The JERICO-NEXT project is funded by the European Commission's
H2020 Framework Programme under grant agreement No. 654410
Project coordinator: Ifremer, France.

Joint European Research Infrastructure network for Coastal Observatory – Novel European eXpertise for coastal observaTories - **JERICO-NEXT**

Deliverable title	Report on Best Practice in the utilization of sensors used for measuring nutrients, biology related optical properties, variables of the marine carbonate system, and for coastal profiling.
Work Package Title	Harmonization of technologies and methodologies - technical strategy
Deliverable number	D2.5
Description	Information on Best Practice in the utilization of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling.
Lead beneficiary	OGS, HZG
Lead Authors	Klas Ove Möller, Wilhelm Petersen and Rajesh Nair
Contributors	Naomi Greenwood (CEFAS), Chris Read, Agathe Laes-Huon, Anne Daniel, Karel Bakker, Daniel Blandfort (HZG), Martina Gehrung (HZG), Pasi Ylöstalo (SYKE), Lumi Haraguchi (SYKE), Jani Ruohola (SYKE), Bengt Karlson (SMHI), Felipe Artigas (CNRS), Fabrice Lizon (CNRS), Melilotus Thyssen (CNRS), Guillaume Wacquet (CNRS), Manolis Ntoumas (HCMR), Veronique Créach (CEFAS), Pierre Jaccard (NIVA), Laurent Delauney (IFREMER), Jukka Seppälä (SYKE), Andrew L. King, Kai Sørensen, Sabine Marty (NIVA), Anna Wranne Willstrand (SMHI), Lauri Laakso (FMI), Carolina Cantoni (CNR), Wilhelm Petersen, Yoana Voynova, Klas Ove Möller (HZG), Michela Martinelli (CNR), Laurent Delauney (IFREMER)
Submitted by	Klas Ove Möller
Revision number	V1.0
Revision Date	10/09/19
Security	Consortium and Public

History			
Revision	**Date**	**Modification**	**Author**
V1.0	10/09/2019	First release, after completion of all sections.	K. O. Möller

Approvals				
	Name	**Organisation**	**Date**	**Visa**
Coordinator	Patrick FARCY	IFREMER	13/09/2019	PF
WP Leaders	Rajesh NAIR Wilhelm Petersen	OGS HZG	10/09/2019 10/09/2019	R. Nair W. Petersen

Table of contents

1. Executive Summary

The coastal area is the most productive and dynamic environment of the world ocean with significant resources and services for mankind. JERICO-NEXT emphasizes that the complexity of the coastal ocean cannot be well understood if interconnection between physics, biogeochemistry and biology is not guaranteed. Such an integration requires new technological developments allowing continuous monitoring of a larger set of parameters. The objective of JERICO-NEXT consists in strengthening and enlarging a solid and transparent European network in providing operational services for the timely, continuous and sustainable delivery of high quality environmental data and information products related to marine environment in European coastal seas.
The best practice of technologies, methodologies and procedures is a vital step in ensuring efficiency and optimal returns from any kind of distributed, heterogeneous, multifaceted, coastal observing infrastructure operating on a transnational level like the JERICO network.

The JERICO network is always striving to increase its suite of sensors to anticipate likely future demands. As part of this effort, it is now implementing a variety of sensors for a number of bio-geochemical measurements. While Task 2.4 of JERICO-NEXT dealt with the harmonization of these sensors and their underlying technologies, this report provides information on Best Practice in the utilization of sensors used for measuring nutrients (lead: CEFAS), biology-related optical properties (lead: SYKE), variables of the marine carbonate system (lead: NIVA), and for coastal profiling (lead: CNR). This deliverable will also inform on the outcome and results of the workshops that were dealing with its topic during the project.

2. Introduction

JERICO-NEXT proposes to strengthen the current knowledge regarding European coastal areas and link biological processes with core physical, chemical and biogeochemical parameters in order to better understand: (1) the interactions between physics, chemistry, biogeochemistry and biology, and (2) how marine ecosystems react to anthropogenic disturbances and global environmental changes. A suite of different sensor systems to observe the above mentioned parameters are applied throughout the project consortium. This report aims to evaluate the best practices for the following sensors:

Sensors for nutrients
Nutrients are important environmental parameters in coastal waters and a number of JERICO partners are employing in-situ nutrient sensors on their installations and/or measuring platforms, in different settings and under diverse conditions. For use in marine waters, nutrient sensors have been developed which work on the basis of (a) wet chemical analysis, (b) optical detection and (c) electrochemistry. The principles of operation for the different nutrient sensors are given in Deliverable 2.2 together with examples of how they are deployed (Report on the status of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling, within the JERICO network and, more generally, in the European context). A review of best practice for operating a submersible optical nutrient sensor (Satlantic SUNA) has previously been given by Nair et al. (2017). Jerico Next partners are routinely deploying only wet chemical sensors and therefore this report focusses on best practice associated with these sensors (a) NuLab (Green Eyes), (b) CHEMINI (Ifremer), (c) NAS (Envirotech LLC) and (d) Micromac C (Systea).

Optical sensors for biological parameters
Optical sensors employing different measuring techniques (fluorometric, spectral irradiance / absorbance / fluorescence, fluorescence induction and scattering), imaging flow systems and scanning flow cytometers can provide valuable information on the biology of coastal marine waters. Many such sensors are standard equipment on many JERICO installations and/or measuring platforms. However, the effectiveness of these kinds of sensors in a networked environment such as JERICO is plagued by a number of problems intrinsic to the measurements themselves (e.g., sample characteristics, measurement technique, calibration, different types of reference material, conversion of measurands to concentrations, etc.). This section describes best practices for optical sensors used in biological measurements within JERICO-NEXT project. The document includes the technologies and instruments used in autonomous and continuous modes in various observatories. Many of the examples presented for a specific brand of sensors are valid also for other brands in the same technology. To be useful for larger communities, we describe best practices for commercially available instruments only.

Sensors for parameters of the marine carbonate system
At the present time, in-situ commercial sensors are available for mainly two parameters of the marine carbonate system: seawater pCO2 and pH. Our need for improved observation and understanding of the oceanic carbon cycling has been recently renewed due the large uptake of fossil fuel CO_2 and subsequent ocean acidification. Also of importance is the seasonal and annual variability in production and consumption of CO_2 via photosynthesis and respiration, respectively, in coastal oceans. In this report we describe recommended best practices in operating a variety of pCO_2, pH, and total alkalinity sensors. These include practical information related to the installation and operation of the various sensors, accessory and supporting sensors and instruments necessary for calculations and corrections, data handling and flagging, and the use of reference materials to check and calibrate measurements. The recommendations are based on long-term (several years) experience with sensors that are used in coastal environments which introduce challenges with high biological activity, particle load, and at times low salinity.

Sensor systems for coastal profiling
Coastal profiling systems can help to integrate indispensable information on water column characteristics in coastal areas. The most mature system technology, and used within the JERICO network, are coastal profiling ARVOR floats. Despite the maturity of this technology, Coastal profiling ARVOR floats are still used sparingly.

The coastal ARVOR floats are specifically adapted from conventional open sea profilers to be operational in the coastal area. The objective of the coastal float is to perform profiles between “stationary” phases. The “stationary” phases are obtained when the float is landed on the seafloor. The scientific payload embedded on coastal profiling float are up to now quite limited due to the small size of such floats. Besides the proven ARGO based technology, fixed (at the surface or bottom) profilers are also under development in JERICO-NEXT and worldwide. The majority of the systems are research prototypes, quite rough to operate and very different from one to the other. It explains why “Best Practices” for coastal profilers are not very documented and remain difficult to establish. Nevertheless, this chapter is focused on the “Best Practices” for this type of systems and contains information as general guidelines for the design and operation of coastal profiling systems.

The goal for all different sensors and systems above described are to define best practice in the use of similar devices, and investigate their portability (across systems/platforms), interoperability and performances to provide recommendations regarding these issues to users, manufacturers, and industry.

3. Main report

3.1. NUTRIENT SENSORS

A workshop was held between 4th – 5th December 2018 at Ifremer in Brest entitled "Interoperability of Technologies and Best Practices: in situ applications to nutrient and phytoplankton fluorescence measurements". It was jointly organised between the Jerico Next and the Atlantos (EU Horizons 2020) projects to share and compile best practice. The workshop first considered the Continuous Flow Analysis reference laboratory method and best practices implemented for the measurement of nutrients in the laboratory using this technique (see Becker et al., 2018). Participants then presented current practice for operating and deploying in situ nutrient sensors and discussed how best practice from the reference laboratory method can be transferred to in situ sensors. A questionnaire was circulated after the workshop to collect information from partners regarding the maintenance, calibration (laboratory, and in situ), on site performance checks and quality documentation. This has been compiled with information shared during presentations and discussion at the workshop to form the basis of this report.

For use in marine waters, nutrient sensors have been developed which work on the basis of (a) wet chemical analysis, (b) optical detection and (c) electrochemistry. The principles of operation for the different nutrient sensors are given in Deliverable 2.2 together with examples of how they are deployed (Report on the status of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling, within the JERICO network and, more generally, in the European context). A review of best practice for operating a submersible optical nutrient sensor (Satlantic SUNA) has previously been given by Nair et al. (2017). Jerico Next partners are routinely deploying only wet chemical sensors and therefore this report focusses on best practice associated with these sensors (a) NuLab (Green Eyes), (b) CHEMINI (Ifremer), (c) NAS (Envirotech LLC) and (d) Micromac C (Systea). Reference is also made to the lab-on-chip nutrient sensors developed by NOC which were presented at the workshop. In addition to following manufacturer recommendations for maintenance, calibration and data processing, Jerico Next partners have developed and shared best practices for using nutrient sensors based on experience, which have been compiled in this report.

The in situ colorimetric nutrient analysers use chemical methods, to form a coloured reaction product which is detected spectroscopically or to form a product which can be measured using fluorescence detection. The analyser is calibrated through the use of an integrated standard. To be deployed in the marine environment the analyser must be robust to withstand the challenging conditions experienced (including fluctuations in pressure, temperature and salinity, harsh weather conditions, no human intervention during deployment).

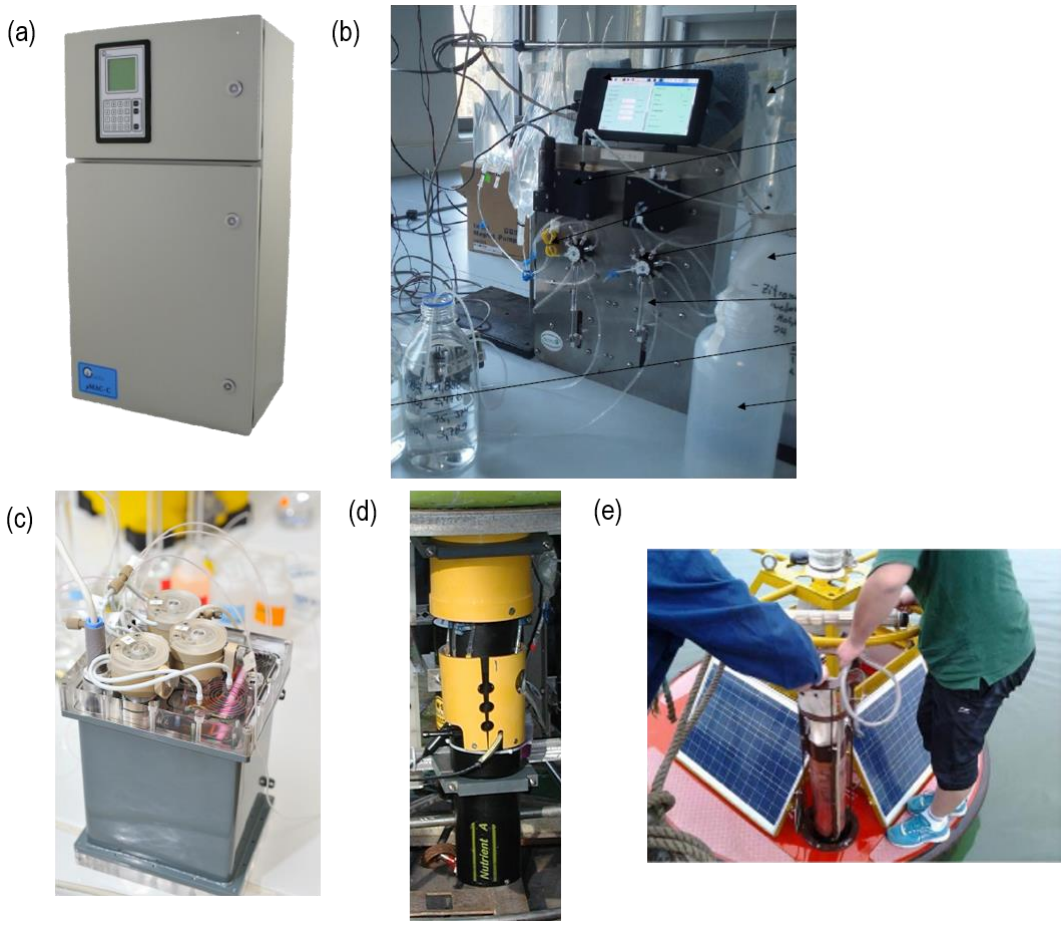


Figure 3.1.1 In situ nutrient sensors deployed by Jerico Next parters (a) Micromac C (Systea), (b) NuLab (Green Eyes LLC), (c) Chemini (Ifremer), (d) NAS-3X (EnviroTech LLC), (e) WIZ probe (Systea)

3.1.1. Method and sensor verification before use

It is highly recommended that method verification is carried out in the laboratory before deployment of the in situ nutrient sensor. Best practice for the determination of inorganic nutrients using Continuous Flow Analysis (the standard laboratory technique) is given in Becker et al. (2018) and where possible and where relevant to the sensor type, the same or similar checks should be carried out as determined for *in situ* nutrient sensors.

1. Reagent stability and preservation

The in situ nutrient sensor may be subject to a range of temperatures during a deployment. The stability of the analytical reagents and standards over the expected temperature range and deployment duration should be tested prior to use. Beaton et al. (2012) use 0.1% chloroform to preserve nutrient standards for in situ nutrient sensor deployments. Some reagents may become coloured over time, particularly if exposed to light and oxygen, which may increase the background absorption (Beaton et al., 2012). Therefore suitable reagent containers for sensitive reagents are required to prevent reagent degradation.

2. Determination of optical blank

The instrument response to a blank sample (artificial seawater or ultra pure water) should be determined, ideally at the start of each sample run so that the sample result can be corrected for the optical blank (Beaton et al. 2012). An optical blank can be determined for all the nutrient sensors listed in section 3.1.

3. Salt effect

The response of the sensor to the nutrient of interest may vary with salinity. If the sensor is deployed in an environment which experiences a large range in salinity (e.g. estuaries) then testing in the laboratory must be carried out to determine the magnitude of the effect so that the results can be corrected post-deployment for variations in salinity. In this case an additional sensor must be deployed to record salinity so that the data correction can happen post-deployment. Figure 3.1.2 shows an example of characterising the salt effect for the Chemini developed by Ifremer.

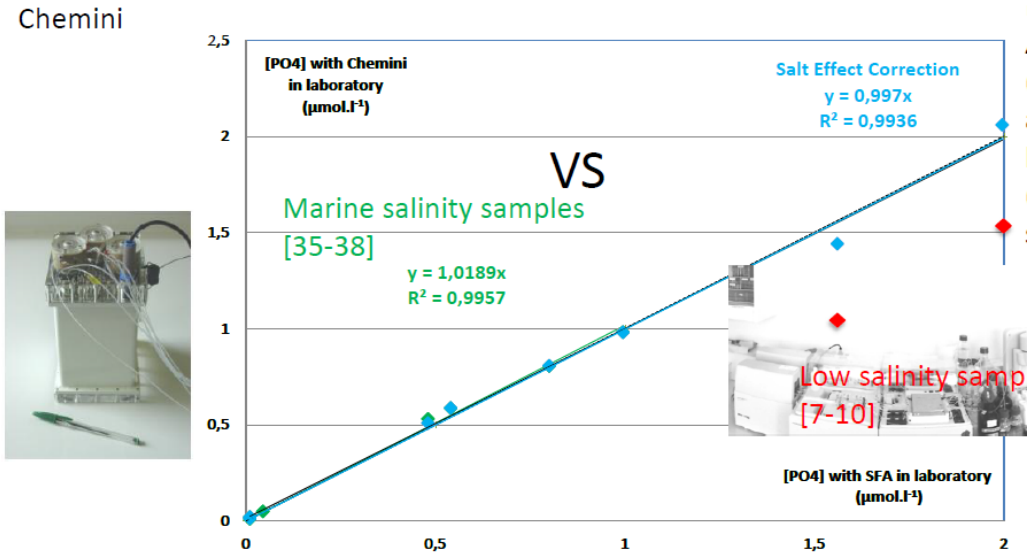


Figure 3.1.2. An example of characterising the salt effect for the Chemini developed by Ifremer. Standards prepared in a low salinity matrix (red points) have a lower response than those prepared in a fully marine salinity matrix (green points). By correcting for the salt effect, the sensor gives a linear response over a wide salinity range.

Calibration standards should be prepared in the same matrix as that of the deployment environment. Therefore for fully marine deployments, standards should be prepared in artificial seawater or low nutrient seawater to match the anticipated salinity during deployment and therefore minimise the salt effect. Figure 3.1.3 shows an example of the change in response of the Micromac C with varying salinity of the calibration standards.

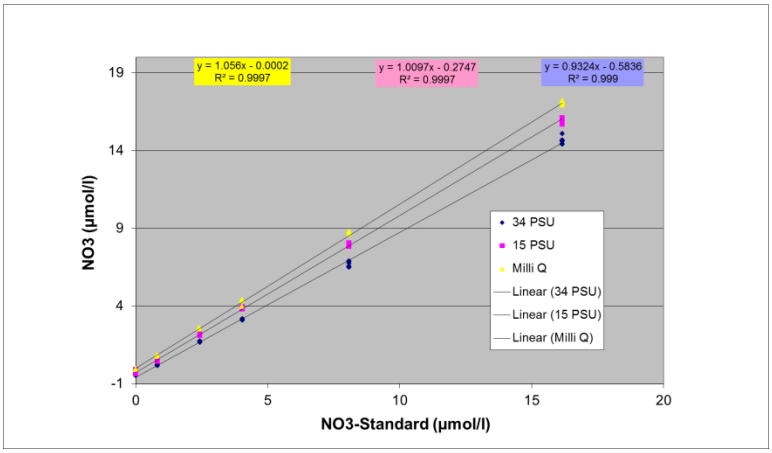
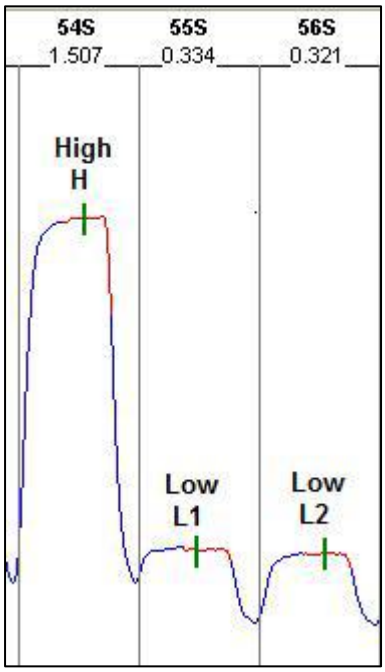


Figure 3.1.3 An example of the change in response of the Micromac C with varying salinity of the calibration standards

4. Sample carry over

Depending on the flushing time between samples, carry over of one sample to the next might occur. This can be observed when running two consecutive identical low concentration standards after a high concentration standard (Figure 3.1.4). If carry over is occuring, the first low concentration standard will have a greater value than the second low concentration standard. This may be eliminated by extending the flushing between samples if the user can adjust this in the sensor settings. If this can not be adjusted, then a carry over correction coefficient can by calculated as discussed in Becker et al. (2018).



Carry over

$k = (L_1-L_2)/(H-L_1)*100$

k = carry over coefficient
L_1 = peak height of first low concentration standard preceded by H
L_2 = peak height of second low concentration standard
H = peak height of high concentration standard

Figure 3.1.4. An example of carry over observed on the standard laboratory nutrients method and how to correct for carry over.

5. Reduction efficiency (nitrate plus nitrite)

Most colorimetric sensors for the determination of nitrate use the Griess assay (diazotization with sulphanilamide and subsequent coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a coloured azo dye). This relies on the reduction of nitrate to nitrite, using a cadmium column, UV photoreduction or vanadium chloride and the efficiency of this reduction should be checked. This is relevant to the lab-on-chip (NOC), NuLAB and NAS-3X which use a cadmium column, the Chemini which uses UV photoreduction and the WIZ probe which has methods for both UV photoreduction and vanadium chloride reduction. The reduction efficiency can be checked by preparing two standards; a high concentration nitrate standard and the same concentration of nitrite. The reduction efficiency can be calculated as the difference in response when both standards are analysed. If reduction efficiency of the cadmium column reduces, the column can be reconditioned (Beaton et al., 2012; Becker et al., 2018).

6. Drift in sensitivity

A change in sensitivity might be due to numerous factors including temperature, salinity, fouling, reduction efficiency of cadmium column (where relevant). The colorimetric nutrient sensors have one or more standards which are run throughout a deployment to calibrate the sample results and therefore allow for change in sensitivity during deployment.

7. Sample filtration

Depending on the suspended load content in the deployment environment, a filter may be necessary to prevent instrument blockage. Filter materials should be checked for contamination. In environments with high suspended load, it may be useful to use a pre-filter before a smaller pore size filter (e.g. Beaton et al., 2018). Filtration is used on the sample inlet for the lab-on-chip and NAS-3X and before the sample chamber for the Micromac C and NuLab. No filter is used when deploying the Chemini.

8. Determination of linear range, limit of detection and limit of quantification

Whilst sensor manufacturers typically provide values for the linear range, limit of detection and limit of quantification, tests should be conducted in the laboratory to validate these under the anticipated deployment conditions (e.g. salinity, temperature), There are international standards providing methods for assessing uncertainty, limit of detection and limit of quantification in relation to water quality (ISO 11352). These methods are widely employed in analytical laboratories and Ifremer have used the same methods for validating the Chemini.

It is recommended that, as a minimum, the following additonal parameters are determined at the same time as nutrients as they are useful for interpretation of the results.
In situ salinity
In situ temperature
In situ chlorophyll fluorescence

3.1.2. Servicing and maintenance

All respondents to the questionnaire carry out the same essential in situ maintenance:

- Visual inspection by knowledgeable personnel (to the degree permitted by the nature of the application, sensor configurations and placement).
- Where possible, run diagnostics to monitor basic functionality (power, telemetry, communications, data transmission, etc.).
- Inspect the state of hydraulic circuit performance (e.g. flow rate, pump functions), if present.
- In-situ calibration during maintenance with reference standards.

In addition, the Chemini and NAS-3X are submersible nutrient sensors which require cleaning of all submersed surfaces after each deployment. All users carry out full maintenance of the nutrient sensors in the laboratory between once and four times per year. The cost of maintenance by Jerico Next partners ranged between €500 and €5000.

3.1.3. Pre deployment laboratory calibration

All Jerico Next partners carry out full laboratory calibrations on the nutrient sensors before each deployment. Those using commerially available sensors only carry out their own calibrations and do not send them to the manufacturer for calibration. The current practice is to use in house reference materials which have been analysed using the standard laboratory method to confirm the concentrations. The number of standards analysed varies between 2 and 6, depending on the laboratory, covering the anticipated concentration range in situ. All users apply an optical blank correction to the calibration data. In addition, Ifremer use working quality control standards to check the Chemini. Whilst not current practice by Jerico Next partners, there was discussion at the workshop about the benefits of also regularly using certified reference materials (CRMs) or samples from laboratory intercomparison exercises as independent quality checks on the nutrient sensor, analogous to the routine practice established for the standard laboratory method. The cost of laboratory calibration by Jerico Next partners ranged between €500 and €7000.

All Jerico Next partners maintain a manual with descriptions of the calibration methods and the measuring procedures it employs, together with details of sample treatment and preparation when these steps are present. The Jerico Next partners who responded to the questionnaire participate in laboratory intercomparison exercises for nutrients for the laboratory reference method for nutrients analysis. All Jerico Next partners actively maintain an archive of sensor calibration reports/certificates for an indefinite period of time.

3.1.4. In situ calibration

The nutrient sensors used by Jerico Next partners have between one and four in situ nutrient standards which provide in situ calibration of the data. All partners prepare their own standards in house which are analysed to determine the actual nutrient concentration using the laboratory reference method. The NAS-3X, the Micromac C and NuLab use a single in situ standard and the Chemini uses four standards. It is recommended to analyse the in situ standards at the end of deployment using the reference laboratory method and the in situ reagents if possible, to check for any decrease in nutrient concentration which will affect the calibration In addition, it is recommended that data from nutrient sensors are compared with results from discrete samples which are collected next to the deployed nutrient sensor and analysed using the reference laboratory method (see Becker et al., 2018). All those responding to the questionnaire carry this out on a routine basis. All Jerico Next partners actively maintain an archive of reports of on-site (field) performance checks of sensors.

3.1.5. Data processing and reporting

The Micromac C, WIZ probe and NuLab provide data in real time whereas the Chemini and NAS-3X provide data in delayed mode. All nutrient sensors used by Jerico Next partners output both raw data and data for the optical blank plus correction for baseline drift for the Chemini. If the concentration of the in situ nutrient standard(s) have changed then a correction will need to be applied for this e.g. by assuming a linear decrease in nutrient concentration in the standard over time. It is recommended that there is cross comparison of data from the nutrient sensor with other sensors and/or discrete sample results. There is guidance available on the quality control (QC) of real-time dissolved nutrient data published by the U.S. Integrated Ocean Observing System (IOOS) available at https://ioos.noaa.gov/ioos-in-action/dissolved-nutrients/.

Becker et al (2018) provide recommendations for the meta data which should be reported for the reference laboratory method for nutrients analysis. Discussion was held at the workshop on the metadata recommended for nutrient sensors. The following is a recommended list:

- General information: investigator (organization, chief scientific, address, e-mail), geographical location (name and GPS positions), cruise (name and number, vessel ID, leg,…) or scientific platform
- Sensor: name and model, serial number, type of analysis (wet chemistry, optical, electrochemical, …), method description, reference to literature,
- Analytical performances: detection and quantification limit, concentration range, linearity, uncertainty, interferences tested in the laboratory,
- Deployment information: measurement duration, depth,/profile, biofouling precautions, sample pretreatment (filtration pore size), extra details (e.g. battery/reagent/standard change),
- Reagents (if used): brand, grade, reference number, batch number, medium used, container type, date of preparation,
- Standardization procedure (if done): stock solution (brand, reference number and grade of the salts, medium, concentration), working standards (concentration, medium, dilution sequence, pipettes,..), container type, number and concentration of working standards, reference material (state batch number, producer, container type),
- Analytical conditions: start/stop cycle procedure (baseline, rinse, standard, sample, blank, QC,..), measurement frequency,

- Quality control result file: QC card control, before/on-board/after deployment calibration, matrix corrections
- Nutrient data csv file: date, time, nutrient name, raw data and unit, voltage of the sensor, correction formula (QC card control, before/on-board/after deployment calibration, matrix corrections), processed data and unit,
- Associated data csv files: date, time, associated parameter (temperature, salinity, pressure), data and unit, name and model of the associated sensors, grab samples (date, time, parameter, concentration and unit, method description, reference to literature,..),

3.1.6. Choice of sensor for different platforms and applications

JERICO-Next partners deploy nutrient sensors on a variety of platforms in the marine environment including buoys, seabed landers and fixed stations. The nature of the platform including size, availability of power and access for servicing influences the choice of nutrient sensor deployed. In addition, the desired range of measurement and limit of detetction will also influence the choice of nutrient sensor. Table 3.1.1 summarises some of the specifications for the nutrient sensors deployed by JERICO-Next partners.

Table 3.3.1. Summary of the specifications for the nutrient sensors deployed by JERICO-Next partners

	Micromac C (Systea)	WIZ (Systea)	NuLab (GreenEyes LLC)	Chemini (Ifremer)	NAS-3X (Envirotech LLC)
Dimensions (mm, length x breadth x height)	420*275*800	140 *790	340*390*200 (2 channel device)	225*146*120	799*246
Materials	Coated steel, silicon, plastics	PVC	steel, glass, tygon tubing	PMMA tap, container stainless steel	uPVC, polypropylene, titanium
Weight (kg)	25	8	6 (2 channel device)	3.5	
Power requirements (VDC)	12	12	10 – 15	12	12
Submersible	no	yes	no	yes	yes
Depth rating (m)	not applicable	10	not applicable	10	250
Current draw (mA) Operation Standby Quiescent state		1500	 1500 90 90	0.3 – 1.7	 285 0.15
Baud rate	9600		9600	9600	19200
Communication interface	RS232	RS232	RS232	RS232	RS232
Parameters measured	Nitrate, nitrite, phosphate, silicate	Nitrate+nitrite, phosphate, silicate, ammonium	Nitrate+nitrite, phosphate, silicate, ammonium (maximum of 3)	Nitrate, nitrite, phosphate, silicate, ammonium	Nitrate, nitrite
Range of measurement in seawater (µmol l-1)	0 - 200	nitrate: 0 – 160 nitrite: 0 - 5 phosphate: 0 - 10 silicate: 0 - 33	not determined	nitrate: 0 – 100 nitrite: 0 - 50 phosphate: 0 - 5 silicate: 0 - 5 ammonium: 0 - 300	nitrate: 0 – 300

		ammonium: 0 - 29			
Accuracy	~ 10%	not determined	not determined	not determined	~ 11%
Limit of detection (µmol l-1)	not determined	nitrate: 0.01 nitrite: 0.02 phosphate0.02 silicate: 0.05 ammonium: 0.06	not determined	nitrate: 0.017 nitrite: 0.004 phosphate: 0.04 silicate: 0.1 ammonium: 0.05	not determined
Limit of quantification (µmol l-1)	not determined	not determined	not determined	not determined	typically 2 - 5
Antifouling measures	none	Copper coil around filter	none	none	none

3.1.7. References

Beaton, A. D., Cardwell, C. L., Thomas, R. S., Sieben, V. J., Legiret, F., Waugh, E. M., Statham, P. J., Mowlem, M. C., Morgan, H. (2012) Lab-on-Chip Measurement of Nitrate and Nitrite for In Situ Analysis of Natural Waters. Environ. Sci. Technol. 46 (17), 9548−9556. http://dx.doi.org/10.1021/es300419u

Beaton, A. D., Wadham, J. L., Hawkings, J., Bagshaw, E. A., Lamarche-Gagnon, G., Mowlem, M. C., Tranter, M. (2017) High-Resolution *in Situ* Measurement of Nitrate in Runoff from the Greenland Ice Sheet. Environ. Sci. Technol. 51, 12518 – 12527. http://dx.doi.org/10.1021/acs.est.7b03121

Becker, S., Aoyama, M., Woodward, E. M.S., Bakker, K., Coverly, S., Mahaffey, C., Tanhua, T. (2018) DRAFT FOR COMMUNITY COMMENT. Update by SCOR WG 147 of The GO-SHIP Repeat Hydrography Manual: Determination of Dissolved Nutrients (N, P, SI) in Seawater With High Precision and Inter-Comparability Using Gas-Segmented Continuous Flow Analysers. http://dx.doi.org/10.25607/OBP-15. Plymouth UK, Plymouth Marine Laboratory, [18pp. & Appendices]. DOI: http://dx.doi.org/10.25607/OBP-169

Nair, R., M. Ntoumas, J. Ma, P. Rigby, T. Trull, and K. Johnson, 2017: Biogeochemical Parameters: Nitrate. In: A user's guide for selected autonomous biogeochemical sensors. An outcome from the 1st IOCCP International Sensors Summer Course [Lorenzoni, L., M. Telszewski, H. Benway, A. P. Palacz (eds.)]. IOCCP Report No. 2/2017, pp. 22-30.

3.2. SECTION ON OPTICAL SENSORS FOR BIOLOGICAL PARAMETERS

This section describes best practices for optical sensors used in biological measurements within JERICO-NEXT project. The document includes the technologies and instruments used in autonomous and continuous modes in various observatories. Many of the examples presented below for a specific brand of sensors are valid also for other brands in the same technology. To be useful for larger communities, we describe best practices for commercially available instruments only.

The complementary information on the status of novel sensors (JERICO-NEXT Deliverable 2.2.; Petersen and Möller, 2017) and their use in automated *in situ* observations of phytoplankton diversity (JERICO-NEXT Deliverable 3.1; Karlson et al, 2017) can be found at JERICO-NEXT web pages [www.jerico-ri.eu/project-information/deliverables/].

3.2.1. LED Fluorometry

a) physical and electrical installation including telemetry

LED fluorometers are the core sensors in many observatories. Most often LED fluorometers are used to track the distribution of Chlorophyll *a* (Chl*a*), but sensors for phycobilin pigments (phycocyanin, phycoerythrin), colored dissolved organic matter (CDOM), hydrocarbons or different tracer dyes are also available. Instruments from different manufacturers differ in materials, dimensions and optical configurations.

Typical power requirement for LED fluorometer is 5-18 VDC with power consumption 0.2-1W during measurement. Instruments provide either analog (0-5V) or digital (RS232/RS422) output, or sometimes both. Gain control may be static or automated, depending on the manufacturer. Digital data resolution varies from 11 to 14 bits. Some instruments also provide internal data storage.

Typically instrument depth ratings vary between 300 m and 6000 m, depending on the pressure housing, which may be plastic, stainless steel or titanium. The weight of instruments in the air varies from 100 g to 1.3 kg and they have a diameter of 2.2 - 6.3 cm and length of 6.7- 28 cm. Operating temperature is typically from 0-30°C to -2 to +50°C.

While practically all LED fluorometers are made for depth profiling, they can also be fitted in fixed depths usually attached in a data logger or a CTD unit and in flow through measurements and some manufacturers supply flow cells. If the flow cell is not available, or for some reason a custom made flow cell is preferred, one has to make sure that the fluorescence readings are not affected by selection of material and that the dimensions of the flow cell do not alter the fluorescence readings, e.g. by light reflections.

b) calibration procedures (user, manufacturer)

Fluorometer output is always in relative units and requires calibration. The aim of the fluorometer primary calibration is to provide a traceable reference point, allowing one to directly compare fluorescence readings collected at different times. The commonly used calibration methods vary. In some cases the calibration is carried out using dissolved chemicals (e.g. Chl*a* in acetone, fluorescein, quinine sulphate, rhodamine) allowing the traceability, while in some cases living organisms (algae cultures) are used without such traceability. Fluorometers should be checked at least once per year and calibrated as needed.

Calibration is often mixed with validation of fluorescence using field samples, and sometimes the importance of primary calibration is questioned. It needs to be noted, that the field validation is the second step in the data processing and should be more straightforward if the instrument primary calibration is done. In field validation,

water samples are collected along with fluorescence measurements and the concentration of the analyte (e.g. Chl*a*) is determined in laboratory using standard analytical methods. The relationship between fluorescence readings and concentrations estimated by laboratory techniques are compared, optimally yielding a conversion factor. Such conversion factors are site-, time- and instrument-specific.

An important part of the calibration activity is detection of zero signal using distilled water. Filtered coastal waters cannot be used to measure the consistent zero signal for fluorometers as such samples may show high and variable background fluorescence. For example, a Chl*a* fluorometer may get high background fluorescence values due to dissolved organic matter. However, it is important to study the magnitude of background signal for the study area, relative to the real signal. Such measurement provides an offset when interpreting the relationship between analyte concentration and fluorescence. Calibration and the measurements for blanks and background signals need to be carried out in a relatively large water container or beaker, following instructions from the manufacturer, to avoid false signals from light reflected or fluoresced from the walls of container (See Figure 3.2.1).

Temperature affects fluorescence intensity. While it may not be an important factor for phytoplankton, with variable fluorescence due to many sources, it need to be taken into account e.g. when using CDOM fluorometers. The temperature correction coefficient varies depending on the instrument type and materials, e.g. CDOM source and need to be studied separately for each case.

Steps towards better definition of fluorometer calibration were taken during joint JERICO-NEXT & AtlantOS Workshop "*Interoperability of Technologies and Best Practices: in situ applications to nutrient and phytoplankton fluorescence measurements*" held in Brest 4-6 December 2018. In the meeting the key challenges in the fluorescence calibration were discussed:

1) There is no commonly agreed methodology for primary calibration of instruments.

2) Due to differences in optical setup of instruments (from different manufacturers) they are not giving directly comparable results.

3) How to perform automated QA/QC for fluorometry.

c) maintenance and checks

Biofouling affects fluorescence readings and frequent cleaning of instruments and flow cells is essential. The required cleaning intervals and methods vary depending on the installation and coastal water types. Especially, if the sensor stays mostly in the deeper layers, out of the productive photic layer, the need for cleaning is typically much less than if the sensor stays all the time close to surface. As the sensors vary largely on the materials used, it is important to check the compatibility of cleaning agent (e.g. acid, solvent) from the instrument manual or from manufacturer. Optical windows should be cleaned using soft tissue paper. Optical windows should be inspected for scratches.

Biofouling is typically seen as an increase of the fluorescence signal and can be difficult to separate from it. Therefore it is important to perform the maintenance cleaning regularly. It is also useful to check occasionally instrument blank (distilled water) and performance using solid secondary standard (if available for the model) or known standard solution, to get information if instrument re-calibration or factory-maintenance is required.

If the sensor is equipped with an antifouling device such as bio-wiper in the sensing optics windows/elements, the maintenance procedures should include the mechanical parts of the wiper and the defective parts should be replaced. Hardware failures of the bio-wipers would affect the sensor data, in some cases a damaged antifouling device instead of protection can cause damage to the sensor itself.

In some specific conditions (e.g. at FerryBox systems with warm instrument and flow of cold water) water condensation may occur at optical surfaces, deteriorating the results. This may be avoided by cooling the instrument or increasing the amount of desiccant inside the instrument housing.

d) data handling and QC/QA routines

QC steps for fluorometry, before setting up the measuring system, include i) selection of suitable sensor with appropriate wavebands and material, ii) sensor performance evaluation and calibration and iii) planning of installation and selection of locations and materials to avoid interferences.

QC steps during the measurement cycle include i) cleaning of sensor, ii) measuring solid secondary standard and iii) taking reference measurements for laboratory analysis. All these actions need to be well documented in a logbook.

Fluorescence raw data should be stored. Raw data may also be used for QC. Like for any sensor, the data should be inspected for outliers and malfunctioning of the sensor or the whole platform. This could include inspection of fluorescence data together with measured adjacent variables (e.g. flow rate, temperature, correlating variables).

Principles of data QC are given by Jaccard et al (2018). If the calibration is done properly, regional climatologies may be used to find out-of-range values. Other criteria for flagging bad data include missing value, frozen value, frozen profile. Spike test is also presented in Jaccard et al (2018) but it is in reality slightly difficult to perform, as the distribution of biomass does not follow normal distribution. Tests using log transformation, suggesting heteroschedastic distribution of biomass, seem to perform better in peak detection, but need to be further studied.

QC protocols, based on Python coding, for automated flagging of the data are currently available, see e.g. JERICO-NEXT D5.12 Software (GISMO Toolbox) for QC of biochemical data from FerryBox and fixed platforms [https://github.com/sharksmhi/gismo_gui_tkinter].

An important part of the data validation is the comparison with field samples. As fluorescence is measured in relative units, the conversion factor to convert fluorescence to concentrations should be determined using a set of field samples. Typically a linear relationship is preferred. However, large deviations from linearity can be observed (e.g. in phytoplankton fluorescence) due to photochemical and non-photochemical fluorescence quenching processes for sensors near the sea surface. At the surface, night time Chl*a* fluorescence is often higher than at day time for the same amount of Chl*a*. To understand and model the effect of a such non-photochemical quenching, irradiance levels should be taken into account when processing Chl*a* fluorescence data. If irradiance data is not available, the time of day may be useful to take into consideration during the data checking.

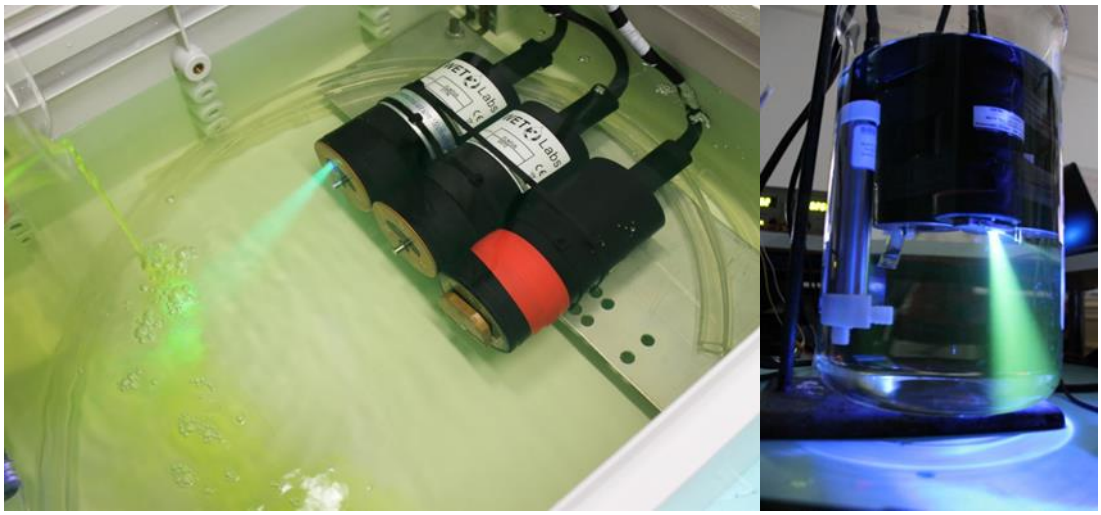


Figure 3.2.1. Experimental set-up for LED fluorometry sensors calibration

3.2.2. Spectral fluorescence

a) physical and electrical installation including telemetry

Integrated spectral LED fluorometers, with several excitation wavebands, are manufactured by bbe Moldaenke GmbH (Fluoroprobe) and JFE Advantech Co. Ltd (Multiexciter). Recently also Trios GmbH (MatrixFlu) and Chelsea Technology Group (V-lux) have launched their multi-waveband fluorometers. The aim of spectral LED fluorometers is to distinguish differently pigmented taxonomic phytoplankton groups. Some emerging sensors (e.g. MatrixFlu, V-lux) in preparation or in the early phase of operational use, have also specific models that are designed to target different CDOM components.

Spectral fluorometers are in larger size and higher in power consumption, than single waveband fluorometers, limiting their use in some platforms. Multiexciter weight 1.6-1.8 kg and has dimensions 79 mm x 244-301 mm, depending on the model. FluoroProbe weight 4.5-7.2 kg depending on the model and has dimension 140 mm x 450 mm. Depth range of the instruments vary from 0-50 to 0-1000 m depending on the materials of pressure housing. Instruments are either battery driven or require input of 12-24 V (dc). Multiexciter is available either as a logger-type or a cable-type device, while FluoroProbe can be configured for both modes. Instruments use RS485 standard to transmit the data to PC.

b) calibration procedures (user, manufacturer)

Instruments come with factory calibrations. Fluoroprobe is calibrated at factory using standardized algae cultures, grown in controlled conditions. Multiexciter is calibrated using 100ppb Rhodamine solution at 570 nm excitation. There are no commonly agreed calibration methods to follow. Users may perform in-house check-ups of calibration using known fluorophores.

To analyse the spectral data, instruments use reference spectra of different phytoplankton classes. These are provided by factory, but should be checked by the user, for the typical species most likely occurring at study site. Similarly, users should check the spectral fingerprints for distilled water and CDOM (Figure 3.2.2), these both are also used in the spectral decomposition algorithm. Instrument manuals give instructions how to perform such measurements.

c) maintenance and checks

As for LED fluorometers, spectral fluorometers are affected by biofouling and countermeasures have to be taken similar to LED fluorometers. It is important to perform occasional check-up measurements using distilled water. Another simple checkup is to check LEDs with known fluorophores. For example, if Fluoroprobe is equipped with Workstation and 25 mL cuvette, the instrument may be checked using known concentration of pure Chl*a* in acetone or ethanol. Large fluctuations indicate that instrument needs recalibration. It should be noted, however, that not all LEDs can be checked using single fluorophore, as the wavebands do not match.

d) data handling and QC/QA routines

QC/QA routines are principally similar to those of LED fluorometers.

Raw data need to be stored. Estimation of the abundance of various phytoplankton pigment groups is done using various algorithms and to allow recalculation (e.g. with different type of algorithm or different fluorescence fingerprints) raw data need to be available. Key issue so far is that calibration of fluorometers is not traceable and thus sharing fingerprints between instruments is challenging and should not be done unless instruments are clearly intercalibrated.

If the build-in detection of algae classes is used, it is important that the user selects the proper algae fingerprints i.e. those matching the type of algae in the water. If new fingerprints are created it is important to study their collinearity.

When algae classes are determined using any calculation method, the goodness of fit should be evaluated and presented along with the calculated estimates for algae classes.

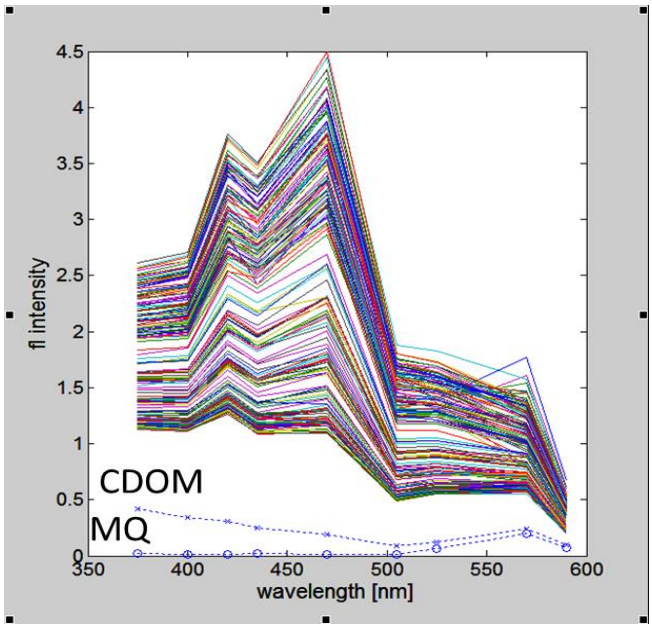


Figure 3.2.2. Example of spectral fluorescence of natural waters with phytoplankton (continuous lines, with different colours), background signal of filtered water from the study site (CDOM) and blank signal due to distilled water (MQ). Measurement is done using Multiexciter spectrofluorometer at Tångesund, Sweden, during JERICO-NEXT workshop 29.9.2016.

3.2.3. Fluorescence induction

a) physical and electrical installation including telemetry

There are two main techniques in measuring variable fluorescence: single turnover technique like Fast Repetition Rate Fluorometry (FRRF) and multiple turnover technique like Pulse Amplitude Modulation (PAM) fluorometry. For continuous and automated online measurements mainly the FastOcean FRRF system (Chelsea Technology Group) is used. It is designed for profiling systems and moorings but when coupled with Act2 system, it can be used in laboratory or in flow through applications, allowing automated measurements of Fluorescence Light Curves (FLCs). The single instrument has dimensions 88 x 284 mm and weight of 2.9 kg. When coupled with dark channel, allowing estimation of electron fluxes, the Ambient Plus Dark (APD) system has dimensions 316 (w) x 292 (d) x 685 mm (h) and weight of 20.3 kg. Power requirements are 18-36 V operating range with consumption of approximate 5 W. The system can be configured in real time (with cable up to 200 m) or battery configurations with internal data logging (8h battery charge is required). To be run, instruments require software package from manufacturer. For flow through applications, Act2 system can be used. It provides a complete package driving FLCs and a solenoid system for sample exchange between runs and also possibilities for cleaning cycles. Act2 operates at 28V and has dimensions of 198 mm (w) x 108 mm (d) x 62 mm (h).

b) calibration procedures (user, manufacturer)

Fast repetition rate fluorometer can be in principle calibrated in terms of instrument-specific calibration coefficient (K_R) (Silsbe et al 2015). This is a tedious procedure, still with some uncertainties, and should be the task of manufacturers or very experienced users.

c) maintenance and checks

To efficiently define the FLC protocol in Act2, users need to be aware of the basic theoretical aspects of variable fluorescence. Steps for selecting values for protocol are clearly presented in the instrument manual. For longer deployments automated functions (to adjust LED energy, photomultiplier voltage and FLC shape) should be used to adjust induction fluorescence measurements and FLC protocols based on observed values. When measuring with APD system in an autonomous way (without sea cable), automated optimization of PMT gain need to be used. Its optimization should be done at an intermediate depth between strong and weak light by the users in the first three minutes of in situ measurements. Recommendations and measurements protocols for field measurements with the profiler are defined in the manufacturer handbook.

The sample chamber water jacket should be filled with deionized water. Temperature regulation of the water within water jacket need to be arranged separately, especially if there are differences in the temperature of sample water and instrument installation location. This could be arranged using automated system adjusting temperature of thermostatic water bath, based on online measurements of sample water temperature.

The solenoid system of Act2 allows users to setup a frequent (e.g. once per day) cleaning cycle using for example mild acid and distilled water.

The current version of Act2 requires frequent check-ups (e.g. daily), especially because there are no means to get feedback from the sample flow rate. The software is not able to track if there is a fail in the pumping unit and if the sample properly exchanged.

Users need to collect discrete samples for the determination of blanks (filtered on 0.2µm), measured separately as single samples for Act2 (3 ml is required) and profiler APD system (150 ml required). The blanks should cover the expected range of background signals.

d) data handling and QC/QA routines

Raw data is saved automatically and need to be stored. Instrument software performs the basic calculations, but user needs to decide on some parameters of the fitting procedure. Spectral corrections need to be done for $sigma_{PSII}$ especially if measured with the Act2 system. Such corrections require measurements of Chl*a*-specific absorption spectra ($a^*(\lambda)$), in situ spectral light distribution and excitation spectrum of the FRRf fluorometer. Spectral correction allows comparison between different $sigma_{PSII}$ estimates different instruments or with independent estimates (as bio-optical parameters) (Suggett et al 2004, Moore et al (2006).

The data fitting software provide the basic fitting. The linear regression step of the fitting provide also calculation of quality standard error, allowing the user to set threshold for quality ratio determining if data fitting is done or not (in the case of too noisy data).

Data fitting results in a large number of derived parameters, and they are affected by selected measuring protocols. Therefore the need for storing the metadata is imperative.

3.2.4. Spectral absorption

a) physical and electrical installation including telemetry

Online hyperspectral integrating cavity absorption meter OSCAR (Trios GmbH) has become commercially available recently, as follow-up of the Hyperspectral Absorption Sensor (HyAbS) (e.g. Wollsclager et al 2013). The other sensor types for spectral absorption measurements, using e.g. reflective and non-reflective tubings, are also available, but not used within JERICO-NEXT community.

OSCAR has a cavity (50 or 80 mm) covered with reflective material (TFE) (Figure 3.2.3). Multi-LED light source is connected to a small scattering quartz-glass sphere, locating inside the cavity. Light is detected using a tip of optic fiber, which is connected to spectrometer. Measurements cover the wavelength range from 360 to 750 nm in 3.3 nm steps. Minimum measurement interval is approximately 1 sample per minute.

OSCAR weigh 6.15 kg (stainless steel version) and has a dimensions of 450 mm x 135 mm. It is a submersible sensor but can also easily be connected to flow through systems. Instrument operates with voltages between 12 and 24 VDC and has power consumption less than 4 W. Sensor has an ethernet connection and supports EIA-232 and EIA-485 and also various protocols like Modbus RTU. Sensor is configured through web interphase. Instrument has internal memory of 2 GB.

b) calibration procedures (user, manufacturer)

The known issues in the calibration include determination of inner radius, reflectivity of the cavity and determination of light transmission, the latter being affected by light source and spectroradiometer. The errors related to the reflectivity have strongest influence and it need to be known in high accuracy. Calibration step, to estimate reflectivity, include measuring spectra of purified water and Nigrosin dye. Calibration step requires that absorption of Nigrosin is determined spectrophotometrically (i.e. using a reference instrument). During the calibration process, it is important to measure temperature of each sample at each measurement. After the calibration the cavity need to be thoroughly cleaned using NaOCl and thereafter rinsed with purified water.

Instrument is provided as pre-calibrated in the factory. However, user is expected to perform frequent calibration measurements. Detailed steps of the calibration are given in the instrument manual.

c) maintenance and checks

For reliable measurements, it is important to keep the cavity clean and perform frequent calibrations as noted in previous section. The frequency of required cleaning and calibration steps depend on the sample water properties and need to be studied in each case separately. For example, during continuous measurements in the Baltic Sea, we have observed the need to perform cleaning of the cavity at least once per day. Cavity can be cleaned using 0.1% NaOCl and with pure ethanol and lint-free tissue. Each cleaning occasion should include measurement of the spectra of purified water before and after the cleaning. It is important to perform all cleaning steps very carefully as any physical damages or any dirty on cavity surfaces or light guides will alter the readings.

It is important to perform parallel measurements of temperature and salinity of the water, as these are required in the calculation of absorption coefficients. As well it is important to record temperature of reference measurements of purified water and Nigrosin.

d) data handling and QC/QA routines

Procedures for data handling are not yet well established for large amounts of data. User community is currently working towards shared tools for data handling. Sensor is rather new on market and not many operational data sets are available. Therefore also the QC/QA routines are still pending.

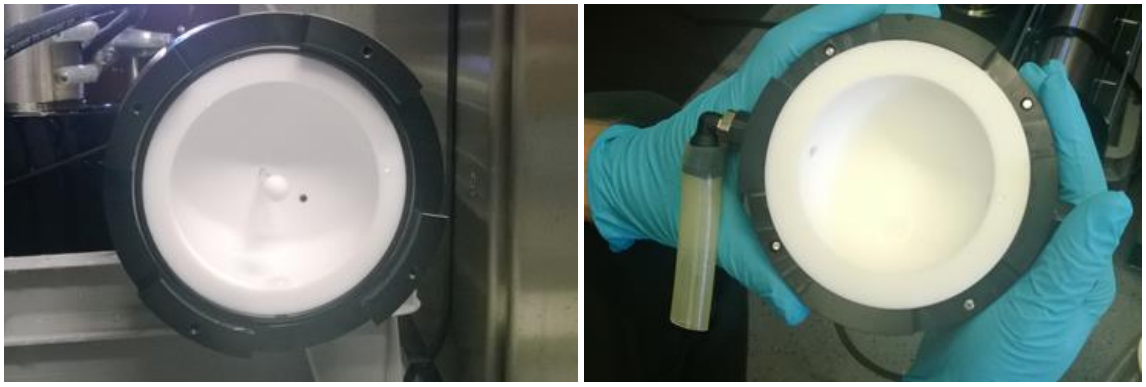


Figure. 3.2.3. The measurement cavity of absorption meter OSCAR is covered with reflective material (TFE). It need to be frequently cleaned with NaOCl, pure ethanol and lint-free tissue.

3.2.5. Turbidity sensors

a) physical and electrical installation including telemetry

Like LED fluorometers, turbidity sensors are provided by several manufacturers. Although the ISO standard EN-ISO 7027:1999 defines the degree of scattering angle (90° +/-2.5°) and use of 860 nm (+/- 10-15 nm) wavelength, some other configurations are also available.

Typical supply voltage is 5 to 25 VDC and power consumption less than 1W. Instruments provide either digital or analog output. Sensors could be rather small, down to weight of 100g, length of 11 cm and diameter of 25 mm. Measuring range could vary, typically from 0-100 to 0-4000 NTU with resolution of 0.1 NTU and accuracy of 2%.

Sensors are available for profiling, stable and flow through applications. Several manufacturers also provide turbidity measurements in the same sensors along with fluorescence measurements.

b) calibration procedures (user, manufacturer)

Turbidity sensors need to be calibrated frequently, e.g. once per year, depending on the use of sensor. Formazin is the most used standard for turbidity calibration. Besides Formazin, some alternative commercial alternatives exist but use of Formazin is most often recommended. It is important that calibration is done according to manufacturer's recommendations, e.g. when selecting a beaker or cup for calibration. If sensor is used in low turbidity areas it is important that the water used for zero readings is really free of turbidity.

Even if the calibration is done according to best practices, instruments with different measuring geometries may result in different results for samples. This is due to specific optical characteristics of various components of suspended solids. Such differences are hard to overcome and therefore it is important that metadata of the instrument used is reported along with the measurement data.

c) maintenance and checks

Light scattered from air bubbles affects turbidity measurements. Thus the measurement site need to be selected in such a way that air bubbles cannot reach the instruments field-of-view and for example in flow through systems the debubbling chamber should be used. When mounting the turbidity sensor on platform or when using custom built flow through cells, it is important to verify that the reflections from surfaces do not influence the turbidity readings.

Turbidity measurements are also prone to biofouling and frequent cleaning should be carried out. The cleaning frequency need to be studied for each case separately. The method of cleaning need to be selected based on manufacturers recommendations. It would also be good to make occasionally reference measurements using purified water or known standard.

Online turbidity records may be verified using measurements of another online sensor or by taking water samples for laboratory based turbidity measurements. The latter may provide controversial results if the measuring geometry of the online sensor and reference sensor differs, if there are changes in the aggregation status of materials during sample storage or if the suspended solids show high variability in the samples. Also the within sample variability is different between sensors, due to differences in the field-of-view.

d) data handling and QC/QA routines

Data handling is rather similar to fluorometry data, given above. Spike tests for turbidity data may be even more difficult than for fluorometry, as distribution of large particles, causing high turbidity readings, may be very patchy and difficult to assess with any other methods. Separation of real spikes, caused e.g. by large particles, are difficult to separate from e.g. water bubbles.

Turbidity readings are often used to assess the amount of total suspended matter and relationships between them can be established. It should be noted that such relationships may be rather site specific.

3.2.6. Imaging and pulse shape-recording flow cytometry

a) physical and electrical installation including telemetry

Flow cytometry records fluorescence and scattering signals of each particle passing the field-of-view. For coastal and open water applications three commercial systems, with slight variations in their operationality have been used by JERICO-NEXT community.

Automated pulse shape-recording flow cytometry (Cytobuoy b.v., the Netherlands) records several optical parameters related to fluorescence and scattering at the single particle level. It also offers the possibility to record the optical pulse shape of every particle (when triggered by scattering) or of auto fluorescent cells (i.e. phytoplankton, when triggered by Chl*a* fluorescence). The optical profiles reflect particles characteristics such as size, shape and pigments (depending on the instruments laser and detectors settings). The most recent versions also provide possibility to take images of the particles at a resolution of 3.6 pixels per µm. Optical configuration (laser wavebands, laser power, detector wavebands) is determined depending on the application. The overall size range of the particles measured is from ~0.5 to 800 µm in width and up to 4 mm in length for chains forming cells, and the volume analysed can be > 5 mL. The instrument is available as a benchtop machine (CytoSense), which could be also used in flow through applications, as a submersible one (CytoSub) connected by power and data cables or as a "CytoBuoy" for moored operations. The CytoBuoy includes wireless connection for instrument connection and data transfer. The basic instrument has a size of approx. 31 x 55 cm and weight of 20 kg. The instrument is controlled by a company software. The CytoSub/Sense can be standalone for several months when installed on a buoy or on a ferry, respectively. An additional sheath cleaning and automated beads analysis is then plugged.

The installation of a Cytosense flow cytometer within the FerryBox system requires a dedicated area, power and flow through sea water outlet filling a dedicated sub-sampling unit of several hundreds of ml, or not, if the measurement is made in continuous mode. The time of analysis and the speed of the inlet pump vary. After analysis, the sub-sampling unit as well as the tubing needs to be flushed before the next analysis. Remote control of the sampling and the internal pressure and temperature sensors can be followed and controlled remotely when internet is available. Power consumption is not more than 700 Wh/d including the imbedded laptop and with a sampling run of 20 min every hour. This power consumption will depend on the sampling strategy, the computer power consumption and the configuration of the Cytosense (laser power, imaging-in-flow device, amount of PMTs).

The Imaging FlowCytobot (IFCB, McLane Research Laboratories Inc., USA) is a submersible imaging flowcytometer. The instrument can also be connected to flow through systems or used as a benchtop instrument. Images are triggered by laser induced Chl*a* fluorescence or scattering. Automated analyses of the images are used to identify the organisms and also to calculate cell volumes. A result in near real time is a list of species or genera with cell abundances and cell volumes for the different taxa observed. The cytometric information is limited and used mainly for triggering purposes. The IFCB has a resolution of 3.4 pixels per µm and is usable for particles in size range 10-150 µm. Instrument can take 5 mL samples approximately three times per hour. IFCB has a depth rating down to 40m and can be used in wired deployments unattended for 6 to 9 months. IFCB operates at 18-36 VDC with power consumption of 35 W. It weight 32 kg and has height of 102 cm and diameter of 26 cm. There is a dedicated open source software, running on Matlab, for images identifications using deep learning algorithms based on identified phytoplankton libraries.

The FlowCam (Fluid Imaging Technologies Inc., USA) is an imaging-in-flow instrument, an automated microscope with the principal aim of providing digital images of each detected particle. One main difference from the IFCB and the CytoSense is that sheath fluid for focusing the water flow with the particles is absent in the FlowCam. For particles detection, two operation modes can be used: « AutoImage » or « Trigger ». For the first one, the particles in the field-of-view are imaged and captured at a regular user-defined interval. For the “Trigger” mode, when a particle passes through the laser, the scattering of laser light is measured and a value of fluorescence is calculated and compared with a fluorescence threshold value. If the obtained value is higher, the camera is triggered to take an image. The fluorescence and scattering have mainly a function to detect particles from the flow, to trigger imaging but fluorescence and scatter information provided is rather limited. The FlowCam can be equipped with different flow cells and microscope objectives providing ability to image particles from 2µm to 2 mm in diameter. However, the chosen optical combination (4X-300µm, 10X-100µm or 20X-50µm), associated to the fluidics parameters, could have a significant impact on the acquisition time for a same imaged volume. By selecting the image triggering mode it is possible to selectively image all particles, only those having Chl*a* fluorescence or those having phycobilin fluorescence. Typical mode of operation for FlowCAM is to apply discrete samples. The benchtop version of FlowCam operates at 100-250 VAC, portable one with 12 VDC. The size of the FlowCAm is approximately 55 cm x 40 cm x 30 cm and 25 kg for Bench top instrument, while slightly less for portable one. FlowCam operates using company software. The FlowCam is available in different configurations. One version is named the Macro FlowCam and is aimed for larger particles than the standard instrument. This was not used in JERICO-NEXT.

b) calibration procedures (user, manufacturer)

In all imaging flow cytometer types, analysis of blanks for the sheath and the sample core should be done. This is possible by shutting down the sample peristaltic pump during acquisition in order to analyse only the sheath, and for the sample blanks, by analysing with the cytometer the sample filtered through 0.2 µm. The background noise of the cytometer has to be identified and will correspond to the lowest level of recording capabilities of the instrument within its specific configuration, and build the sampling settings on behalf. A good noise/signal ratio has to be identified by each user for each mission.

The stability of the instrument is monitored by analysing fluorescence beads. If the beads are used as internal standards, their size and fluorescence need to be as stable as possible and their properties should be different from the organisms found in the study area.

The size calibration of the scatter signal from the flow cytometer can be approximated using beads with best refractive indexes such as silica beads that still have a higher scatter value than phytoplankton cells. A second way is to combine the analysis by flow cytometry and by microscopy of successive filtering of a sample containing a natural community. And a third way is to sort a dedicated population and analyse them with a size estimated device such as the counter coulter.

The counting validation has to be checked regularly by comparing with bench top flow cytometer, known beads concentrations or microscopy counts. It also depends on the good functioning of the peristaltic pump and the sheath speed, and on the volume analysed. Until electronics gets fast and strong enough to both acquire

fluorescing and non-fluorescing particles within the natural sea water, it is advised to either trigger on the red fluorescence or if a trigger is done on scatter, to check the ratio between acquired and rejected volume. This should not be below 50%. Within a cluster, counts < 100 particles generate an error of 10 % (1/sqrt(n)).

For FlowCAM instrument, the manufacturer recommends to perform a standard calibration check with Count-Calibration bead standards, with known size. These beads can also be used to focus the optical system. With the first generation of FlowCAM, this focus is performed manually thanks to a micrometer screw. However, the new-generation of FlowCAM is equipped on an autofocus feature which allows an algorithmic focus of the field-of-view without operator intervention, ensuring repeatable focus position and, therefore, measurements.

Data analysis includes creation of image libraries (for imaging-in-flow instruments) and creating clusters from optical signals (for pulse-shaped cytometry). Standardization of such operations are enduring tasks. This need to start by creation of common vocabularies, as already started within SeaDataCloud project towards ingestion of CytoSense data. For IFCB data a global network of users work together. It is critical to develop classifiers for identifying plankton for regional seas since the phytoplankton communities differ in different sea areas. Standardization of the nomenclature of image libraries should follow either regional conventions or official taxonomic reference list WoRMS.

c) maintenance and checks

Maintenance procedures for the imaging and pulse shape-recording flow cytometers are explicitly given in the instrument manuals and they need to be followed. Basic maintenance includes changing sheath fluid from salt to fresh waters in order to prevent salt crystals formation when the cytometer is not in use for more than one week, keeping the fluidics system free of contamination and biofouling, in some cases this requires the use of biocide and active cleaning cycles (Figure 3.2.4). The flow rate of the peristaltic pump has to be checked regularly in order to prevent decrease in pumping rate due to the stretching of the tubing. The pressure sensors and temperature gauges are there to validate the healthiness of the optical unit and the fluidics. Beads or cells from algae cultures should be used to verify that images provided by the instruments are in focus, and the optics need to be aligned as needed.

Sheath tubing's has to be removed when they start to evidence a change in color, or every year. The flow rate of the sheath pump should be checked regularly. The filters should be changed after a certain amount of analysed samples depending on the turbidity conditions of the sample, or when the sheath pressure increases. Lenses and cuvette from the optical unit can be cleaned with an optical wipe and ethanol when dirt is suspected.

In the automated operations, there may be a need for inlet filter to avoid clogging, this filter need to be replaced depending on the conditions. If the instrument is using sheath fluid, bead solutions, cleaning agents or biocides, it is important to renew fresh stocks when needed. In continuous operation, there may be a need for additional remotely controlled option (e.g. using controllable multivalve) to run additional cleaning reagents (e.g. milliQ, NaOCl, cleaning agent) when clogging or bubbles are observed.

While starting the instrument, user should inspect that there are no air bubbles in the system. User should also follow the data flow frequently, to verify that the observed particles are well in focus and their abundance is as could be expected.

For FlowCam, the most important maintenance step concerns the cleaning of the system after each use. Immediately after use, the analyzer system must be rinsed with appropriate cleaner. This rinse will help to keep the flow cells clear and free from contamination for day-to-day use. If necessary, the rinse can be preceded with a solvent or surfactant to remove particles.

d) data handling and QC/QA routines

The end results from automated imaging systems are in principal not very different from data obtained by light microscopy. The main differences are the amount of data (much larger for automated systems) and the procedures handling imaging in flow, image processing and for quality control.

Flow cytometer data without imaging relies on identifying clusters of organisms with similar fluorescence and/or scattering properties. Here functional groups such as red fluorescing picoplankton, orange fluorescence picoplankton, red fluorescent nanoplankton, orange fluorescent nanoplankton and microplankton represent the common groups which abundance can be comparable to classical flow cytometry and microscopical counts.

Flow cytometer data handling includes storing of raw data, removal of false objectives (e.g. air bubbles), machine noise and extraction of various particle specific features. These features may contain basic flow cytometry signal (intensity of fluorescence or scattering), pulse-shape of the signal (optical profile of the particle) and features of the image (various dimensions of the particle). This results in multivariate data for each particle, to be used in the classification of the particles. No dedicated QC/QA routines are built for flow cytometry data so far.

About FlowCam data, when an analysis finishes, the list data containing all parameters computed on the image of each detected particle is exported (CSV format) and stored in a dedicated directory. Moreover, even if it is also possible to save the raw camera images and the binary images during a run, the storage of collage images into the working directory is preferred in order to save memory space. As for flow cytometry, no dedicated QC/QA routines are built for FlowCam data.

Data processing is evolving, while the basic processing software are CytoClus (for manual clustering), and Easyclus and two dedicated R-packages named RclusTool and flowCARS (for automated classification) for CytoSense and CytoSub data. Concerning the FlowCam, the manufacturer's software VisualSpreadsheet is provided with the instrument. But the ZooImage R-package and the EcoTaxa web-application represent some interesting alternatives for data analysis. Image classification for Imaging FlowCytobot is typically done using Random forest algorithm developed with Matlab environment by Sosik and Olson (2007). The software has been updated continuously since 2007. All software for analysing IFCB data is available open source on a GitHUB repository at https://github.com/hsosik/ifcb-analysis.

Figure 3.2.4. Replacing pumps, tubing and renewing reagent bags for biocide and calibration beads are occasional tasks for Imaging FlowCytobot user.

3.2.7. References

Jaccard, P. et al. (2018) Quality Control of Biogeochemical Measurements, v6. Copernicus Marine Environment Monitoring Service. http://doi.org/10.13155/36232

Karlson, B., Artigas, F., Créach, V., Louchart, A., Wacquet, G., Seppälä, J., (editors) (2017). Novel methods for automated in situ observations of phytoplankton diversity. JERICO-NEXT-WP3-D3.1, 4 Oct. Sep 2017 http://www.jerico-ri.eu/download/jerico-next-deliverables/JERICO-NEXT-Deliverable-3.1_V9.pdf

Moore, C.M., Suggett, D.J., Hickman, A.E., Kim, Y.N., Tweddle, J.F., Sharples, J., Geider, R.J., Holligan, P.M. (2006) Phytoplankton photoacclimation and photoadaptation in response to environmental gradients in a shelf sea. Limnology and Oceanography, 51, 936-949.

Petersen, W., Möller, K.O. (editors) (2017) Report on the status of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling, within the JERICO network and, more generally, in the European context. JERICO-NEXT-WP2-D2.2-28062017-V1.2 http://www.jerico-ri.eu/download/jerico-next-deliverables/JERICO-NEXT-Deliverable-2.2.pdf

Silsbe, G., Oxborough, K.D.J.S., Suggett, D.J., Forster, R., Ihnken, S., Komàrek, O., Lawrenz, E., Pràsil, O., Röttgers, R., Sicner, M., Simis, S.G.H., Van Dijk, M.A., Kromkamp, J. (2015) Toward autonomous measurements of photosynthetic electron transport rates: an evaluation of active fluorescence-based measurements of photochemistry. Limnol. Oceanogr. Methods 13, 138–155.

Sosik, H.M., Olson, R.J. (2007). Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. Limnol Oceanogr- Meth, 5(6), 204-216.

Suggett, D.J., MacIntyre, H.L., Geider, R.J. (2004) Evaluation of biophysical and optical determinations of light absorption by photosystem II in phytoplankton. Limnology and Oceanography Methods, 2, 316-322.

Wollschläger J., Grunwald, M., Röttgers, R., Petersen W. (2013) Flow-through-PSICAM: A new approach for determining water constituents absorption continuously. Ocean Dynamics 63: 761-775.

3.3. CARBONATE SYSTEM SENSORS

The oceanic carbonate system is composed of key environmental variables such as carbon dioxide (CO_2) and pH. Our need for improved observation and understanding of the oceanic carbon cycling has been recently renewed due the large uptake of fossil fuel CO_2 and subsequent ocean acidification. Also of importance is the seasonal and annual variability in production and consumption of CO_2 via photosynthesis and respiration, respectively, in coastal oceans. Below we describe recommended best practices in operating a variety of pCO_2, pH, and total alkalinity sensors. These include practical information related to the installation and operation of the various sensors, accessory and supporting sensors and instruments necessary for calculations and corrections, data handling and flagging, and the use of reference materials to check and calibrate measurements. The recommendations are based on long-term (several years) experience with sensors that are used in coastal environments which introduce challenges with high biological activity, particle load, and at times low salinity.

3.3.1. pCO2 systems

3.3.1.1 GO pCO_2 system

The system is composed by a deck box, a dry box and a wet box. The deck box contains of a Druck barometer, GPS, and iridium modem. The dry box contains the Non-Dispersive Infrared Analyser (LICOR; LI-7000), computer and valves for the measured gases and the calibration gases. The wet box contains of the main equilibrator, the second equilibrator and the cooling system for the gas to be measured. Seawater is circled through a main equilibrator (EQU) at a flow rate of about 2 L/min and a pressure of 4 psi. Water enters the EQU through a spiral nozzle, creating a conical spray which enhances the CO_2 gas exchange between the water and the overlaying air in the EQU. The water is then gravity drained out of the system. A siphon break in the middle of the EQU effectively isolates the headspace gas from the outside air and greatly minimizes any gas loss due to air entrainment from the water flow.

The headspace gas is circulated through the system and back to the EQU with a pump at about 100 ml/min. It is first dried by going through a Peltier cooling block operating at about 5 °C and then a Permapure Nafion tube. The dry gas is then sent to the LICOR where the mole fraction of CO_2 and H_2O is measured. Atmospheric air is also being measured by the system. A dedicated pump constantly draws outside air, which is dried in a second channel of the condenser.

a) Physical and electrical installation including telemetry
The sensor should be installed in a dry environment and relatively close to the water inlet in order to keep a similar temperature *in situ* as in the equilibrator. The GO system can be equipped with an own setup of FerryBox parameters or installed next to one. Because of the need for regular seawater flow, a dedicated pump that is reliable in nature is required (centrifugal or peristaltic pump recommended by GO). A regular 220V power supply is needed, and a serial or ethernet connection to a PC is desirable. The system stores all data in txt format on the computer used for the GO software. The setup comes with a possibility to transfer data with iridium connection.

b) Calibration procedures (user, manufacturer)
The calibration gases are transferred to the GO system and distributed with a VICI valve. The amount of samples analyzed and the frequency of the standard measurements is set by the user in the GO software. The standard procedure when starting up the system is that a zero and span test is done, i.e the system analyzes a standard gas with zero CO_2 and then a sample of the highest concentration of standard gas. After this zero-span, the standard gases are analyzed in order of their concentration; 200, 400 and 800 ppm CO_2. The gases are provided from Deuste Gas Solutions in Germany.

c) Maintenance and checks
The GO system needs to have manual service and maintenance every 6 weeks. During this interval it is necessary to monitor the data, if possible, to have a knowledge of the status of the system. During regular service the main filter is rinsed with water and cleaned. The main filter is a trap for organic substances in order to keep the equilibrator as clean as possible. If the equilibrator is contaminated with organic matter it needs to be rinsed and cleaned, and a manual for demounting the equilibrator from the wetbox is provided by GO.

d) Data handling and QC/QA routines
The GO system presents raw data, xCO_2, in the data output when the system is not equipped with it's own salinity and temperature sensor. If the salinity and temperature is measured by an external FerryBox, the two data files need to be merged in order to calculated pCO_2. General Oceanics recommend a set of calculations in their manual that is referenced (Takahashi et al., 1993). The primary QC/QA check is the difference between the inlet temperature and equilibrator temperature - a difference larger than ~ 2 deg C indicates a problem with the water flow.

3.3.1.2 Franatech pCO_2 system

The system is composed of a deck box and pump. The pump supplies a constant flow (1.2-1.4 L/min) of seawater to the deckbox which houses an equilibrator unit – wet and dry chambers separated by an air-permeable membrane. Carbon dioxide in the water stream equilibrates with the dry side of the chamber, and this air mass is then introduced to an infrared detector.

Figure 3.3.1. An example of a Franatech pCO_2 sensor system. The leftmost part contains the electronics, datalogger and display. The circular object in the middle is the equilibration chamber. And the detector lies along the bottom of the case. Water inlet and outlet on the right side of the unit with water pressure and water detector mounted. On the top the gas interface for calibration.

a) Physical and electrical installation including telemetry
The sensor should be installed in a dry environment, ideally close to the same temperature as seawater that it is measuring. This has only be operated as part of a FerryBox setup. Because of the need for constant seawater flow, a dedicated pump that is reliable in nature is required (e.g., Iwaki magnetic drive pump, Model MD-6). A regular 220V power supply is needed, and a serial or ethernet connection to a PC is desirable. The system is capable of self-logging a limited amount of data in ASCII format or transferring data to a PC for recording.

b) Calibration procedures (user, manufacturer)
All calibrations are carried out by the user. Three or more air:CO_2 mixtures are humidified and pumped directly to the detector for calculation of slope and intercept. Alternate calibrations can be made by pumping humidified air into the water chamber, etc. High quality and certified air:CO_2 mixtures are available from the NOAA ESRL GMD lab (https://www.esrl.noaa.gov/gmd/ccl/), and custom air:CO2 mixtures can be acquired from local specialty gas suppliers and compared to ESRL gases. In Norway we have found specialty gas supplier AGA to make reliable air:CO_2 mixtures (within +/- 5 ppm) (https://shop.aga.no/shop/en/no-ig/specialty-gas-107-specialtygas). The calibration procedure typically takes >30 minutes per calibration gas to ensure a stable signal.

c) maintenance and checks
The pump, water connectors, and data output should be regularly checked (during each visit to the FerryBox) to ensure that water flow is constant and achieving the desired 1-2 L/min. If water connectors are quick-connect style (e.g., Swagelok QC style), the connectors must be checked regularly that they are not blocked and flow is adequate. The membrane should be visually assessed for fouling. Any cleaning or replacement of the membrane should be accompanied by a new calibration check. While the change in membrane has should not affect CO_2 equilibration, we have been unable to corroborate this in practice. The temperature sensor in the water chamber is critical for calculating in situ pCO_2, so this sensor must also be regularly checked with a traceable thermometer.

d) Data handling and QC/QA routines
The pCO_2 sensor requires auxiliary data for calculating in situ temperature. This includes in situ temperature (from the FerryBox inlet temperature sensor), water temperature in the water chamber, and air pressure in the air chamber. Fugacity of CO_2 (fCO_2) is calculated using the measured pCO_2 and the above variables using calculations presented in Dickson et al. (2007) SOP 5. The primary QC/QA check is the difference between the inlet temperature and water chamber temperature - a difference larger than ~ 2 deg C indicates that the either the water flow rate was low or there was an overheating issue, and these data are flagged. Further QC/QA can be conducted by comparing fCO_2 calculated using CO2SYS and CT/AT measured in discrete samples or other carbonate system variables measured by other sensors (e.g., overdetermination).

3.3.1.3. Sunburst SuperCO_2 system

a) Physical and electrical installation including telemetry
Finnish Meteorological Institute is operating two SuperCO_2 analyzers (Sunburst Sensors, Fig. 3.3.2). One instrument is placed on M/S Silja Serenade travelling between Helsinki and Stockholm, one direction per every 24 hours, the second instrument is placed at Utö Atmospheric and Marine Research Station on Utö Island. Both instruments have double shower head equilibrators, which ensure the measured CO_2 is properly equilibrated relative to the continuous stream of seawater. CO_2 molar fraction is observed using an NDIR (non-dispersive infrared) gas analyzer (LI-COR 840A CO_2/H2O). The system at Utö is connected to the FMI measurement intranet and receives electricity through an online UPS to protect it against lightning.

Water sampled on Silja Serenade comes from the FerryBox system operated by SYKE. At Utö, water is pumped from the depth of 5 m down to 23 m depth and then led to the measurement cabin through 250 m long pressure pipe. The residence time in the inlet is approximately 5.5-8 minutes, with a total water flow of 45-65 lpm. Currently, SuperCO_2-system has an extra pump inside the cabin providing the double showerhead equilibrator a waterflow of 3.5-4.0 L/min.

Figure 3.3.2. SuperCO_2-system at Utö (the setup at Silja Serenade is similar, except the system for water outflow requires an additional pump, which is placed after the "exhaust water" tank). After testing several different setups, FMI has found the current pCO_2 measuring setup operating with little maintenance required. No plans to change the instrument planned. During the summer 2019, FMI plans to test the influence of inlet tube system on observed pCO_2 concentrations. As labeled: A) Reference gases, B) Instrument with LI840A gas analyzer inside, C) Tablet computer for operating the system, D) Water separator unit, E1) air filter (sample air in), E2) air filter (sample air out), F) Equilibrator chambers, G) Rotameters, H) washing pump, I) washing liquid storage bottle.

b) Calibration procedures (user, manufacturer)
The system measures four standard (reference) gases with concentrations of approximately 0, 200, 400 and 1000 ppm every four hours. These standard gases are ordered from AGA company, with original accuracy of ±2%. The exact CO_2 concentrations with accuracy higher than 0.1 ppm are obtained by measuring the concentrations (with Picarro gas analyzer) against the ICOS reference standard gases used at FMI.

These reference points are used to correct the data. If major differences between the measured gas concentrations and the expected concentrations are observed, the optical bench of LI-840A is cleaned and the analyzer is recalibrated. In case major differences remain, LI840A is replaced with a spare sensor and the other serviced either by FMI GHG specialized technician or sent to LI-COR Inc. for maintenance.

c) Maintenance and checks
The system automatically measures reference gases every six hours. If major deviations are observed (data is visible through internet, with basic warnings for e.g. reduced flows, clearly wrong readings etc.), the instrument is serviced. The wet part of the measurement system is automatically washed daily (interval depend on biological activity) with hydrogen peroxide solution. For regular maintenance, we have 6 pages long Finnish SOP's for weekly (checking the values, bottle pressures, state of the filters etc), monthly (manually cleaning the equilibrators) and annual maintenance (changing e.g. all tubings).

d) Data handling and QC/QA routines
Data from the instrument is sent to a server at FMI. On the server, we run FMI-made Python 2.7. program to check the data for errors and to take into account the standard gas observations. After that the data is stored on the server and visualized on Utö www-page (http://swell.fmi.fi/Uto/graphs_pco2both_1d.html). Data from Silja Serenade go through a similar process. QC/QA program is built in that way it shows the warning messages with service suggestions on Utö www-page and also emails the warning messages to key personnel, when the variables (e.g pressures, water and air flows, temperatures) are out of the pre-set ranges.

3.3.1.4. Contros HydroC CO_2 system

a) Physical and electrical installation including telemetry

The Contros HydroC® CO_2 is an optical, headspace-based underwater pCO_2 sensor, that operates within a temperature range from +3°C to 30°C, and is hosted in a titanium housing available for different operating depths (down to 6000 m depth). Power can be supplied by external batteries or by a cable connected to an external power source (from 12 to 24V). A version for flow-through systems such as FeryBoxes are also available (HydroC® CO2 FT).

Dissolved gas diffuses from the water through a thin film hydrophobic composite membrane into an internal headspace where all the partial pressures equilibrate. A gas pump continuously circulates air between the membrane equilibrator and thegas concentration is measured by non-dispersive infrared spectrometry (NDIR) within the gas circuit; xCO_2 data along with temperature, pressure and relative humidity are measured and used by the software to calculate in situ pCO_2 values. Regular zero gas measurements can be carried out to calculate the zero drift of the NDIR detector. All the data are saved on an internal data logger and/or transmitted by cable.

For use in coastal waters it is mandatory the installation of a pumping system on the head of the sensor and all the copper antifouling protections available from the manufacturer, that effectively protect the membrane from hard-fouling incrustations. The main concern for the presence of fouling is membrane damage: even a small scratch could lead to an instrument flooding and to serious damage to all the electronics.

Figure 3.3.3 shows the condition of the membrane after about the same time of deployment, with and without the antifouling system. Copper tape can be used to protect also the external part of the instrument head; to avoid damages due to corrosion, it must be positioned over insulating material, not in direct contact with the instrument housing. Another aspect that deserves attention is the sensor alimentation. The use of external battery packs should be limited to installations where is not possible to provide an external power source, as the energy consumption is quite high. Even if the sensor can use an external power source of 12V, we suggest a 24 V alimentation to minimize the power loss if 10 – 20 m long instrument cables are used.

(a)  (b) 

Figure 3.3.3 Growth of fouling on the membrane of HydroC-CO2II using only the copper filter for the inlet of the pump (a) and also the antifouling copper sensor head (b).

b) Calibration procedures (user, manufacturer)

To ensure good results, it is suggested to perform a factory calibration and standard maintenance every year. It is not possible to perform a user calibration. The factory calibration is performed in a calibration tank and is based on four different pCO_2 values chosen according to the expected pCO_2 range in the field, using a SPRINK underway instrument with LiCOR LI7000 as a reference (Fietzek et al., 2014). The instrument is equipped with an internal CO_2 scrubber to check the "zero CO_2" signal and correct the data for the baseline drift after deployment.

c) Maintenance and checks
A good laboratory practice is to use pre and post deployment checklists to ensure standard protocols even if the instrument is handled by different operators. Their keeps track of basic information in an organized and easy accessible way that is useful for future troubleshooting and data QC. An example from the ones used at PALOMA station in the N. Adriatic is attached in Annex I.

When the sensor is equipped with a standard membrane, it should be recovered and the membrane changed by the user, every 3-6 months according to the environmental conditions. A new tougher membrane is also available from the manufacturer that requires less frequent exchanges, even in high fouling environments. As soon as raw data are available, it is recommended to check for correct instrument functioning. The basic checks are: alimentation problems, internal sensors failures (nonsense data), drift in the sensor temperature and very high humidity (it's a warning for possible flooding).

d) Data handling and QC/QA routines
The instrument automatically acquires all the data needed to calculate pCO_2 values at in situ conditions. During long term deployments, the instrument usually presents some drift in the results: both as an increase in the baseline (the "zero CO_2" level) and in the signal for a given CO_2 concentration (the "span" drift). To allow an effective correction it is important to frequently register the baseline level, ideally at the beginning of every measurement cycle, and to perform a "post cruise calibration" when the instrument is serviced at the manufacturer. Data can hence be post-processed according to Fietzek et al. (2014). The instrument performance can be checked comparing the measured values with results from discrete sample analysis. If pCO_2 cannot be measured directly, the best couple of carbonate system parameters that should be used to validate pCO_2 data are pH and total alkalinity (AT) or pH and total dissolved inorganic carbon (CT) (Orr et al., 2018).

3.3.2. pH systems

3.3.2.1 NIVA pH system

a) Physical and electrical installation including telemetry
The NIVA pH sensor system (Fig. 3.3.4) spectrophotometrically measures absorbance ratios at wavelengths corresponding to protonation of an indicator reagent (e.g., thymol blue or m-cresol purple). The sensor is primarily intended for operation in surface waters and is currently in use with FerryBox systems. Some limited deployments on fixed buoys and autonomous surface platforms have been performed, but future work on reducing power consumption and optimising sampling strategies could support deployment on other autonomous vehicles. The system's central processing unit, LED sources, pumps, spectrophotometer, etc. requires 12V via a 220V power supply - it can optionally be operated via a battery pack. The sensor is controlled by open-source Python code on a Raspberry Pi, and this allows connection for data transfer and operation via Ethernet, serial (RS-232), or wireless connection (802.11). Seawater sample supply can either be via external pump (e.g., either the general FerryBox pump or dedicated external pump), or through a small internal peristaltic pump.

b) Calibration procedures (user, manufacturer)
Sensor validation and calibration is carried out by comparison with analysis of discrete samples and measurement of AT and CT according to standard operating procedures. Seawater pH (total scale) is calculated using absorption spectra and calculations presented in Zhang and Byrne (1996) and Dickson et al. (2007). A new set of coefficients will soon be available for thymol blue at salinities <30 (B. Bryne, pers. comm.). Overdetermination is possible through fundamental stoichiometry and thermodynamic equations, given salinity, temperature and pressure in situ. Accuracy obtained with laboratory analytical instrumentation is traced by the use of certified reference material (CO2 CRMs), CO2SYS, and Tris-buffered seawater (Dickson laboratory, Scripps Institution of Oceanography; Pierrot et al., 2012; DelValls and Dickson, 1998).

The thermistor must also be regularly calibrated due to the strong dependence of pH on temperature. A traceable digital thermometer and a warming/cooling circulating bath can be used to for generating a multipoint calibration curve.

Figure 3.3.4. NIVA spectrophotometric pH system. The system includes (from top left to bottom right: touch screen display of Python GUI, gas impermeable foil reagent bag for indicator dye, cuvette with stir bar, and pump for indicator dye. Not shown but present in the top compartment: Raspberry Pi, spectrophotometer.

c) Maintenance and checks

Regular physical and data checks are required to ensure the optical and wet reagent components are operating properly. This includes opening the cuvette to evaluate fouling of the light source and spectrophotometer windows, the stir bar assembly, and thermistor. If fouling is observed, a physical wipe or assistance with weak solvents can be used for cleaning. And this cleaning is also performed on a regular basis regardless of visual check. The dye reagent (thymol blue or m-cresol purple) are supplied via gas impermeable bags – these must be checked periodically for structural integrity and refilled regularly. Additionally, the data can be evaluated for decrease in signal over time, especially in the 730 nm wavelength that is indicative of fouling or loss of LED intensity. While this does not affect pH measurements because dark pre-dye measurements are made, severe decrease in signal output or increase in signal to noise ratio can result in larger uncertainties in each measurement.

d) Data handling and QC/QA routines

Once the reagents have been characterized and stability assessed, there should not be any need of re-calibration, provided that ancillary data are properly collected (temperature and salinity). Dye reagent manufacturer and lot numbers should be recorded, as well as batch numbers for each solution batch for traceability. Further purification

of dyes are also possible and are being carried out at a number of labs. Data obtained can be validated with carbonate chemistry models available (CO2SYS; Pierrot et al., 2012). The 730 nm absorption can further be used to detect particle interference in the signal beam during measurements - because particles can create artifacts in the dye absorption measurements, these data points are compromised and must be flagged and omitted from the dataset.

3.3.2.2 Contros pH system

a) Physical and electrical installation including telemetry
The CONTROS pH analyzer (CONTROS HydroFIA pH; Fig. 3.3.5) is a flow through system for determination of pH in seawater. The system is designed for operation in the lab or for autonomous operation in underway measuring systems such as FerryBoxes. The determination is based on spectrophotometric determination using 0.01 mol/kg m-Cresol Purple (mCP) as indicator dye and measuring the change of color depending on pH. For each measurement a small volume of indicator dye is injected into the sample stream (principle of flow injection analysis (FIA)). The color changes are determined by measuring the absorbance spectra of the dye as changes in the peak absorbance ratio. All pH measurements are related to 25°C independent of the temperature of the water sample. Therefore, the system interior is kept a 25°C. In principle, the analyzer is calibration free and suitable for long-term applications. The calibration is based on Dickson et al. (2007). The pH data are only valid for a salinity range of 20-40 psu within a range of pH = 7 - 9. New publications also describes the application and corrections for lower salinities from 5-20 (Müller et al., 2018).

Specification of the manufacturer: Accuracy +/-0.001, precision /+-0.001, ambient temperature range 5 – 30 °C, measurement cycle ~1 min, power supply 100-240 VAC. Communication via RS-232 or Ethernet connection.

Figure 3.3.5. HydroFIA pH analyzer.

b) Calibration procedures (user, manufacturer)
The analyzer works with a solution of mCP for pH determination and a solution of 0.1N HCl for flushing and cleaning. The manufacturer delivers the prepared solutions as changeable cartridges. One cartridge of mCP (500ml) is suitable for ~16000 measurements. Assuming one measurement in two minutes (an actually achievable frequency) the system can be continuously operated for approximately 22 days. The calibration of the instrument is certified by the manufacturer. The manufacturer calculates an individual offset for each device based on pH measurements of certified reference material (seawater CRMs) from Scripps Institution of Oceanography, University of California, San Diego with a certain batch of mCP.

c) Maintenance and checks
The calibration can be checked by the user by measuring certified buffer solutions of TRIS (2-amino-2-hydroxymethyl-1,3-propanediol) in synthetic sea water (Nemzer & Dickson, 2005). The system has two inlets, one for continuous flow and another for discrete measurements such as a CRM. Fouling should be minimized by

regular acid flushes which can be automatically performed. Intensive cleaning with 0.1N HCl should be performed after longer deployment. After replacement of the mCP cartridge there might be an offset of the measured pH due to changes of the indicator dye composition (different dye supplier, different impurities of the dye, etc.). This must be checked by measuring reference samples.

The instrument must be checked for air inside the flow-through system. If air gets inside, the system has to be flushed completely until the air is gone. As the internal pump doesn't work properly with air in the tubes of the system has to be flushed with acid before flushing with sample water.

d) Data handling and QC/QA routines

The device can be connected via RS-232 for remote control and real time data transfer. Internally the data including the spectra are stored in a database which is available via FTP server or a web interface (RJ45 connector). The device needs sample salinity for calculation of the correct pH. This can be provided automatically via serial interface or entering these data manually. The system has some internal control mechanism to assess the quality of the measured pH value. This might be a deviation of the sample temperature from 25°C, distorted (invalid) spectrum (e.g. by air bubbles), invalid salinity, etc.. These errors are marked by different flags.

The quality of the pH measurement depends on spectrophotometric quality and on calibration of the properties of the used indicator dye. The manufacturer uses as standard dye non-purified mCP which might cause uncertainties (Liu et al., 2011). The temperature control has sometime problems to keep the measuring temperature stable at 25°C especially at low sample temperatures (Fig. 3.3.6). The measuring temperature varies around ±1.2°C.

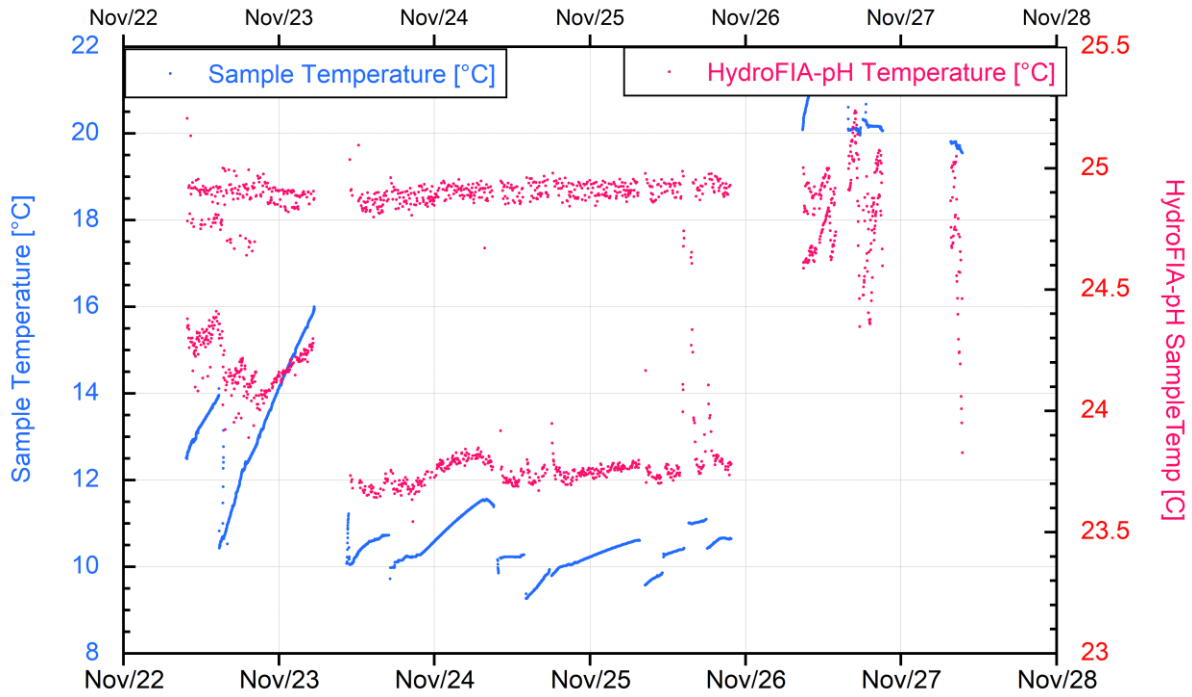


Figure 3.3.6. Sample temperature of pH measurement (HydroFIA Temperature) depending on temperature of sample water.

In principle this can be compensated by recalculating the measured values for an exact temperature of 25°C. However, the temperature control should be improved.

Compared to pH measurements with a state of the art Isfet sensor (Endress & Hauser, Germany), the reproducibility of the measurements of the Isfet sensor seems to be a little bit better. Figure 3.3.6 shows the comparison of the data from a Isfet sensor compared with the data from the pH-HydroFIA analyser. The reproducibility of the pH-HydroFIA analyser is in the order of ±0.0015 pH units.

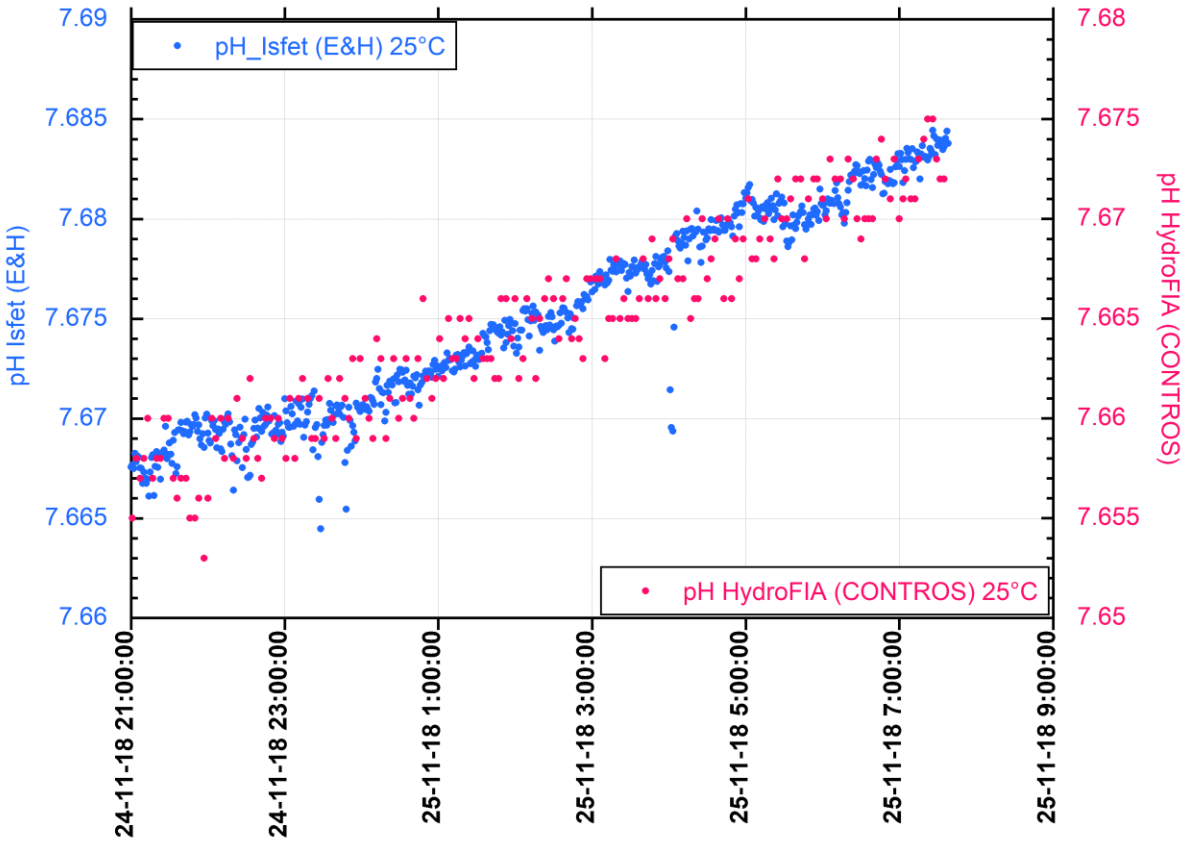


Figure 3.3.7. *Comparison of pH Measurement from Isfet (Endress & Hauser) vs. HydroFIA (CONTROS).*

3.3.2.3. Sunburst AFT-pH system

a) Physical and electrical installation including telemetry

AFT-pH (Sunburst Sensors LLC, U.S.) is a spectrophotometric reagent based pH analyser designed for flowthrough applications. When connecting to water flow, it should be noted that while the flowchamber itself has been pressure tested for 12 psi (0.8bar), the solenoid pump within AFT cannot work against pressures higher than 1 psi (0.07 bar). For that reason the recommended flow rate is from 2 to 4 L/min. The manufacturer recommends to drain the unit in open sink. In addition, the normal setup includes that the dye is purged back to the main flow, thus possibly influencing the downstream measurement. The solutions for the latter problem have been elaborated in recent models (and could be easily custom made for older ones), by applying an additional port for purging the measured sample with dye along with outflowing water, thus providing possible contamination of follow-up measurements.

The measuring principle is similar to Contros pH system, using m-Cresol Purple (mCP), described above. pH measurements are related to in situ temperatures, as the optical cuvette and tubing for reactions are located inside the flowchamber immersed in water.

The instrument supply voltage range is 10-13 V and it needs to stay plugged in for the entire measurement. Connection to PC is required at the start of the measurement, but not during the deployment.

For the reliable measurement it is important to minimize the effect of external temperature sources to both the device and inlet flow. The inlet tube may be insulated in order to minimize the warming of the sample.

b) Calibration procedures (user, manufacturer)
The original AFT-pH sensor is designed for pH range 7-9 at salinities 25-40 and it is practically calibration free. Manufacturer gives the accuracy of instrument as +/- 0.003 pH units, precision as <0.001 pH units and long term drift as <0.001 pH units over 6 months. The AFT-pH sensors used by SYKE have been validated to extended salinity range 4-8 for use in the Baltic Sea.
Purified mCP is available through manufacturer. One reagent bag lasts for 15 000 measurements. If the sampling frequency is set to 15 min, the reagent bag last for approx. 200 days. The minimum sampling frequency is 4 minutes. The user is expected to check the calibration occasionally using certified reference materials such as CO2 CRMs.

c) Maintenance and checks
AFT-pH flowchamber need to be cleaned along the other components of the flow-through system. Daily automated rinsing with Triton-X (0.1%) has been applied by SYKE, combined with occasional thorough cleaning by hand and using solvents and acids as needed. The instrument needs to be flushed after each deployment, including draining any remaining water from the flow chamber, manual cleaning of the flow chamber and flushing the fluidics thoroughly by ultrapure water.

d) Data handling and QC/QA routines
Data may be viewed online if the AFT-pH is connected to computer, using SAMI_Client software. A salinity correction is required and is done during offline data processing.
The AFT-pH used by SYKE is validated for low salinities and the standard software cannot calculate the correct pH. For this purpose, the raw data logged by the instrument need to be downloaded and aligned with salinity information (e.g., from thermosalinograph). A Matlab routine, provided by manufacturer, is then used to calculate the correct pH. QC/QA routines need to be developed.

3.3.3. Total Alkalinity Sensors

3.3.3.1 Contros HydroFIA TA system

a) Physical and electrical installation including telemetry
The CONTROS HydroFIA®TA is a flow-through analyser for AT, which uses wet chemistry for AT determination (Assmann et al. 2011). A known amount of seawater sample is acidified using dilute hydrochloric acid (HCl), after which the sample is degassed in an open-cell titration. The change in pH is measured using an indicator dye (bromocresol green (BCG), Breland and Byrne (1993)) and VIS absorption spectrometry. Temperature of sample is kept constant at 25°C during titration, and external input of salinity is necessary for precise determination of AT. The instrument has a manufacturer specified accuracy of ± 25 umol/kg (successfully tested in the lab), and precision of ± 5 µmol/kg (specified by manufacturer). In the laboratory, the precision varied up to ± 20 µmol/kg.

The instrument needs a power source: 13.4 A power supply, 100-240 V AC for 15V DC. It can be operated directly, via a touch screen, or remotely using RS-232 data interface (live data, Baud rate 115200, data bits 8, parity, none) or Ethernet (batch download). After considerable improvements, and software updates in 2016-2018, the DHSP and FTP servers can be used for communication to the instrument.

The instrument was deployed as a flow-through AT analyser in October 2016, on the Cuxhaven stationary FerryBox. In 2017-2018, it was also operated on two moving platforms, the Hafnia Seaways, and the Lysbris Seaways, externally controlled via the FerryBox software.

b) Calibration procedures (user, manufacturer)
Calibration of the HydroFIA®TA is done using an external certified reference material (CRM, Dickson et al., 2003; Dickson et al., 2007). Calibration is required anytime new reagents are installed. Sometimes glitches with the Hamamatsu spectrometer interferes with proper calibration of the instrument. That is why it is recommended to use a separate well characterized AT reference sample to test for instrument stability.

During deployment, it is recommended that check samples are collected for CT and AT measurements, as an external check of the carbonate system measurements. An experiment was done in spring 2016, when the HydroFIA®TA was still in the laboratory. Water samples in duplicates (not preserved) were collected aboard the MV Hafnia via an autosampler, and CT (on an AIRICA DIC analyser, Kiel, Germany) and AT (on the HydroFIA®TA) were measured in the lab. CO2SYS (Pierrot et al., 2012) was used to calculate pH and pCO_2, and the calculated values were compared to the measured pH and pCO_2 values (Table 1). The measured and calculated values compared well (pCO_2, y = 0.86x + 22.64, R^2 = 0.96 and pH, = 0.808x + 1.609, R^2 = 0.95). However, the fact that the samples were only processed when the ferry arrived at port (4-8 hours after collection) may have resulted in some measurement artifacts due to biological activity or gas exchange. While It is better to fix the samples with $HgCl_2$ immediately after collection, a recent publication demonstrated that unfiltered water samples collected via an autosampler (refrigerated in the dark), fixed within 10 hours of collection, and measured in the laboratory, can be successfully used to check the HydroFIA TA measurements (Voynova et al., 2018).

Table 3.3.1. Salinity, temperature, CT, AT, pH (calculated and measured with two different techniques), and pCO2 (calculated and measured) from samples collected on MV Hafnia in spring 2016.

Bottle	salinity	temp	CT	HydroFIA®TA	pH	pH	pH	pCO_2	pCO_2
number		°C	µmol/kg	µmol/kg	Calc	Meas (Clark)	Meas (ISFET)	Calc	Meas
4	34.689	6.799	2128.64	2288.3	8.045	8.124	8.105	393	357
7	34.438	6.869	2127.79	2334.9	8.151	8.227	8.193	305	289
10	34.196	6.662	2118.42	2334.4	8.176	8.272	8.234	285	282
13	33.635	6.238	2117.14	2350.3	8.223	8.275	8.240	254	229

c) Maintenance and checks
It is necessary to filter the water samples before measuring AT with the HydroFIA®TA. This requires an in-line filtration (e.g., a crossflow filter), or the measurement of whole water discrete samples, in which particles have been allowed to settle. It is advised to clean the system with dilute HCl regularly (about every couple to a few months, depending on frequency of use).
Currently data issues have been observed related to the Hamamatsu spectrometer: faulty spectra may affect the calibration, which in turn will produce faulty AT levels.

d) Data handling and QC/QA routines
The HydroFIA®TA software flags AT measurements out of range, as well as issues with the spectra. However, it is important to review the raw files for such flags, to properly evaluate each calibration. It is recommended that a reference sample (CRM, and/or an additional seawater reference) is regularly used to check the performance of the instrument during deployment.

A break of more than 4 hours between measurements could lead to instrument drift (Voynova et al., 2018), a problem that has not yet been resolved by the manufacturer. Typically, the drift is observed by higher than expected AT measurements, which decrease exponentially as the instrument measures continuously the same reference water sample. Eventually, the AT measurements reach a plateau around the expected value. This drift is a significant problem for deployments aboard moving vessels, which usually remain at ports for anywhere between a few hours to a few days. Therefore, it is important to test the stability of the AT measurements on moving vessels and apply suitable filters to remove questionable data. For long-term measurements, regular measurements of CRMs during deployment are recommended, as the instrument tends to show a small linear drift after several hundred measurements probably caused by fouling in the cuvette. This is possible with a newer version of the instrument with a second inlet for CRM measurements, which allows correcting the drifts after deployment. Furthermore regular flushing the instrument with acid minimizes this fouling effects.

3.3.4. Summary and conclusions

A variety of carbonate system sensors that measure pCO_2, pH, and total alkalinity are currently in use by the JERICO-NEXT. Most of them are designed to be operated on a moving vessel, in most cases a FerryBox. One of the pCO_2 sensor can be deployed in situ on a fixed platform such as a buoy. In terms of best practices, a rather common task is maintenance and repair – a visual check, data quality check, and cleaning/replacing consumables whether they be reagents, membranes, or plumbing. A best practice that is more specific to carbonate system sensors (pH and AT sensors) is the recommended use of a CRM or Tris seawater buffer. The most readily available CRMs and Tris solutions are from Andrew Dickson's lab at Scripps Institution of Oceanography, although efforts are currently underway to produce similar reference materials in Europe for easier shipping and logistics). For pCO_2 sensors, it is recommended to use zero CO2 reference gases for baseline correction, as well as reference CO_2 gases at various environmentally-relevant concentrations (either procured or produced and verified) for performing a span calibration.

Data QC/QA is clearly an important facet of sensor operation and use. A general best practice that is common to all sensor measurements is the transfer and safe storage of data to institutional or other facilities. Also quite general is the identification of data compromised by sensor malfunction or artifacts – the procedures are quite sensor-specific a can range from sampling waters that are low in salinity (an issue unique to coastal measurements), issues with flow rates delivering adequate volumes of water to sensors (especially CO_2 sensors), fouling by detritus or biological growth, and high particle load for sensors using optical techniques. No unified system of identifying and flagging suspicious data is currently in place for the sensors described above. Most QC/QA operations also recommend the use of CO2SYS – a program that calculates carbonate system parameters using a series of carbonate system constants – to check and overdetermine variables to check for precision of measurements. While this is a reasonable and widely used approach, one must be cautious when using CO2SYS with coastal samples in which high organic matter load (both dissolved and particulate) can significantly affect the accuracy of program output.

Several other reports and best practice documents (especially for laboratory-based measurement of carbonate system variables – Dickson et al., 2007) are also available from: the Alliance for Coastal Technologies, the International Ocean Carbon Coordination Project, the Integrated Carbon Observation System, and the FP7 Fixed-Point Open Ocean Observatories. The use of carbonate system sensors in ocean observing is progressing quickly and providing the much-needed support of future observations related to oceanic C cycling and the pervasive impact of anthropogenic CO_2 emissions.

3.3.5. References

References:

Assmann, S., C. Frank, A. Körtzinger. 2011. Spectrophotometric high-precision seawater pH determination for use in underway measuring systems. Ocean Science 7: 597-607, doi:10.5194/os-7-597-2011

Breland, J.A. and R.H. Byrne. 1993. Spectrophotometric procedures for determination of sea water alkalinity using bromocresol green. Deep Sea Research I, 40(3): 629-641

DelValls, T. A., Dickson, A. G., "The pH of buffers based on 2-amino-2-hydroxymethil-1,3-propanediol('tris') in synthetic seawater", Deep-Sea Res. I, 1998, 45:1541-1554

Dickson, A.G., J.D. Afghan, G.C. Anderson. 2003. Reference materials for oceanic CO2 analysis: a method for the certification of total alkalinity. Marine Chemistry, 80(2-3): 185-197, http://dx.doi.org/10.1016/S0304-4203(02)00133-0

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication 3, 191 pp.

Fietzek, P., B. Fiedler, T. Steinhoff, A. Körtzinger. 2014. In situ quality assessment of a novel underwater pCO_2 sensor based on membrane equilibration and NDIR spectrometry. J. Atmos. Oceanic Technol., https://doi.org/10.1175/JTECH-D-13-00083.1

Honkanen, M., Tuovinen, J.-P., Laurila, T., Mäkelä, T., Hatakka, J., Kielosto, S., and Laakso, L. 2018. Measuring turbulent CO2 fluxes with a closed-path gas analyzer in a marine environment, Atmos. Meas. Tech., 11, 5335-5350, https://doi.org/10.5194/amt-11-5335-2018.

Liu, X., Patsavas, M.C., Byrne, R.H. 2011. Purification and Characterization of meta-Cresol Purple for Spectrophotometric Seawater pH Measurements: Environ. Sci. Technol., 2011, 45 (11), pp 4862–4868. doi: 10.1021/es200665d

Müller, J. D., F. Bastkowski, B. Sander, S. Seitz, D. R. Turner, A. G. Dickson and G. Rehder. 2018. Metrology for pH measurements in brackish waters - Part 1: Extending electrochemical pH_T measurements of TRIS buffers to salinities 5-20. Front. Mar. Sci. 5: 176, doi: 10.3389/fmars.2018.00176

Müller, J. D. and G. Rehder. 2018. Metrology of pH measurements in brackish waters - Part 2: Experimental characterization of purified meta-Cresol purple for spectrophotometric pHT measurements. Front. Mar. Sci. 5: 177, doi: 10.3389/fmars.2018.00177

Nemzer, B.V. & Dickson, A. 2005. The stability and reproducibility of Tris buffers in synthetic seawater. Marine Chemistry. 96. 237-242. doi.org/10.1016/j.marchem.2005.01.004

Orr J. C., Epitalon J-M, Dickson A. G., Gattuso J-P. 2018. Routine uncertainty propagation for the marine carbon dioxide system. Marine Chemistry, Vol 207, pp. 84-107 ISSN 0304-4203, https://doi.org/10.1016/j.marchem.2018.10.006.

Pierrot, D., Lewis, E. and Wallace, D. W. R.. MS Excel Program Developed for CO2 System calculations. 51 2006, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center v 2.1,2012

Voynova, Y.G., W. Petersen, M. Gehrung, S. Aßmann, A.L. King. 2018. Limnology and Oceanography, 9999: 1-15, https://doi.org/10.1002/lno.11103

Takahashi, T., J. Olafsson, J. G. Goddard, D. W. Chipman and S. C. Sutherland. 1993. Seasonal-Variation of CO2 and Nutrients in the High-Latitude Surface Oceans - a Comparative-Study. Global Biogeochemical Cycles 7(4): 843-878

Yao, W. and R.H. Byrne. 1998. Simplified seawater alkalinity analysis: Use of linear array spectrometers. Deep-Sea Research I, 45: 1383-1392.

Zhang, H., and Byrne, R. H. 1996. Spectrophotometric pH measurements of surface seawater at in-situ conditions: absorbance and protonation behavior of thymol blue. Mar. Chem. 1996, 52, 17–25.

3.4. COASTAL PROFILING SYSTEMS

3.4.1. Introduction

Coastal profiling systems can help to integrate indispensable information on water column characteristics in coastal areas. The most mature system technology, and used within the JERICO network, are coastal profiling ARVOR floats. Despite the maturity of this technology, Coastal profiling ARVOR floats are still used sparingly. The coastal ARVOR floats are specifically adapted from conventional open sea profilers to be operational in the coastal area. The objective of the coastal float is to perform profiles between "stationary" phases. The "stationary" phases are obtained when the float is landed on the seafloor. The scientific payload embedded on coastal profiling float are up to now quite limited due to the small size of such floats. Besides the proven ARGO based technology, fixed (at the surface or bottom) profilers are also under development in JERICO-NEXT and worldwide. The majority of the systems are research prototypes, quite rough to operate and very different from one to the other. It explains why "Best Practices" for coastal profilers are not very documented and remain difficult to establish. Nevertheless, this chapter is focused on the "Best Practices" for this type of systems and contains information as general guidelines for the design and operation of coastal profiling systems.
Furthermore, another emerging technology for coastal profiling used within JERICO and JERICO NEXT is the one based on the use of fishing vessels as vessels of opportunity. This application has recently been extended in various world regions (e.g. recent application in EU waters: http://berringdatacollective.com) but the most mature examples are the Italian FOOS and the French RECOPESCA (see JERICO D2.2, Falco et al. 2007, 2011, Leblond et al. 2008, Patti et al. 2016). For these reasons, the best practices suggested at present time for this application are those developed within these contexts (see JERICO D2.2 and Martinelli et al 2016).
In general based on recent studies (e.g. Aydoğdu et al 2016, JERICON NEXT JRAP#6) it is easy to reckon the worth of fishing vessels as volunteer observing ships (and of VOOs in general) in the field of operational oceanography. Data series obtained through this kind of approach show a frequency in space and time that cannot be reached by research vessels unless huge expenditures in terms of shiptime and operators.

3.4.2. Coastal profiler systems

Apart from the ARVOR Argo floats there are two main types of coastal profilers:

- Buoy profilers deployed on the sea surface and that automatically raise and lower oceanographic instruments at pre-programmed intervals using an onboard winch. A typical buoy profiler consists of a buoyant housing that contains the winch, wire drum, batteries, and communications equipment. For example, the EOL buoy that raises and lowers a SBE CTD from the surface down to a predefined depth.

- Bottom mounted profilers also use an automatic winch but, unlike buoy profilers the winch is anchored on the bottom and is used to raise oceanographic instrument embedded in a buoyant housing. Bottom mounted profilers under development in the JERICO network are the IFREMER Mastodon and the IMR YOYO system. The Mastodon system is a passive water column profiler (chains of static sensors) that provide profiles made up measurements from discrete depths above the seafloor while the YOYO is an active automated system that continuously profile a specified portion of the water column above the seafloor.

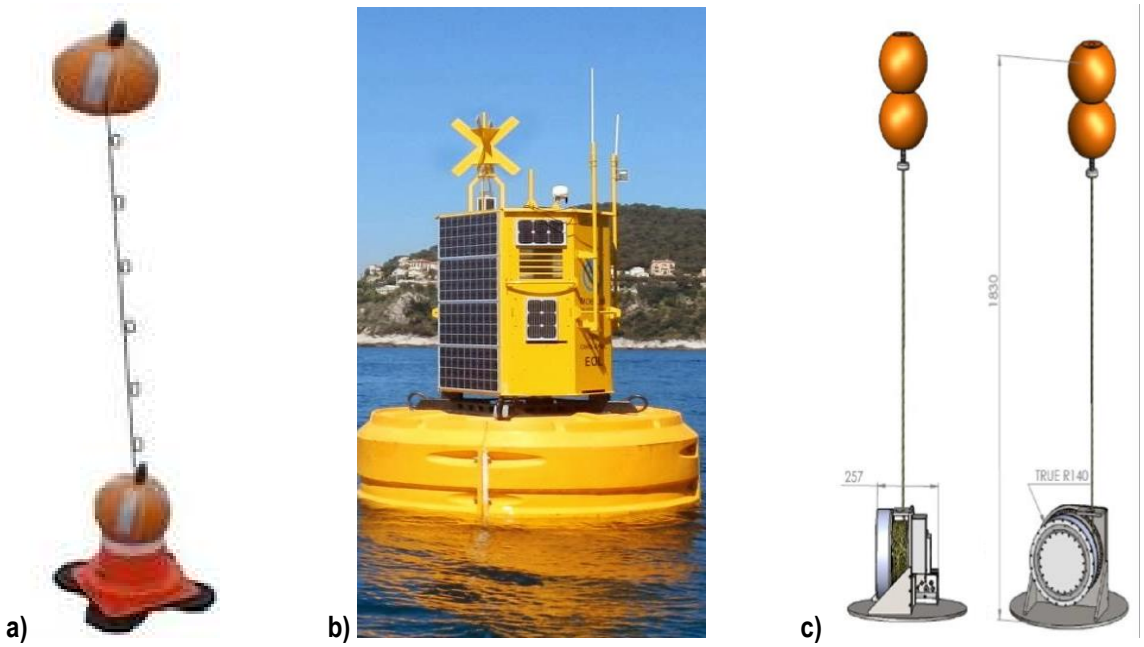


Figure 3.4.1. Example of coastal profilers: (a) Mastodon 2D, (b) EOL buoy, (c) YOYO

Specifications and technologies used for the operation for coastal profilers are summarized in the table below:

System	Deployment	Power	Fouling	Telemetry	Maintenance
Buoy Profiler	Deployed at the surface but exposed to winds, waves, floating objects and marine traffic.	The buoy is usually equipped with solar panels, wind generators and can operate for a long period	The surface immersed components are exposed to high fouling pressure.	The surface components can provide bidirectional telemetry as fixed stations.	All the modules of the system apart the mooring line are accessible for field maintenance.

Bottom mounted Profiler	Deployed at the sea bottom and secured from surface exposure but vulnerable to fishing and anchoring activities.	The winch equipment consumes large amounts of energy. To be deployed for a long period requires cable to shore connection.	The oceanographic payload can be parked between profiles below the photic zone so the fouling effect is minimized.	The real time communication with the system is limited. If there is no cable up to the surface or the shore the data are obtained only if the system comes to the sea surface between profiles or after recovery.	The system needs to be recovered totally for maintenance.
ARVOR Argo Coastal Profilers	Deployed at sea from a (small) boat. Vulnerable to fishing activities. Risk of beaching according to currents.	Limited embedded power and very constraint by floatability of the float that need to be very well adjusted.	The oceanographic payload can be parked between profiles below the photic zone so the fouling effect is minimized. And, the deployment duration is often limited to several weeks.	Data transfer and M2M communication can be achieved between profiles when the float is on surface. Satellite communication is used.	No maintenance during deployment. The float can be and refitted if recovered.

3.4.3. Installation design for coastal profilers

The surface buoy systems are following the general specifications for the design of the oceanographic mooring lines described in several BP documents (ESONET, JERICO, FIXO3). But there are significant differences because the profiling payload must be able to move vertically attached on the mooring line or parallel to it. The solution adopted in the EOL buoy is an advanced anchoring system with three mooring lines distributed in a radius relative to the buoy itself, that allows the profiling equipment to move freely in a dedicated line just below the buoy hull. The main concern for the design of the bottom mounted profiler is the protection from fishing activities (trawlers) and anchoring maneuvers from ships. The underwater cable operators and industry reports that (source: ultra-map.org, telegeography.com).

- Around 70% of all cable faults are caused by fishing and anchoring activities (man-made) and around 12% are caused by natural hazards (current abrasion or earthquakes).
- Over a 3rd of all cable damage is caused by fishing activity. Trawling and fixed nets including stow nets cause damage.

- Damage from anchors - 25% of subsea cable damage arises from ship anchors. Large anchors of fishing vessels and merchant vessels cause cable damage. Anchoring outside designated areas causes damage. Dragging anchors in error during passage causes damage.

These facts stand for the coastal underwater oceanographic equipment so the deployment site should be chosen considering the ship traffic and the fishing activities of the area. A technical solution to protect the equipment are the Trawl-Resistant Bottom Mounts developed by some companies and are mainly for the protection of bottom placed ADCP, waves and current meters. Other issues one should consider when using a bottom mounted winch driven profiler are the environmental conditions at the deployment site such as current speed, softness of bottom sediment, bottom slope, and typical weather. Important engineering issues include the buoyancy of the carrier and payload and the type of the electric power supply.

Winches and equipment

There is a variety of commercial underwater winches that are available in the market developed mainly for the oil and gas industry. In order to choose one to be operated as coastal profiler for oceanography there are considerations to be considered:

- dimensions and weights of measurement sensors to be hosted,
- power requirements,
- profiling speeds,
- maintenance/deployment requirements,
- necessity to absorb the swell,
- technological simplicity,

The other parts of the equipment such as underwater connectors, mooring components, wires and ropes should follow the already existing documentation of Best Practices for the design and the deployment of marine monitor stations especially coastal seafloor observatories.

Buoyancy

The most common solution to raise the equipment from the sea bottom are the flotation spheres that are used widely in the oceanography. This type of equipment is used occasionally as housings too where the controller and the data loggers of the scientific payload can be hosted. More sophisticated approaches are the variable buoyancy mechanisms where the driving unit is using Archimedes' law, without changing its weight they modify their volume leading to increased or decreased buoyancy. This technique is widely used in controlling Argo floats, gliders and can be used in coastal profiling systems.

Telemetry

The development of cell-phone, satellite and RF telemetry has made real-time data flow available to the oceanographic community. The scientific observatories can be located in remote areas, the cost of laying cable becomes prohibitive and the mobile network coverage can be limited. Thus, there is a need to seek for other data transmission solutions. Bidirectional telemetry is desirable since sensor configurations and platform operation modes can be remotely modified. Telemetry systems should be tested as much as possible; prior to the installation or deployment in laboratory, on the way to the deployment site, while a pre-deployment realistic configuration and testing is desirable. The available technologies used for data transmission are summarized in the table below.

System	Pros	Cons
Underwater cable	Robust and reliable. Nearly unlimited power and bandwidth.	Very expensive (purchase and installation). Environmental permission required. Maintenance. Vulnerable to human activities.
Satellite	Robust and reliable. Global coverage. Limited bandwidth.	Can be expensive if too large bandwidth needed.
Radio frequency	Free of charge. Quite large bandwidth. Reasonable power.	Requires line of sight or repeaters.
Cell phone telemetry	Low power.	Geographically restricted.
WiMAX	Huge bandwidth.	Power requirements. Necessity to deploy specific communication infrastructure.

Power

The surface oceanographic systems are powered using power generator systems such as solar panels and wind turbines. Normally, photovoltaic panels power rechargeable secondary batteries that directly drive the buoy systems. However, photovoltaic panels may be damaged at sea or during buoy servicing, so a small number of primary batteries should be used as a reserve supply until a service visit can be carried out. Power requirement depends largely on the number and types of sensors used, the sampling frequency and the consumption due to frequency of data transmission. The bottom mounted systems, unless there are cabled, are more demanding in power storage. Battery purchase is a large investment and the proper size and type of battery is critical in the operation of submerged infrastructures. There are a large number of batteries available such as AGM, Supercapacitor and lithium-ion and the operator should consider not only the load requirements and the longevity but the safety regulations for handling power storage units.

Sensor maintenance

Components that would benefit from maintenance, apart from the components of the system are also the sensors. Each sensor has dedicated maintenance procedures, usually described in the manufacturer's manual or already available in “Best Practices”. Sensor maintenance often requires specific spare parts and equipment and can be time consuming. Consequently, it can only be carried out in laboratory conditions. That’s why a good practice is to have a second set of serviced and calibrated sensors, so the ones operating in the field can be easily and swiftly replaced. The sensors of the coastal profilers have a specific operation scheme compared to the ones at fixed depths. Indeed, the profiler payload will be exposed very frequently to a large pressure variation during ascending or descending. Then, this is why special attention should be given in the sensor housing and connectors. When possible, a pressure test should be performed in order to make sure that the sensor response is not affected.

3.4.4. Best Practices for the JERICO-NEXT Mastodon-2D coastal profiler

Deployment

The Mastodon-2D coastal profiler do not have a buoy on the surface but on the sub-surface for several reasons: resistance to the heavy swells frequent in coastal areas and to limit at its best looting risk.
The target depth for buoyancy is 10 m below the surface. This implies that the deployment must be done with the help of a depth sounder to know the exact ocean bottom depth. This will prevent the buoy from being neither too close to the surface (visibility and risk of breakage by passing ships) nor too deep (risk

of insufficient reserve of rope in the frame). If possible, a visual inspection should be conducted a few tens of minutes after dropping the line in order to localize the line and ensure that the buoyancy depth is good (e.g. in the Mediterranean Sea when the sea is calm).
The buoy and the line with the probes chain must be deployed at sea before the frame. Its falling speed is about 1m/s, which will make a fall of 1'40" for a depth of 100m. In the event of strong currents, a drift will therefore be possible and should be anticipated as far as possible.
The programming of the probes and of the recovery date should preferably be done on shore and doubly controlled.

Measuring frequency

In order to keep the low-cost spirit of the Mastodon-2D coastal profiler, the probes and the temperature sensor are packaged in plastic pouch filled with oil. This increases the thermal inertia of the system to about 1-2'. Therefore, a maximum sampling frequency of 1' is recommended.

Metrology

The uncertainty of temperature measurement is linear and does not depend on temperature. It is recommended to check the bias before and after deployment. We have not noted any significant evolution of this bias in the range of precision sought (0.1°C).
The pressure sensor does not have absolute calibration. It is recommended when processing the data to remove the measured offset before or after deployment (the same). An absolute accuracy better than 5 cm can thus be obtained.

Fishing protection

The mooring is very vulnerable to trawling (experience shows that a small mark on the surface does not reduce the risk). As far as possible, areas with low traffic, either for administrative reasons or in the vicinity of areas avoided by fishermen (e. g. wrecks) should be selected. Also, fishermen association may be warned of the presence of the lines with their characteristics.

Recovery

It is recommended to paint the buoy with a bright colour (avoid white, which creates confusion with birds or waves) so that it can be easily spotted on the surface. Once the buoy is spotted, grab it, wind the rope on a winch and pull it up again, taking two precautions: detach the probes when they come out of the water and, above all, drive the ship to put it in a vertical position from the mooring so that the frame can detach itself from the bottom vertically. Avoid pulling the rope if it is tilted (especially because of the ship's drift). The resistance of the rope is limited to 300 kg.

Best Practices for sensors installed on fishing vessels

Martinelli et al 2016 suggests that sensors to be used for application on fishing gears need to be tested in order to determine the accuracy of the datasets produced and then allow the comparison to traditional oceanographic ones (e.g. CTD transects). Martinelli et al 2016 proposed specific procedures to follow for the testing phase and the definition of the offsets that were then as well used in the EU FP 7 NEXOS project (Martinelli et al 2017; Memè et al. 2017). Furthermore, before the use, optimal operational conditions should be defined and then followed for each kind of sensor (depending on the parameters measured, the manufacturing of the sensor and the technical characteristics). Various companies are producing sensors properly thought for this application (eg. NKE) or adaptable to this, but the most appropriate to be used are those allowing to continuously and automatically record oceanographic parameters and send them directly to a remote server.

References

Aydoğdu A., Pinardi N., Pistoia J., Martinelli M., Belardinelli A., Sparnocchia S. 2016. Assimilation Experiments for the Fishery Observing System in the Adriatic Sea. Journal of Marine Systems. 162:126-136.

Falco P, Belardinelli A, Martinelli M, Cingolani N, Arneri E, Russo A, Santojanni A. 2011. A new observing system for the collection of fishery and oceanographic data. In: Marine Research At Cnr, Fishery and Sea Resources. ISSN 2239-5172 Volume DTA/06-2011: 1889–1895.

Falco P, Belardinelli A, Santojanni A, Cingolani N, Russo A, Arneri E. 2007. An observing system for the collection of fishery and oceanographic data. Ocean Sci. 3:189–203.

Leblond E, Berthou P, Laurans M, Woerther P, Quemener L. 2008. The Recopesca project: a new example of participative approach to collect in-situ environmental and fisheries data using fisheries vessels of opportunity. ICES Annual Science Conference, 22–26 September 2008, Halifax, Canada,ICES CM 2008/R:16.

Martinelli M., Belardinelli A., Penna P., Croci C., Domenichetti F., Campanelli A., Betti M., Paschini E., Sparnocchia S., Guicciardi S. Fishing vessel and EAF.4/EAF.6 sensors in Østerhus et al. Work Package 8 – Deliverable 8.2 – Validation Report - FP7 Ocean 2013 NeXOS project "Next generation Low-Cost Multifunctional Web Enabled Ocean Sensor Systems Empowering Marine, Maritime and Fisheries Management". 2017.

Martinelli M., Guicciardi S., Penna P., Belardinelli A., Croci C., Domenichetti F., Santojanni A., Sparnocchia S. Evaluation of the oceanographic measurement accuracy of different commercial sensors to be used on fishing gears. Ocean Engineering 111: 22–33, 2016.

Memè, S.; Delory, E.; Felgines, M.; Pearlman, J.; Pearlman, F.; del Rio, J.; Martinez, E.; Masmitja, I.; Gille, J.; Rolin, J.F.; et al. NeXOS—Next generation, cost-effective, compact, multifunctional web enabled ocean sensor systems. In Proceedings of the OCEANS 2017—Anchorage, Anchorage, AK, USA, 18–21 September 2017; pp. 1–10.

Patti B., Martinelli M., Aronica S., Belardinelli A., Penna P., Bonanno A., Basilone G., Fontana I., Giacalone G., Gallì N. G., Sorgente R., Angileri I.V.M., Croci C., Domenichetti F., Bonura D., Santojanni A., Sparnocchia S., D'Adamo R., Marini M., Fiorentino F., Mazzola S. 2016. The Fishery and Oceanography Observing System (FOOS): a tool for oceanography and fisheries science. Journal of Operational Oceanography 9(1): s99-s118.

4. Conclusions

The best practice of technologies, methodologies and procedures is a vital step in ensuring efficiency and optimal returns from any kind of distributed, heterogeneous, multifaceted, coastal observing infrastructure operating on a transnational level such as the JERICO network. This is because such harmonization in best practice leads to an intelligent use of resources across the network, adds to the consistency of its services and products, and standardized procedures.

A characterization and evaluation of best practices for the different sensors and systems as described above has been performed in this report and is supposed to faciliate consensus on methods in the utilization and deployment of sensors *used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling*

5. ANNEX

5.1. Apendix Carbonate System Chapter

CONSIGLIO NAZIONALE DELLE RICERCHE –CNR
ISTITUTO DI SCIENZE MARINE - ISMAR
sede di TRIESTE
PALOMA station QA/QC

HydroC/CO2 II – Contros/Kongsberg

Pre – deployment checklist

Laboratory control, date: (dd/mm/yyyy) Operator
Expected deployment date: (dd/mm/yyyy) Operator

New membrane Y N
Antifouling wrapping collect a picture

System check and programming:
Is data/time correct? Y N
Is the memory empty? Y….N
Are all the configurations and sample collecting interval correct? Y N
Take note or a screenshot
Is the pump turned on “operating”? Y N
Double-check the settings of the timetable Y N

Delivered to the diver, date: (dd/mm/yyyy) Operator

CONSIGLIO NAZIONALE DELLE RICERCHE –CNR
ISTITUTO DI SCIENZE MARINE - ISMAR
sede di TRIESTE
PALOMA station QA/QC

HydroC/CO2 II – Contros/Kongsberg
Post – recovery checklist

Recovery from the site: date (dd/mm/yyyy) Operator
Picture form in situ recovery?: Y N
Delivered to the laboratory: date (dd/mm/yyyy) Operator
Visual inspection: date (dd/mm/yyyy) Operator
Presence of damages, if yes what:
Fouling amount:
Fouling type:
Collect a picture
Disconnect and remove pump, remove antifouling protections
Remove the sensor head and take a picture of the membrane and of the inner sensor head
Membrane status: hard fouling Y N; soft fouling Y N; extended bacterial biofilm Y N; scraches or damages Y N.
Clean the membrane with running tap-water and MQ water, dry gently with a Kleenex
The membrane should be changed Y N
Notes:

Data download and system check: date (dd/mm/yyyy)
PC used:
Display status and configuration check, screenshot
Data download
Data backup
Notes:

Cleaning stage 2: date (dd/mm/yyyy)
Remove antifouling wrapping and clean the external part of the instrument with tap water, with the help of small brushes; the membrane must not be touched.

Let the instrument dry, functioning outside water for at least 1h.
Notes:

5.2. Apendix Coastal Profiler Specifications

Coastal profiler	EOL profiling buoy	MASTODON-2D profiling system	ARVOR C float
Energy	solar panel	alkaline cell	alkaline cells (300 profiles for 200 meters depth)
Measuring frequency	twice a day	every 1 second (faster rate)	one profile every 24 hours
Measuring period during ascent and descent	depending of the CTD model (SBE19+).	none	no measurement during descent, one measurement every meter during ascent.
Metrology and pressure compensation for specific sensors	data are processed with SBE data processing procedure. No P compensation.	calibration on sensors should be performed before and after deployment. P compensation: It is recommended when processing the data to remove the measured offset before or after deployment (same value). An absolute accuracy better than 5 cm can thus be obtained.	metrology and pressure compensation is performed by manufacturers before deployment (Seabird CTD).
Anti-fouling	electro-chlorination chamber system	not needed due to the limited deployment duration and due to the parameters involved (temperature and pressure).	no protection (except well-known Seabird CTD biofouling protection based on TBT rings and pumping)
Data transfer (telemetry)	by GSM to the lab	After recovery up to a contact less link (radio link).	Iridium SBD, bidirectional
Mooring lines	3 moorings for anchorage	One line that supports the temperature chain and that is attached to the bottom structure and to a subsurface floating element.	none
Fishing protection	none	none	none
Recovery for untethered systems	some sensors are standalone in surface (fixed mode) and decoupled from profiling sensors	from a boat… MASTODON-2D comes to surface at a pre-programmed date and must be localized manually.	recovery is possible if float is maintained on surface by remotely changing it's programme, then the GPS information is received every 5mn (faster rate).

5.3. Annex White Paper draft

From Coastal to Open Sea observations

Interoperability of Technologies and Best Practices: in situ applications to phytoplankton fluorescence measurements

Workshop held in Brest the 5-6 dec Dec. 2018

White paper preparation (ideal 5 pages, very max 10p)

White paper draft

Authors: Jukka Seppälä, Seppo Kaitala, Jani Ruohola (SYKE)

Paula Oset Garcia (VLIZ)

Ingrid Puillat, Laurent Delauney (IFREMER)

Et al, TBD

1. Introduction to challenges in Chlorophyll *a* fluorescence of living phytoplankton cells

This White Paper provides background and basic requirements for future developments towards more consistent phytoplankton data, based on Chla fluorescence. The White Paper propose next steps which the user community need to take, with support of sensor manufacturers, getting into the agreement how the primary calibration of fluorometers should be done and reported, and in setting guidelines for data QA/QC and conversions. Such actions could improve drastically the consistency of fluorescence records, obtained by different users, and increase the reliability of phytoplankton data for various uses.

Phytoplankton play a key role in aquatic ecosystem functioning transferring energy to higher trophic levels. In global biogeochemical cycles, phytoplankton drives the marine cycles of key elements like carbon, nitrogen, phosphorus and silica. Based on current knowledge, approximately half of the global photosynthesis, i.e. carbon fixation, takes place in aquatic environments, and is primarily carried out by phytoplankton (Falkowski and Raven, 2007). However, despite very high production, the actual biomass of phytoplankton is low, compared to land plants, as the turnover rate of phytoplankton is very high. The biomass of phytoplankton, though one of key components in global biogeochemical cycles, is hard to measure accurately and Chlorophyll *a* fluorescence techniques are often used to get such estimates.

Phytoplankton are a diverse group of aquatic photosynthetic microorganisms. Different groups, like diatoms, dinoflagellates, coccolithophores and cyanobacteria, have different life strategies and functional traits. Phytoplankton abundance and community composition is governed by interplay of abiotic and biotic factors. Temperature, light, salinity, mixing and nutrient availability are the major factors affecting the productivity of phytoplankton cells and modifying the phytoplankton diversity as species and groups differ in their optima and tolerance for environmental factors. On top of those, the interactions with other organisms - like viruses, bacteria, zooplankton, and other phytoplankton species – affect the lifecycle of cells.

Assessment of phytoplankton biomass, productivity and diversity is the key in understanding how marine ecosystems function. As the phytoplankton growth and decline processes are very dynamic and often interlinked to physical and chemical factors, the observations should be carried out at relevant scales of phytoplankton events. The traditional monitoring, with few annual samples at each monitoring site, combined with laboratory analyses, provides valuable information for long-term trends, used e.g. studying the eutrophication status of water bodies. However, more frequent observations are needed to understand the cause-effect relationships in more detail, to get estimates on the spatiotemporal variability and on the scales of events, to provide early warning of harmful algae blooms, and to provide supporting information for marine ecosystem models and for interpretation of satellite ocean color data.

Direct observation and counting of phytoplankton cells, or measuring their production is challenging, though various technologies based on flowcytometry and imaging have recently emerged. Such technologies, with huge potential, are still rather rarely used as they are costly and require skilled users. Most often the phytoplankton biomass is inferred from pigment based measurements, and Chlorophyll *a* concentration is commonly used proxy for phytoplankton biomass.

Common to all phytoplankton, Chlorophyll *a* (Chl*a*) is the central pigment for photosynthesis. Concentration of Chl*a* is also the most widespread method to estimate phytoplankton biomass, though large variability in Chl*a* content of phytoplankton cell dry weight is well recognized. Analytical laboratory methods to quantify Chl*a* are reliable, but have high cost, require water sampling, and only limited number of observations can be obtained. Satellite ocean color can give trustworthy estimates of Chl*a* at the ocean-basin scale, but does not work always well in optically complex coastal waters. Further limitations of ocean color are due to cloudiness, adjacency of land areas and the fact that ocean color reflects only the surface of water column, while phytoplankton populations may form maxima at deeper layers as well.

The most widespread automated online methods for phytoplankton observations are based on measuring optical properties of water and sorting out the signals of phytoplankton pigments from the other optically active compounds. Fluorescence methods have proven especially sensitive to detect even low amounts of phytoplankton, and phytoplankton autofluorescence is relatively little overlapped by signal from other compounds, like humic matter.

Chl*a* is the major pigment showing autofluorescence in living phytoplankton cells. Other chlorophylls and carotenoids within photosynthetic machinery transfer the energy they have captured from sunlight towards Chl*a* and have no fluorescence of their own. Phycobilin pigments, especially abundant in cyanobacteria, show also autofluorescence, while their wavebands for fluorescence excitation and emission are easily distinguished from those of Chl*a* by selecting appropriate optics for sensors (Seppälä et al. 2007).

Detection of phytoplankton abundance using Chla fluorescence of living cells has been done for more than 5 decades already (Lorenzen 1966). Method is extensively used in research vessels, FerryBox systems, buoys, gliders, fixed platforms, cabled observatories and as simple hand-held devices. Various technical modifications are available for field studies, and most technologies rely on blue LEDs and detection of emitted red light using photodiode. Their prevalent use is due to relatively low price (even less than 2000€), small size (e.g. weight 100g and length 10 cm), low energy consumption (sometimes below 1W) and ease to interphase and use.

The limitations of the Chl*a* fluorescence method are well known and can be presented as five interdependent challenges, discussed further in forthcoming chapters:

- **Challenge 1:** There is no commonly agreed methodology for traceable primary calibration of Chl*a* field fluorometers
- **Challenge 2:** Due to differences in optical setup of Chl*a* field fluorometers (from different manufacturers) they are not giving directly comparable results
- **Challenge 3**: Conversion factor between Chl *a* concentration and fluorescence varies due to phytoplankton physiology & community structure and environmental conditions (e.g. light and nutrient availability). It is typically determined case-by-case using field validation samples, but the various details and steps in validation are seldom reported
- **Challenge 4:** There are no widely agreed Best Practices how to perform QA and QC of Chl*a* field fluorometer data, and especially how to report the primary calibration data, optical setup, and use of conversion factors as noted in Challenges 1-3
- **Challenge 5**: Chl*a* fluorescence data, stored in various databases, is not consistent and comparable, as a consequence of challenges 1-4

To conclude, Chl*a* is a widespread method to analyze phytoplankton abundance and to study scientific questions related to phytoplankton or to study the effects of anthropogenic pressures on aquatic ecology. Chl*a* records are used. e.g. as an indicator for Good Environmental Status in MSFD Descriptors and to assess the status of regional seas (e.g. by HELCOM, OSPAR). *In situ* methods, like Chl*a* fluorometry, provide large amounts of online data cost efficiently for various uses and at the relevant spatio-temporal scales of biological processes. Such data most often provides a proxy of true concentration, or measurements at relative scale, and therefore special attention is required when results from different sources are compared.

The compilation of coherent Chl*a* fluorescence multisource data, so far poorly comparable, will improve especially our ability to perform reliable ecosystem assessments and will largely support satellite Ocean Color and ecosystem modelling applications by providing consistent data on phytoplankton abundance.

2. Background

2.1. *In vivo* Chlorophyll *a* fluorescence – complex response from biological system

Analytical measurement of Chl*a* concentration using standard laboratory techniques is considered as reliable ground-truth for optical online measurements. The critical steps in the analytical procedure include representative sampling, storage of water samples, efficiency of filtration, efficiency of extraction, storage of samples and various instrument-specific issues in the used method (photometry, fluorometry, or chromatography). These challenges are thoroughly reviewed e.g. by Jeffrey et al 1997 and Roy et al 2011. As the laboratory analysis are laborious and not appropriate for continuous measurements, *in vivo* Chl*a* fluorescence is largely used to track phytoplankton abundance. These online Chl*a* fluorescence measurements are practically free from the drawbacks of analytical laboratory measurements described above, but they come with totally other types of challenges, shortly described below.

First, the Chl*a* fluorescence quantum yield (ratio of light absorbed to light emitted as fluorescence) is not constant for living cells. In the analytical laboratory method, when Chl*a* molecules are extracted from the cells using organic solvents, pigment molecules are isolated from each other and behave as any dissolved chromophore obeying Beer-Lambert law and showing constant fluorescence yield (for pure Chl*a*, quantum yield varies from 0.23 to 0.32, depending on the solvent; quantum yield is also affected by temperature). In contrast, for the living cells, Chl*a* fluorescence is much more complicated as pigments in the cells are not as free molecules but bound to various proteins. In the cells, pigments are found in functional packages and the absorbed energy is efficiently transferred from photosynthetic accessory pigments (other Chlorophylls, carotenoids and phycobilins) towards central pigments in the photosynthetic reaction centers. These reaction centers, where Chl*a* has a key role, are the sites for photochemical reactions, fueling primary production. Photochemical processes are very efficient, consuming large part of the energy absorbed by pigments and less energy is available for fluorescence, decreasing fluorescence yield. As photosynthesis is a dynamic process, affected by environmental factors, the share of energy used for photosynthetic reactions vary and this is mirrored in the amount of energy available for other pathways, like fluorescence. In addition, during times of excessive light, there are rapid dissipation pathways protecting photosynthetic machinery from excess energy (and follow-up oxidative stress and damage), quenching fluorescence even more. As a result, the quantum yield of in vivo Chl*a* fluorescence is variable and low, with approximate quantum yield reaching values in a range from 0.005 to 0.05.

Another complication, affecting the determination of simple conversion factors between Chl*a* concentration and *in vivo* fluorescence intensity is due to sites where Chl*a* locates within the photosynthetic machinery. This machinery consists of two functionally different units, photosystem II and photosystem I. Both of them contain Chl*a*, but with different optical properties (due to different protein complexes). In photosystem II (called also P680), there is overlap between absorption and fluorescence maxima (5-10 nm) and the excitation energy may travel back and forth in Chl*a* pigments located in the photosynthetic antenna and in reaction center and the probability for fluorescence reaction increases. In photosystem I

(P700), the absorption maxima for Chl*a* in reaction centers is around 700 nm, and once energy is received by these pigments, it can no more turn back to photosynthetic antenna and probability of fluorescence is low. Thus, *in vivo* Chl*a* fluorescence mainly (90-95%) arises from photosystem II, while Chl*a* located at photosystem I is not much showing up in fluorescence. The amount of fluorescently inactive Chl*a*, located in photosystem I, varies between taxonomic groups. Especially cyanobacteria has low amount of Chl*a* in photosystem II, roughly 10-20% of the total amount, and therefore their Chl*a*-specific fluorescence (i.e. Chl*a* fluorescence normalised to concentration) is very low. Thus, standard Chl*a* field fluorometers are virtually blind for cyanobacteria. Here it should be noted that during extraction of pigments, as it happens during analytical laboratory methods, the pigment-protein binds break down and thereafter all Chl*a* show equal optical properties and also Chl*a* of cyanobacteria becomes fluorescent. Thus, the two hurdles of fluorescence, presented above, are valid for living cells only.

2.2. Chlorophyll *a* fluorescence – relation to concentration

As a conclusion from the two facts described above – low and variable Chl*a* fluorescence quantum yield in living cells and variable amount of Chl*a* in fluorescing and non-fluorescing photosystems – it is clear that the fluorescence intensity measured from living samples will not directly estimate the amount of Chl*a* concentration.

Because of the measuring technology and geometry of light sensing, fluorometers are providing results primarily at relative units. It is not known what is the total amount of excited light provided to the sample or what is the amount of light the sample will fluoresce. Thus, field fluorometers do not provide results with real physical units (though often results are recalculated using some laboratory validation and represented as µg/L – see below). If the measuring geometry remains stable and measurements are done with optically dilute samples (obeying Beer-Lambert law), the measured fluorescence can be calibrated with known amount of standards. Such is easily demonstrated e.g. for pure Chl*a* dissolved in organic solvent and the sensor may be used to analyse concentrations of unknown samples using the calibration curve. This will not work ideally, however, for Chl*a* in living cells as their concentration-fluorescence relationship (i.e. quantum yield) is not stable. As presented above, the pure dissolved Chl*a* does not serve as calibrant for Chl*a* in living cells as their quantum yield differ largely.

A common way to circumvent the problem described above is to use so-called field validation. While phytoplankton Chl*a* fluorescence is recorded in field conditions, simultaneously sampling is done for laboratory analyses. From the water samples, the Chl*a* is extracted and analysed according to standard laboratory methods, and then these results are compared with fluorescence records. Aim is to create a conversion factor between two measurements, typically using linear regression model between measurements. If one is lucky – or, in reality, if measurements come from relatively homogeneous phytoplankton populations (physiology and community composition) and if the biomass range in the samples is relatively large – the two measurements may correlate very well. But if the relationship is not linear, as often is the case, it does not actually mean that one of the measuring technologies has failed, but rather indicates that data comes from heterogeneous populations or the concentration range is

narrow (or there are relatively large changes in background fluorescence signal, to be discussed in forthcoming sections). Thus, the relationship between *in vivo* Chl*a* fluorescence and concentration is not random, but mostly reflects the phytoplankton physiology and community composition.

2.3. Primary calibration of fluorometers – improving the concentration estimation

Primary calibration of fluorometers stands for measuring a fluorometer response in a traceable way. This is a very basic metrology operation in any analytical measurement, but despite 50 year history in measuring *in vivo* Chl*a* fluorescence, the actual protocols have not been agreed for this specific method. For this reason, the raw fluorescence data obtained with different Chl*a* fluorometers – even within same brand of sensors – is not consistent and traceable; e.g. if one sensor shows value x fluorescence units for a given water sample, the other sensor may show something very different for the same sample. Both sensors may work well, and have been properly serviced and calibrated, but the actual method of calibration may differ as we have not defined the traceability of calibration.

This rise a question, why such situation is taking place, is it important, and if it is, why traceable calibration methods have not yet been implemented? Answer is not simple, but there are at least two obvious interlinked explanations.

First, Chl*a* fluorescence is considered as a semiquantitative tool only, to study relative phytoplankton distribution. Relationship between fluorescence and real concentration has been analysed with field validation and real fluorescence values have not been of interest as they are not seen as important. Thus, field validation has been seen as "*the method*" to convert relative fluorescence readings to something meaningful, with low or even unreliable information content of their own. But there is a serious oversight in this logic. Fluorescence is very sensitive and precise measurement, and its relationship to Chl*a* concentration is not random (though we are not always able to describe why they do not match). More, this relationship is following patterns, like day-night cycles and shifts due to community composition, or it is influenced by environmental gradients in light and nutrient concentrations affecting photochemical reactions within cells. Such patterns may be predictable, thus indicating that we may learn from previous measurements of Chl*a*-specific fluorescence. But this learning (and eventually predicting) is only possible, if we know that our instruments primary fluorescence response is consistent, i.e. similar from year-to-year, place-to-place, or sensor-to-sensor. This is obtained only if we have our sensors calibrated in always similar manner, and thus calibration needs to be traceable. So far jointly agreed best practices or protocols for such traceable calibration have not been established by manufacturers and users and the raw fluorescence values are not comparable.

Second explanation is related to sensor technology itself. Field Chl*a* fluorometers are manufactured by several companies worldwide and there is no standardization of wavebands or measuring geometry. The optical windows of excitation and emission vary between sensors, and therefore they integrate the optical signal from different portions from the sample fluorescence excitation-emission matrix. Between different phytoplankton groups, and thus between communities, the spectral shape of this excitation-emission matrix varies, especially regarding to excitation spectra. This is due to group-specific accessory pigments

harvesting light for photosynthesis. As a result, if we calibrate two brands of fluorometers (with different optical setup) to show equal fluorescence intensities for phytoplankton community “A”, for the community “B” they already show different readings as the sample fluorescence properties have been changed. Thus, the matchup between fluorometer detection wavebands and sample signal wavebands vary between samples. Standardization of the instrument optics may decrease the discrepancies between sensors, but this may not be the appropriate way forward. Sensor technology progress continuously, light sources being one branch of development, and the requirement for standardising the optical setup is not compliant with such emerging improvements. Resulting from the differences in the optical setup of sensors, their traceable calibration will not eventually lead to overall consistency of fluorescence readings, though improving the current situation.

2.4. Spectral fluorometry – calibrating spectral responses

Besides measuring the bulk fluorescence with single LED instruments, spectral sensors have been developed to study abundance of various, differently pigmented, phytoplankton groups. Evolutionary distinct phytoplankton groups show qualitative differences in their pigmentation. In addition to Chl*a* found in all groups, these pigment groups may be briefly listed as Chlorophyll *b* containing green algae, Chlorophyll *c* containing diatoms and dinoflagellates, phycobilin containing cyanobacteria (in different variants) and Chlorophyll *c* and phycobilin containing cryptomonads. The differences in the pigmentation are reflected in their spectral properties, and these major groups may be distinguished based on their fluorescence excitation spectra. Spectral fluorometry of living phytoplankton populations use such differences as a starting point, and often the aim is to decompose the observed spectra of natural community into components of single groups, thereby estimating their relative contribution in the community.

Not going into details of various ways of analysing spectral fluorescence data, the implicit assumption of all techniques is that the spectral response of the sensor is stable. To exemplify, in the methods using the spectral fingerprints of different phytoplankton groups, both fingerprint and sample spectra need to be measured using the same instrument settings, otherwise the basic assumptions of the spectral analysis fail. As fluorescence is a relative response, all shifts in the sensor optics are reflected in the spectral output measured. When analysing the spectra, each spectrum used in the analyses need to be measured consistently and thus all factors affecting the spectral response (excitation light, filters, lenses, cyvette, detection) need stay constant. Eventually this means that such spectra are highly instrument specific, as the optical setups vary between sensors. As an outcome, spectra measured with two instrument brands are not implicitly interchangeable and comparable. As well, if the optical setup of individual sensor is altered, the spectral response is shifted. To overcome this principal difficulty, there need to be a traceable calibration of sensor spectra output, which can be used to compensate spectral shifts caused by sensor itself.

The implications of the heterogeneity of spectral responses between sensors are manifold. First, spectral analyses can be done only including the spectra measured with the same spectral responses. Spectra measured with various sensors cannot be pooled unless their

interdependency is demonstrated. Second, so called spectral libraries of fingerprints, used in some analyses, cannot be shared between users. It may even happen that if instrument maintenance affects the spectral response, new fingerprint spectra need to be recorded, which may be a tedious job including cultivation of some algae species in controlled conditions. Third, the spectral results reported cannot be pooled, thus their inclusion into joint databases will not actually increase the knowledge but rather creates confusion.

The obvious solution, as for single waveband fluorometers, is that each sensor is characterised by traceable calibration factor. In the case of spectral fluorometry, this mean that each LED is independently calibrated. Using such calibration factors, one can start pooling larger datasets from various sensors (with same LED bands) and aggregate data for joint data analysis.

3. Proposed actions towards consistent phytoplankton fluorescence data

In the previous chapter the challenges in Chl*a* fluorescence measurements are described, as related to primary calibration of sensors. The obvious reasons why the question is still open are because simply there is no solution to solve the whole issue and even the partial solutions are not necessarily trivial. The need to elaborate at least a partial solution is evident, databases are filling up with fluorescence records and to analyse the biological state of the global ocean and transnational areas, we need to have these records comparable.

3.1. Primary standard for Chl*a*

The obvious solution to the challenges described above is to find a commonly agreed primary standard for sensor calibration. Though this sounds simple, it may not be, and below we elaborate the requirements for such a solution.

Primary requirement for calibration standard is the traceability. To fulfill this requirement, the standard needs to be chemically stable and have consistent optical properties. To be applicable for fluorescence calibration, the standard need to have constant fluorescence yield. As such this already rules out using algae cultures as a standard, as their fluorescence properties are largely determined by the growth conditions and physiological state of cells (even affected by time of the day). Algae cultures may be used to understand the magnitude of fluorescence to concentration relationship, but their use in instrument primary calibration will not result in consistent outcome. Along the traceability, the standard should obviously have optical properties closely matching the sensor, high absorption of the excitation light followed by fluorescence emission peak well overlapped with detected waveband. Violations of this may bring so called inner filter effects, where secondary absorption (not leading to fluorescence) or re-absorption of fluoresced light cause non-linearity in the fluorescence response. To be practical to use, the standard should show minimal temperature and pH dependency of fluorescence yield.

The selected standard, apparently a chemical substance, needs to dissolve in a solvent which is compatible with the available field fluorometers. For the safe use of the method, the standard needs to be nontoxic and noncorrosive. To be available for larger communities, the standard needs to be easily available, relatively inexpensive and simple to use. If possible,

to allow its use in the field conditions, the standard should be relatively stable for longer periods (e.g. weeks).

One obvious suggestion as a primary standard has been to use Chl*a* in organic solvent, as it complies with the most requirements stated above. The are two drawbacks however. First, some field fluorometers, or their plastic parts, are not compatible with organic solvents, and then Chl*a* dissolved in acetone or ethanol cannot be used. The second drawback is the use of different LEDs for Chla excitation in field fluorometers. LEDs with peak at 470 nm are still quite commonly used, and there is not much absorption by Chl*a* in organic solvent in that region, making the response of fluorometer very weak.

Finding out, and agreeing upon, the perfect calibrant for field Chl*a* fluorometers is not trivial. One solution may be to select a traceable standard and measure its output with a calibrated laboratory spectrofluorometer, by normalising the response to excitation light intensity and detector sensitivity. For example, quinine sulphate has been proposed as such material. Then, a second fluorescing compound may be measured with the same instrument and represented as fluorescence equivalents of the traceable standard (e.g. quinine sulphate units). Field sensors may then be calibrated using this second fluorescing compound (as quinine sulphate does not show fluorescence signal at wavebands of Chla fluorometers), and taking into account the spectral characteristics of the sensor and above mentioned measurements, a traceable calibration will be yielded in units of Quinine sulphate equivalents. Such a calibration is not entirely removing the sensor specific differences, but provide a method for more direct comparison of sensor outputs. The downside of the method is that it requires a dedicated high quality lab spectrofluorometer for calibration, which is not available in normal laboratories. As well, determination of spectral characteristics of sensors is not trivial task. Though potentially effective, this solution requires still more detailed documentation and tests.

For spectral fluorescence applications finding a suitable primary standard is even more complicated than for single waveband sensors. The output of fluorescence should be reasonable for all excitation wavebands, allowing consistent calibration of all wavebands. Most likely solution is to use several dyes, maybe each of them calibrated as shown above for quinine sulphate equivalents.

As a conclusion from this section, the solution for primary calibration is not trivial. As it certainly takes time to agree on the common solution, it is recommended that various laboratories have their intermediate solutions. Such a solution may be the use of pure Chl*a* in organic solvent, using fluorescein in well described solutions (especially pH) or other dye with traceable fluorescence properties. It is recommended to share such practices with larger communities, to get comparable fluorescence results. One possibility is also to use reference sensors, calibrated in traceable manner, and use them to transfer calibration to a series of other sensors (of the same model), using e.g. algae cultures.

3.2. Steps in quality control of Chla field fluorometers

Besides primary calibration, steps in the quality assessment and quality control need to be harmonised to get reliable and comparable data from various oceanographic missions recording Chla fluorescence missions. It is obvious that oceanographic data needs to be accompanied by metadata, but for measurements like Chl*a* fluorescence, with relative units, metadata is absolute necessity to understand sensor calibration and possible data transformations.

Quality assurance (QA) of Chl*a* field fluorometer measurements inevitably start by planning of installation and selection of materials and location adjacent of sensor, like defining if measurement is done in flowthrough mode, and how does the measuring chamber affect the readings. Sensor calibration, as discussed in length above, need to be decided and documented. QA include also planning, which adjacent variables may be required when data is quality controlled (QC) and interpreted. These may include measurements of the flow rate and temperature among the most common factors providing information if the installation is functioning as it has been planned. Additional environmental variables may be recorded - like light, turbidity, phycocyanin and coloured dissolved organic matter - depending on how the Chl*a* fluorescence data is used and analysed, and which are the most obvious variables explaining the natural variability in relationship between Chl*a* concentration and fluorescence.

QA/QC actions may thus include steps when adjacent variables are i) checked to understand if the Chl*a* fluorescence records are usable or not (e.g. is there water flow in the flowthrough system), ii) inspected for correlation to see if Chl*a* fluorescence values are realistic (e.g. do the high readings show any correlation with turbidity), or iii) used in transforming Chla fluorescence records to Chla concentration estimates (e.g. using multiple regression by including turbidity or phycocyanin data in the analysis). The basic principles of Chla fluorescence QA/QC are given in Jaccard et al (2018). As a specific note, however, relating to the spike test analysing whether the high/low readings are outliers, it must be taken into account that distribution of biological components in aquatic environment is typically heteroschedastic and tests should be done using log-transformed data. In addition, the spike detection is rather dependent how the original data is filtered (averaged or not) and what is the time/space resolution of data.

Important component of QA/QC is sensor verification before, during and after the deployment. This cannot be always carried out using methods for primary calibration (and which are still under scrutiny). Some Chl*a* fluorometers can be provided with “solid secondary standards” or “calibration kits”, simply utilizing a piece of fluorescent plastic/glass in a holder. This holder can be attached directly to the sensor, in a unified way, producing a constant fluorescence reading. Such systems can be used to check performance of individual sensors, track their changes. But they do not provide direct means for calibration, not even for transferring calibration from one sensor to another. This is because the fluorescence reading from these solid systems is largely affected by the actual distance between sensor optical components and surface of fluorescing materials, then even slight modifications in sensor optics will influence the reading. Thus, if sensors are properly calibrated using fluorescence

dyes in liquids, they do not necessarily show similar readings with such “solid secondary standards”. But such systems are very useful for quick field inspection of sensors sensor.

Yet another important component of the interpretation of Chla fluorescence readings is the background fluorescence and its variability. Although coloured dissolved organic matter (CDOM) does not show clear fluorescence at Chl*a* emission wavebands, in CDOM rich waters background may be notable and should be taken into account when interpreting the signal as phytoplankton biomass. It is recommended that the magnitude of CDOM background is measured for each study area, and measuring system, using 0.2µm filtered seawater samples. Similarly, highly turbid waters may influence the readings. Along with these concerns, it need to be also noted that high quality purified water (e.g. bidistilled or Milli-Q) must be used in sensor blanking.

Metadata associated to the Chla fluorescence readings should include fluorometer model, calibration information and inspections carried out. Additional information on background fluorescence and blanks may be useful. In the case fluorescence values are transformed to present values of Chl*a* concentrations, typically using field validation samples with known Chl*a* concentration, it would be beneficial to know the model used.

4. Harmonization of the optical biological data flow

As discussed above, the inter-comparability between field Chl*a* fluorometers still remains a challenge (and same applies to several other optical sensors). Significant research will be needed to establish commonly agreed best practices for fluorescence measurements, and this will then be followed by studies of conversion factors between Chl*a* fluorescence and concentrations. Already now, adequate data management strategies are necessary to safeguard data traceability and ensure that the data currently being collected can be re-distributed and used in the future with confidence. In practice, this means the development, adoption and implementation by the fluorescence community of (meta)data standards that contain detailed information on the sensor characteristics together with pre- and post-measurement procedures.

Significant efforts have already been made to explore existing sensor metadata and data standards that can be applicable to the ocean sciences. The EU Ocean of Tomorrow calls initiated several projects with a focus on innovative marine sensing technologies in which (meta)data standards were investigated and implemented. These standards included the internationally recognized Open Geospatial Consortium (OGC) Sensor Web Enablement (SWE) specifications. SWE standards are now widely used in the European marine data infrastructures, such as EMODnet Physics and SeaDataNet.

SWE are a set of OGC specifications to standardise sensor data and metadata flow so that these can be discoverable, accessible and useable via the web. Two of these SWE specifications are the XML-based Sensor Model Language (SensorML) and Observations & Measurements (O&M). SensorML is used to encode the characteristics of the instrumentation and its deployment whereas O&M describes the observations and the procedures to obtain

such observations. Both SensorML and O&M profiles define a set of required/recommended properties to be provided in a pre-defined schema or syntax. The property types and values can be encoded as “soft typing”. Although this makes these SWE standards very flexible and applicable in a wide range of fields, it may also difficult interoperability within a specific sensor community. To overcome this issue, OGC recommends to define community specific semantics (e.g. online ontologies) that resolve to a persistent URL that can be used as a value to the definition attribute.

In the context of the JERICO-Next project, task 5.3. (Platform registration and metadata management system) has dealt with the adaptation of the current SWE standards to the JERICO-Next observatory network, proposing SWE application profiles for the three different types of observatories considered: fixed station, HF Radar and flow cytometry. Several partners from other marine related projects set up a collaboration group, referred to as the SWE Marine Profiles group or team, to inventorize existing terms and identify and develop new required terms to enrich SensorML for marine applications. These terms and their grouping collections are published in the NERC Vocabulary Server, which is widely used and supported by other European data infrastructures such as EMODnet and SeaDataNet. Within JERICO-Next, the Flow Cytometry community has been actively working to develop required vocabularies. Development of these accurate, community specific vocabularies is crucial not only for sensor data, but also to describe sample-based observations. Lessons learnt from all these experiences should be taken into account by the fluorescence community to develop and implement these data standards and lay the groundwork for future harmonization of the optical data flow into the European infrastructures.

References

Falkowski PG, Raven JA (2007) Aquatic Photosynthesis: Second Edition, Princeton University Press

Jeffrey SW, Mantoura RFC, Wright SW (1997) Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods, UNESCO, Paris, 661 p

Lorenzen CJ (1966) A method for continuous measurement of in vivo chlorophyll concentration. Deep-Sea Res 13: 223-227

Roy S, Llewellyn CA, Skarstad Egeland E, Johnsen G (2011) Phytoplankton Pigments. Characterization, Chemotaxonomy and Applications in Oceanography. Cambridge University Press, 845 p.

Seppälä J, Ylöstalo P, Kaitala S, Hällfors S, Raateoja M, Maunula P (2007) Ship-of-opportunity based phycocyanin fluorescence monitoring of the filamentous cyanobacteria bloom dynamics in the Baltic Sea. Estuarine, Coastal and Shelf Science, 73, 489-500