last measurement done. The (green) traffic light panels indicate if measurements are correct.
The information in the panel below is for scientific and management activities. The concentration and discrimination of the size based and optical (phytoplankton) groups may be followed during the transect of measurement or in time. Variables such as chlorophyll, turbidity, counts, biovolume, particles sizes, cyanobacteria and other phytoplankton groups are measured by FCM. Chlorophyll, light curves, Fmax/Fo, primary production related variables are measured by FRRf.

[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.



Important lessons learned during this TNA

Vibrations:
Extra vibrations or oscillations were expected in the engine room at the lowest part of the ship. Although some vibration could be felt during the first cruise on board, this did not pose any problems with the instruments. Specifically no changes were detected in data or alignment of the FCM on a time-scale that could be related to vibrations. Similar observations (i.e. no effect of severe ship movements on the FCM) have been made in the past during other cruises with stormy weather at research vessels.

Water-in and outflow
Seawater has to be transported towards the instruments. The shorter the route, the less changes in the phytoplankton community and primary production can be expected. At the ms. Fantasy the sample water from the Ferrybox-pipeline was divided between the Ferrybox and two separate tap points. One was used for the automated sampler and one for the inlet of the flow-through sample chamber, from which both the FCM and the FRRf pump their water. The flow rate through the sample chamber had to be set lower than the desirable rate, due to a limit in output possibility of the waste water. Therefore, there was some concern about the residence time of water in the sample chamber. A faster refreshment rate of the sample chamber will reduce temperature increase due to the high temperature in the engine room.
For applying these instrument in a permanent setup in a ship, it is recommended not to use a separate sample chamber, but use the fixed pipeline of the ship from which the instruments immediately can get their input water.

Temperature conditions in the working space
The instruments involved and usual all optical instruments are temperature sensitive. For the FCM the environmental air is used to cool the internal instrument temperature, which increases by the

electronics and lasers. This is done by a blower on top, but this is only efficient when the external temperature is lower than the internal. Most ideally the FCM is place in a temperature controlled room. Constant temperature conditions are necessary for maintaining stable alignment (the position of the sample core *vs*. the laser beam) and for images in focus, but also to reduce detector (noise) effects.
During this cruise some cooling airflow was used, which was just enough for keeping the alignment at an acceptable level, but this may not be the case later in the season.
For applying the FCM at a SOP, there has to be an adequate temperature control system be installed.

For the FRRf the cooling was realised by connecting the water jacket around the measuring cuvette to a cryostat, which worked adequately.

Data infrastructure
Real-time data must be available for implementation in smart monitoring platforms and also when the data are used in predictive modelling. A reliable data infrastructure is needed for analysing the data, as well as for real-time access of the data for third parties. The FCM data was fully automatically analysed and this data and results were automatically uploaded to a server and the website. In future this can be done in a similar way for the FRRf data.
The development of the data infrastructure will be ship and/or transect dependent and must be organised before applying the instruments.

NL, 30/06/2022 — M.Rijkeboer

Location and date — Signature of principal investigator

Lelystad
30-6-2022