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

Deliverable title	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.
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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 harmonization 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. This is because such harmonization leads to an intelligent use of resources across the network, adds to the consistency of its services and products, and helps to provide uniformed access modes and interfaces to users.

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. Task 2.4 of JERICO-NEXT deals with the harmonization of these sensors and their underlying technologies within the network through four different subtasks, which are described below. The work performed within this Task will provide insights and useful information to active EU projects dealing with the development of new marine sensors such as NEXOS, EnviGuard, MariaBox and Sea-at-ship. Two workshops have been planned to support the activities of the different subtasks during the project. The first workshop (MS10) aimed to review the state of the-art on 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. The outputs are gathered in the present Deliverable 2.2. Afterwards, a second step will allow defining best practices in relation to the different sensor systems, which will be then reported in the follow-up deliverable D2.5.

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. To evaluate the different existing sensor systems within the JERICO network and, more generally, in the European context, two respective workshops have been organized, gathering experts on the following sensors:

Sensors for nutrients
Nutrients are important environmental parameters in coastal waters. Most of the commercially available in-situ nutrient analysers use wet-chemical techniques based on standard laboratory methods for making measurements (e.g. the NAS-2, the NAS-3, the Ecolab, and the SubChemPak systems), though sensors based on an optical spectrophotometric method are available for nitrate (e.g.,- the ISUS and the SUNA sensors), however with less sensitivity. 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. Subtask 2.4.1 aims to review and evaluate the effective capabilities of the nutrient sensors utilized within the JERICO network, describe the current modes of their deployment, and deal with data quality concerns.

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.). Subtask 2.4.2 aims to review and evaluate the capabilities of the optical sensors employed for biology-related measurements within the JERICO network, describe the current modes of their deployment, and deal with data quality concerns.

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. The pCO2 sensors are based either on the equilibration of a carrier gas phase with a seawater sample and subsequent determination of the CO2 that diffuses through by means of NDIR spectrometry (e.g., the CO2-Pro and the Hydro-C systems), on reagent-based colorimetry (e.g., the SAMI-CO2 sensor) or on species-specific solid-state detectors. The pH sensors are based on potentiometric measurements, either employing pH glass electrodes or ISFET technology (e.g., the SeaFET sensor), or are wet-chemical analysers (e.g., the SAMI-pH sensor). pCO2 and/ or pH sensors are being employed by a number of JERICO partners on their installations and/or measuring platforms, in different settings and under diverse conditions. Subtask 2.4.3 aims to review and evaluate the effective capabilities of these sensors, describe the current modes of their deployment, and deal with data quality concerns. Links with other EU initiatives like, for example, the Integrated Carbon Observation System (ICOS) which currently employs an observing network for studying the carbon system - mainly in the terrestrial and atmospheric compartments for now, but with plans for extending coverage to include the marine system in the future will be carefully explored and coordinated.

Sensor systems for coastal profiling
Profiling sensor systems can help to integrate indispensable information on water column characteristics in coastal areas. Two such profiling technologies are being utilized within the JERICO network: instrumented coastal profiling floats and fishing-vessel based, net-deployed sensor systems. Both technologies are relatively new, and are still undergoing development. Subtask 2.4.4 aims to review and evaluate the effective capabilities

of these technologies, describe the ways they are currently being used, and deal with data quality concerns.

The goal for all different sensors and systems 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 manufacturers and industry.

This deliverable informs on the outcomes and results of the workshops for nutrients (MS10-1, Brest, October 10th to 14th 2016) and biology related optical properties, variables of the marine carbonate system, and for coastal profiling (MS10-2, Paris, December 13th to 14th 2016). Current status and experiences with the respective sensors are presented in subchapters and structured into:

- Description of the sensor
- Current modes of deployment
- Reliability of the sensor and technological differences
- Sensor calibration
- Data issues (including biofouling)
- Links with other WPs and other EU initiatives
- Summary
- References

3. Main report

3.1. NUTRIENT SENSORS

A workshop was held on 10th October 2016 in Brest for JERICO-Next partners to discuss the nutrient sensors they deploy on platforms in the marine environment. A questionnaire was circulated prior to the workshop to collect information from partners regarding the following aspects:

1. Which sensors partners use and the parameters they measure
2. Precision, accuracy and concentration range obtained in the field
3. Which platforms they are deployed on, reliability, ease of use
4. Frequency of servicing and calibration
5. Why are they deployed (e.g. monitoring/research)
6. Problems experienced

Information obtained from the questionnaire and during and after the workshop has been compiled in the following report.

JERICO-Next partners deploy nutrient sensors on a variety of platforms in the marine environment including buoys, seabed landers, towers, piers, FerryBoxes and CTD packages. There is experience in using chemical and optical sensors, some commercially available and some developed by laboratories but not commercially available. The nature of the platform including size, availability of power and access for servicing influences the choice of nutrient sensor deployed. Table 3.1.1 summarises the nutrient sensors deployed by JERICO-Next partners.

Sensor type	Cefas	SMHI	SHOM	HZG	Ifremer
submersible chemical	✓	✓			✓
portable chemical				✓	
submersible optical			✓		

Table 3.1.1. Summary of the nutrient sensor types deployed by JERICO-NEXT partners.

3.1.1. Submersible chemical nutrient analysers

3.1.1.1. Description of the sensors

Mills and Fones (2012) and Nightimgale et al. (2015) reviewed the operation of in situ flow injection analysers. These analysers use existing laboratory analytical methods in a miniaturised, submersible instrument. The in situ flow injection nutrient analysers on the market use chemical methods, which form a coloured reaction product is detected spectroscopically. 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).
Within JERICO-Next, three different in situ flow injection nutrient analysers are currently in operation. Cefas has deployed the NAS-3X nitrate sensor (EnviroTech LLC) on the SmartBuoy platform (Mills et al. 2005) and its predecessor, the NAS-2E since 2001. It uses a well-established chemistry (Griess assay) for the determination of dissolved oxidised nitrogen (i.e. nitrite plus nitrate). Nitrate is reduced to nitrite with a copperized cadmium column and reacted with reagents to form a highly colored azo dye, which is measured colorimetrically. Reagents and the on board nitrate standard are placed in bags within the analyser housing. The analyser uses scripts to control the reaction chemistry using a rotary valve and syringe pump to mix reagents. It uses a

colorimeter to measure the absorbance of the coloured reaction product. The user can programme the analyser to analyse samples at the frequency desired and to start sampling at the time required. The analyser runs an on board nitrate standard regularly during the programme to calibrate the deployment data. EnviroTech LLC is no longer trading but NAS-3X analysers were also designed to run different chemistries for the determination of dissolved ammonia, phosphate and silicate. The analysers are submersible up to 100m depth with a configuration allowing deployment up to 250m depth. SMHI operate an EnviroTech LLC MicroLab for the determination of dissolved phosphate although it is not currently deployed in the marine environment. The principle of operation is the same as the NAS-3X.

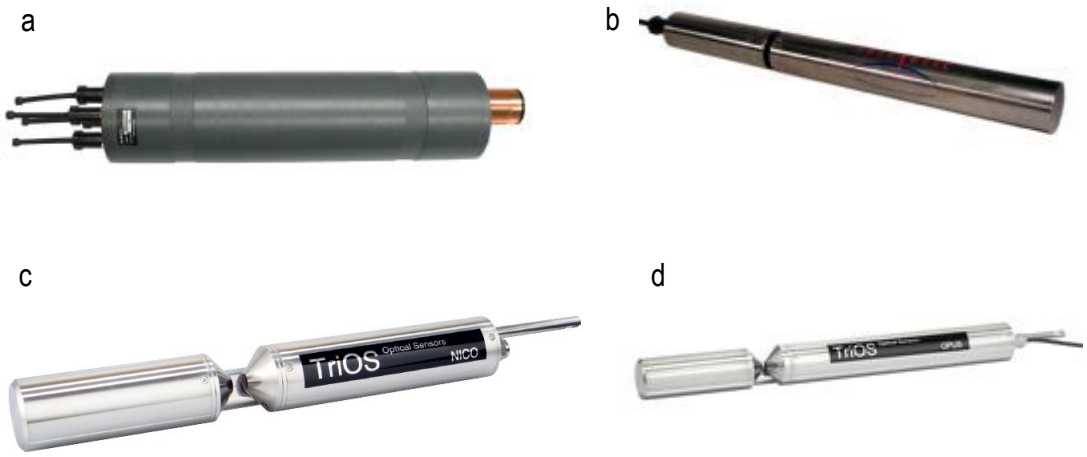
Ifremer has developed its own in situ chemical miniaturized analyser, CHEMINI: CHEmical MINIaturized analyser (Vuillemin et al 2009). It is a single parameter in situ chemical flow injection analyser with colorimetric or fluorimetric detection. A coastal version (submersible at 10m with specific structure) has been elaborated for the analysis of nutrients (nitrate, nitrite, ammonium, silicate, phosphate). Ifremer has also developed a submersible version (up to 6000m depth) of the CHEMINI for the analysis of iron and sulfides.
The deep sea version of the CHEMINI is made of two modules: a hydraulic module and a detection module. The hydraulic module is composed of a pressurized titanium tank filled with dielectric oil. It contains a mechanically polymethylmetacrylate engraved manifold, two in-house designed peristaltic pumps and eight solenoid valves for the circulation of various fluids. The detection container is a titanium tank kept under slight depression (-0.2bars). It contains the electronic cards for driving the various actuators recording the LED detection system and storing data. The quartz flow cell is located outside the tank and is connected to the LED detection system via optical fibers and special waterproof titanium optical pass through. The coastal version is made of only one container including all the actuators, flow cell and electronic cards.

Analytical methods
The first prototype produced by Ifremer was a fluorimetric analyser for the measurement of ammonia concentrations on the basis of the method finalized by Aminot et al. (2001). The sample is mixed with a mixture of orthophtaldialdehyde and ethanol in the presence of sulfite (reductor). The reagent mixture is maintained at a temperature of 40°C during 4 minutes. The excitation is made by LED at 370 nm and the detection is performed at 425nm. The intensity of the fluorescence is proportional to the concentration of ammonia present in the sample.
The nitrate method applied on the CHEMINI is based on the Griess assay except for the reduction of nitrate into nitrite which is performed using photoderivation. In fact the copperized cadmium column is replaced by UV lamp. As described in Motomizu et al., (1995), the sample is mixed up with a chemical activator: DTPA (diethylene triamine pentaacetic acid). Sodium hydroxide or a buffer is used to adjust the pH. The mixture is then UV- irradiated during a specific amount of time using a low pressure mercury lamp. Under light illumination, nitrates are transformed into nitrite. Then the sample is mixed up with classical reagents SAN (sulfanilamide) and NED (N-naphtyl-ethylenediamine). The intensity of the colour of the azo dye compound formed, proportional to the amount of nitrite and transformed nitrite in the sample, is then recorded.
The silicate Method is based on the traditional laboratory method. The ammonium molybdate is reacting with the silicates in the presence of sulphuric acid to form a silicomolybdic complex. After reduction using the ascorbic acid, the intensity of the blue absorbance peak is recorded at 480 nm, which intensity is directly linked to the silicate concentration (Treguer and Le Corre, 1975).
The determination is based on the reaction of phosphate with acidic molybdate to phosphomolybdate, which builds non-fluorescent ion pairs with rhodamine 6G. The remaining rhodamine fluorescence is detected at 550nm with an excitation at 470 nm (Frank et al. 2006). The intensity of the fluorescence is proportional to the concentration of phosphate present in the sample.
Reactive iron concentrations (Fe(II) + Fe(III)) are obtained using the ferrozine method (Stookey 1970). The ferrozine molecule reacts with Fe(II) to form a magenta complex in the presence of a buffered ascorbic acid solution. The colorimetric detection consists in dual-wavelength signal treatment by recording the reference signal (810 nm) and the measurement signal (562 nm). The dual wavelength measurement using LEDs was performed to limit any matrix effects such as turbidity variations.

For all the CHEMINIs, in situ calibration is performed in order to avoid any sensitivity drift. Reagents and the in situ standard are placed in bags located close to the analyser. For the deep sea version, temperature and pressure tests are performed in hyperbaric tanks before any deployment at sea. The analysers can be set serially on a sampling line and controlled by a single software. (Laes-Huon et al. 2016)

A range of inorganic compounds in seawater absorb light at wavelengths in the ultraviolet range. These compounds have different overlappting absorption spectra which can each be characterised. In situ uv absorption sensors have been developed for the detection of nitrate and are available commercially. These are solid state sensors which do not require chemicals. Multispectral instruments are available which use a stable light source and a stable, high resolution spectrometer to make multi-wavelength measurements of samples. See Johnson and Coletti (2002) for details of the technique. Examples of such instruments include the Satlantic ISUS (http://www.satlantic.com/isus) and the Satlantic SUNA (http://www.satlantic.com/suna) and the TriOS OPUS UV (http://www.trios.de/en/products/sensors/opus-uv.html) which can also be used to measure a range of dissolved organic compounds. In addition, TriOS make the NICO, a single wavelength sensor for the determination of nitrate (http://www.trios.de/en/products/sensors/nico.html). The absorbance spectrum of a sample is compared with reference spectra held on the sensor and algorithms on the sensor are used to compute the nitrate concentration.

All these instruments can be programmed to run autonomously and are fully submersible. In addition they can be integrated into other logging systems and some can be run in flow-through mode for bench top applications. Manufacturers specifications have been summarised in Table 3.1.2. An interface is used to programme the sensor prior to deployment.



(a) Satlantic ISUS (b) Satlantic SUNA (c) TriOS NICO (d) TriOS OPUS UV

	Satlantic ISUS	Satlantic SUNA	TriOS OPUS UV	TriOS NICO
technique	Multispectral	Multispectral	Multispectral	Single wavelength
parameters	NO_3	NO_3	NO_2, NO_3, range of dissolved organics	NO_3
Range	up to 2000 µM	up to 4000 µM	0 – 11 mM (NO_2) 0 – 7 mM (NO_3) Variable for organics	0 – 100 mg L^{-1}
Accuracy	± 2 µM or 10%, whichever is larger	± 2 µM	± (5 % + 0.1)	± (5 % + 0.1)
precision	± 0.5 µM	2.4 µM	± 0.4 µM	N/A
Dimensions (mm)	580 - 608 (l) 114 (diameter)	588 (l) 63 (diameter)	470 (l) 48 (diameter)	370 (l) 48 (diameter)
Weight (kg)	5.26	3.9 to 4.8	3 (SS) 2 (titanium)	3 (SS) 2 (titanium)
Power	6.5W 6-18 V	8-18 VDC	< 8W 12 – 24 V	< 8W 12 – 24 V
Max pressure	1000m	500m	30bar	30bar
antifouling	Optional copper screen	Optional wiper and copper screen	Nano coating on optical window	Nano coating on optical window

Table 3.1.2. Summary of the manufacturer specifications for the Satlantic ISUS, Satlantic SUNA, TriOS OPUS UV and TriOS NICO

The design of the sensors (e.g. weight, power requirements, housing design and depth rating) is suitable for autonomous operation on fixed and floating platforms, profiling systems and towed vehicles. The ISUS technology has been reconfigured and integrated into an ocean profiling float for long term deployment (Johnson et al 2013).

In seawater main uncertainties are caused by the close proximity and the magnitude of the bromide peak (as one of the main components of seawater salt) close to the nitrate signal in the UV. There are further potential interfering species which may alter the optical properties of a sample. These may include suspended sediments which may attenuate the light signal. In addition coloured dissolved organic matter (CDOM) have variable absorbance in the uv range. Some of the sensors have algorithms designed to compensate for such interferences but as the nature of CDOM varies with location, users must verify the in situ performance of the sensors against independent measurements of nitrate. The sensors are calibrated by the manufacturer but some also allow for user calibration. Current data processing methods are optimized to give good results under constant conditions in terms of temperature, salinity, and CDOM concentration. In coastal regions with much higher variability of these infering species postprocessing of the data is recommended (Zielinski et al., 2011, Frank et al., 2014). The sensors have varying antifouling measures to guard against fouling during extended deployments (Table 3.1.2.).

There are other submersible chemical nutrient analysers available which are not currently used by JERICO-Next partners. These include:

1. Lab on a chip sensors developed by National Oceanography Centre (http://noc.ac.uk/technology/technology-development/instruments-sensors). See Nightingale et al. (2015) for a review. Sensors exist for dissolved nitrate (Beaton et al 2012), dissolved silica, dissolved

phosphate (Legiret et al), dissolved organic nitrogen, dissolved organic phosphorous, dissolved iron and dissolved mangenese (Milani et al 2015). These sensors use wet chemical methods but on a miaturised sensor platform therefore reducing the chemical and power reqirements.These sensors are not yet commercially available.

2. WETLabs sell a phosphate analyser - HydroCycle-PO4 (http://wetlabs.com/cycle-phosphate-sensor). See Gilbert et al. (2013).
3. SubChem Sensor Systems Inc. sell a multi channel in situ nutrient chemical analyser called an Autonomous Profiling Nutrient Analyzer for the determination of nitrate, nitrite, ammonium, phosphate, silicate and iron (http://www.subchem.com/Autonomous%20Profiling%20Nutrient%20Analyzer%20(APNA).pdf).

Water sampler for collection of discrete water samples. Samples are analyzed in the laboratory after recovery of the SmartBuoy.

3.1.1.2. Current modes of deployment

Cefas deploy the NAS-3X on the Cefas SmartBuoy network (Mills et al. 2005). Figure 3.1.1 shows the NAS as part of the SmartBuoy payload. It is typically configured to analyse a sample every four hours during a three-month deployment. The SmartBuoy network operates in the North Sea (since 2001) and Liverpool Bay (since 2002) with historical sites in the Celtic Sea (2009 to 2015). The analyser is designed for a deployment of up to three months. The analyser is approx. 25cm diameter x 80cm tall, weighs 14kg in air (5.5kg in water) and requires an external battery pack (12V). These specifications limit the marine applications of this analyser to moorings, piers and towers. The size and weight exclude it from being used on platforms such as buoyancy gliders and autonomous surface vehicles where there are limitations on weight, space and power. Cefas programme the NAS to start sampling prior to SmartBuoy deployment to ensure that the instrument is operating correctly before deployment and to condition the cadmium column. To do this, a pre-deployment nitrate standard is fitted to the sample inlet port and the user verifies visually that the NAS is operating prior to deployment. This pre-deployment standard is removed just before the SmartBuoy is deployed and a filter fitted to the sample inlet.
SMHI do not currently deploy the MicroLab but the same considerations apply to this instrument.

NAS-3X prior to deployment with pre-deployment nitrate standard attached for sensor to run before whilst on deck. Water

Figure 3.1.1. A Cefas SmartBuoy prior to deployment showing a NAS and a water sampler.

Figure 3.1.2 shows total oxidised nitrogen (TOxN) and salinity data from a deployment in Liverpool Bay during December 2007. A clear tidal signal is seen in the TOxN data and episodic rainfall events, as seen in the salinity data, is accompanied by elevated concentrations of TOxN from the increased riverine discharge.

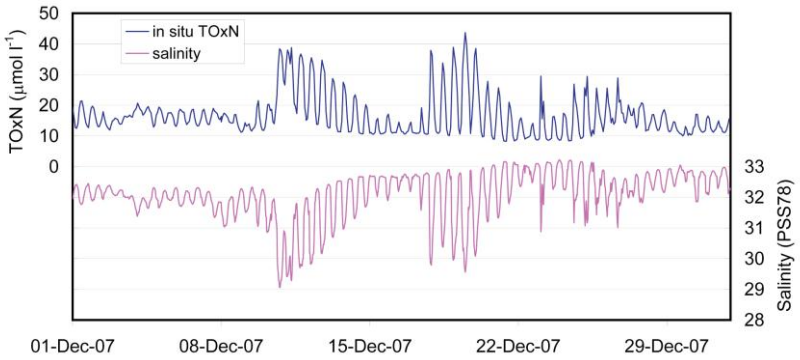


Figure 3.1.2. Data from the Liverpool Bay SmartBuoy shows the strong tidal signal evident in TOxN and salinity, with TOxN concentration showing a four-fold increase over a tidal cycle. An increase in rainfall brings lower salinity water and elevated TOxN concentrations at the site.

CHEMINI can be integrated on different platforms (including land platform, buoys, ROV, seabed station) and be used in a tele-operated mode or in an autonomous way. It requires a battery with a capacity dependant on the measurement frequency.

The coastal CHEMINI has the following specification:
Weight: 5 kg (without reagents); Dimensions: 233mm h x 148mm l x 121mm L; Power supply: 12 V, Consumption: 0,5A - 12V; Remotely controlled by automate; In situ calibration

CHEMINI for ammonium determination was deployed for the first time in October and November 2005, interfaced with the automated MAREL (Mesures Automatisées en Réseau pour l'Environnement Littoral), station Saphyre for analysis of water localised in the shellfish farming experimentation station in Argenton, 30 km from the Ifremer Brest centre. The CHEMINI was remote functioned by automate control of the MAREL station. During one month, successive analyses were performed automatically every 20 minutes in 8 different experimental tanks. The aim of the experiment was to measure the main physiological functions (ingestion, bio-deposition, excretion and respiration) of a population of oysters fed with various concentrations of phytoplankton. The data were recorded in the internal memory of the CHEMINI, the latter being remotely operated.

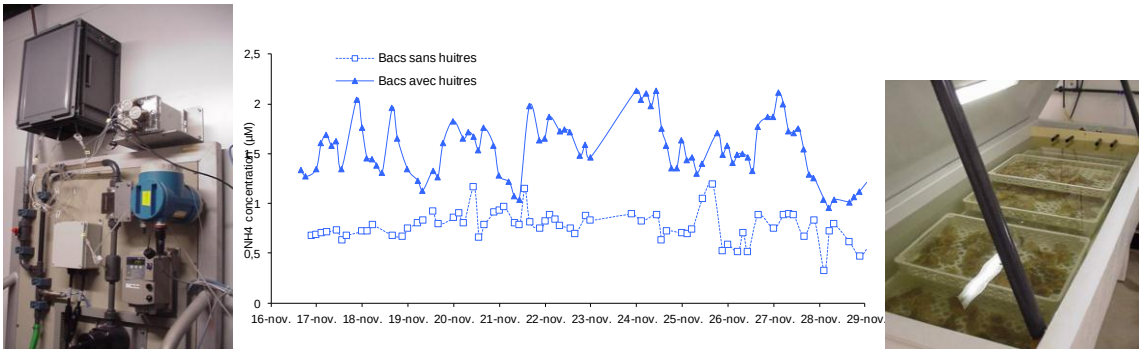


Figure 3.1.3. Implementation of the CHEMINI on the MAREL Saphyre station, variation of the ammonium concentrations (NH3 production, µmol/l) between two populations of oysters and a control tank, for the period of the 15th and 30th of November 2005. Photograph of the sample oysters tank.

As part of the ANR TROPHIMATIQUE project, 2 CHEMINIs were deployed on the MOLIT Buoy for the nutrient monitoring of the Vilaine Bay subject to eutrophication. Two periods of nitrate and silicate monitoring were recorded during 7 months in 2009 and 4 months in 2011. During the deployments, successive analyses were performed automatically every 12 hours at two sampling points of the MAREL MOLIT Buoy (surface and just above the seafloor). In situ data show more fluctuations than the discrete sampling, which may underestimate the input of nitrate to the Vilaine Bay. The data were recorded in the internal memory of the CHEMINI, the latter being remotely operated. Some of the data were transmitted to the Ifremer Brest lab via GSM.

Figure 3.1.4. Molit Buoy, Model of two CHEMINIs inside Molit silicates concentrations in µmol/l during the 2009 deployment in the Vilaine Bay.

The deep sea CHEMINI has the following specification:
Hydraulic tank (POM) H 130 x L 148 x l 120; Electronic tank (Titanium) H 264 x D 140; Weight: 13,5 kg; Power supply: 24V; Consumption: 1.5A - 24V; Remotely controlled by automate; In situ calibration

The iron in situ chemical analyser 'CHEMINI' has been implemented on the deep-seafloor observatory in the Atlantic Ocean MoMAR (Monitoring the mid-Atlantic Ridge) since 2010. This observatory is part of the EMSO-Azores marine research infrastructure (EMSO: European Multidisciplinary Seafloor and Water Column Observatory): this platform consists of ocean observing systems for long-term, high-resolution, (near) real-time monitoring of environmental processes. The system was then successfully serviced and redeployed annually between 2011and 2016. The results can be followed on the website (http://www.emso-fr.org/EMSO-

Azores/Instruments/CHEMINI-Fe, Sarrazin et al. 2014, Laes-Huon et al. 2016). The data were recorded in the internal memory of the CHEMINI, the latter being remotely operated. Some of the data were transmitted towards the surface buoy via acoustic link and then transmitted to the Ifremer Brest lab via satellite link.

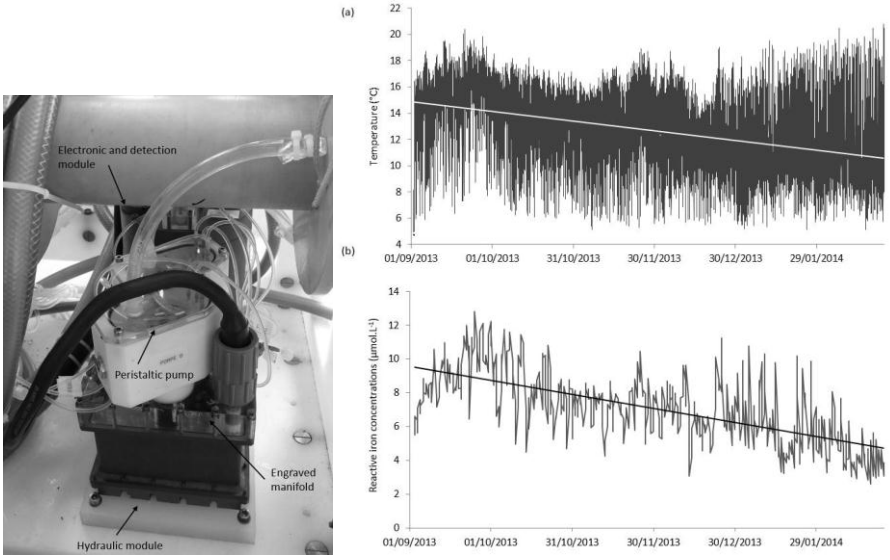


Figure 3.1.5. Hydraulic module of the CHEMINI and its implementation on the Tempo module and variation in (a) temperature (°C, 16,108 data points, sampling period: 15 minutes, [26]) and (b) reactive iron concentrations (µmol/l, 517 measurements, sampling period: 24 hours, [25]) from 31 August 2013 to 23 February 2014 (T°C: y= -0.0247±0.0004 + 1041.5± 18.2, R^2= 0.1656, n=16108 p<0.001; [Fe]: y= -0.0278±0.0014 + 1164.7± 57.7, R^2= 0.4373, n=517, p<0.001)

Since their manufacturing, iron and sulphide deep-sea versions of CHEMINI have been deployed on 17 oceanographic cruises installed on ROV Victor 6000. They usually performed 10 - 15 dives per cruise spending 6 to 24 hours underwater. The aim is to identify the chemical conditions influencing the biological assemblage at various hydrothermal sites (Martins et al. 2011). The data were recorded in the internal memory of the CHEMINI, the latter being operated via the ROV communication link.

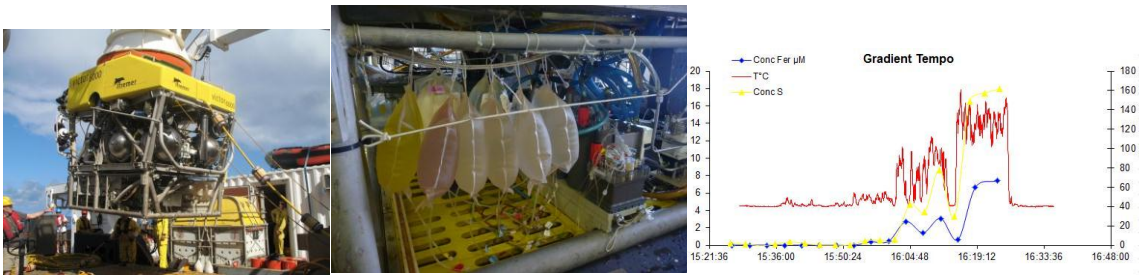


Figure 3.1.6. Photograph of the ROV Victor 6000, the Chemins and the plastics bags containing the reagents placed under the ROV, one example of the variation of sulfide, iron concentrations and temperature, on the Tempo area located on the Tour Eiffel hydrothermal site (North Atlantic ridge).

3.1.1.3. Reliability of the sensor and technological difficulties

The NAS is at technology readiness level (TRL) 9 (actual Technology qualified through successful mission operations) as it is operationally deployed. However, as noted earlier, the NAS is no longer manufactured or

supported. To ensure the long term stability of the on board nitrate standard on the NAS, Cefas autoclave the nitrate standard. They analyse a subsample from the standard in the laboratory before and after deployment to check for stability. It is challenging to deploy this instrumentation in a shelf sea with relative high sediment loads which wear the mechanical parts and means that seals require frequent replacement. In addition, silt can block the sample inlet to the NAS leading to poor data.
The NAS requires an experienced technician to service it every 3 months and to check the linearity before every deployment. The phosphate MicroLab operates over a concentration range 0.02 to 50 µmol l^{-1} but has not been assessed by SMHI for accuracy and precision. The software for these instruments requires some time for familiarisation. The CHEMINI requires an experienced operator for servicing and set up in the laboratory prior to deployment at sea.
The performance of the NAS and the CHEMINI is summarised in Table 3.1.3. The MicroLab has not yet been assessed for accuracy and precision at SMHI.

Instrument	Parameter	Reliability	Precision	Accuracy	Operating range
NAS-3X	TOxN	75%	1.8 µmol l^{-1}	2.6 µmol l^{-1}	0-60 µmol l^{-1}
CHEMINI	Nitrate	57% (during 2009)	3.1% NO_3 (1.5 µmol l^{-1}, n5)	N/A	0-100 µmol l^{-1} NO_3
	Phosphate	N/A	4.5% (2 µmol l^{-1}, n=4)	N/A	0-200 µmol L^{-1}
	Silicate	76% (during 2009)	2.8% (5 µmol l^{-1}, n=4)	N/A	0-200 µmol l^{-1}
	Ammonia	N/A	3% NH_4 (5 µmol l^{-1}, n=5)	N/A	0-300 µmol l^{-1} NH4
	Iron [Dfe]	67-91% (during 2014-2016)	5% (6 µmol l^{-1})	N/A	0-100 µmol l^{-1}
	Sulphide	78-100% (during 2014-2016)	2.3% (20 µmol l^{-1}, n=3)	N/A	0-150 µmol l^{-1}

Table 3.1.3. Summary of the performance of the NAS at Cefas and the CHEMINI at Ifremer

Coastal version of CHEMINI is well suited for long term *in situ* monitoring of the coastal ecosystems. The lifetime of the reagents (1 month) determines the deployment length. The main maintenance interventions except for replacement of reagents were due to clogging, hydraulic connection or reagent precipitation (which has been resolved for the following deployments). Deep Sea version of CHEMINI is well suited for long term *in situ* monitoring of the hydrothermal ecosystems. On ROV of deep sea observatories deployment between 2006 and 2016 they have shown analytical reliability and mechanical robustness. Malfunctions were caused by tubing becoming disconnected, failure of electronic cables and saturation of the memory. The objectives now are to ensure an optimum functioning for a long time series (longer than 6 months, and to minimize system failure).

3.1.1.4. Sensor calibration

Cefas perform a linearity check of the NAS before every deployment using TOxN standards of known concentrations. They analyse 6 or 7 nitrate standards and a minimum r^2 of 0.9 is needed before deployment. An example is shown in figure 3.1.7. The NAS uses the on board nitrate standard to calibrate the data collected during the deployment. The NAS analyses an on board standard after every five sample analyses.

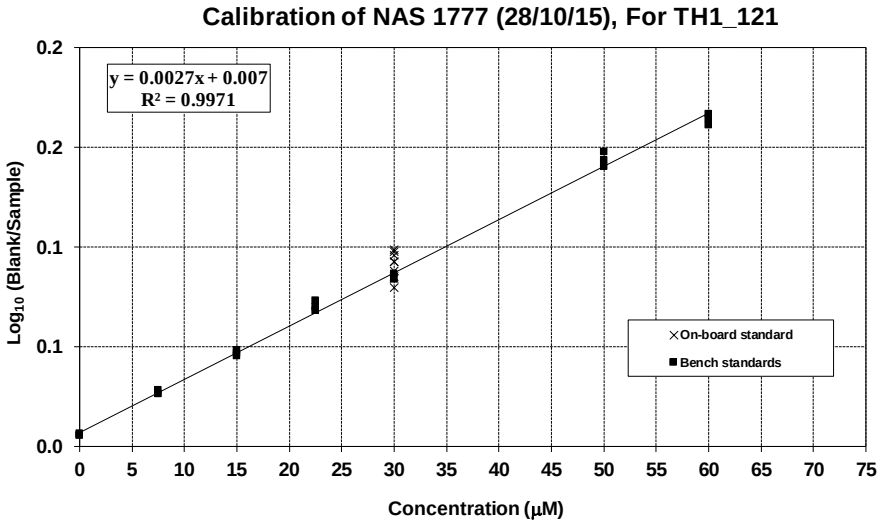


Figure 3.1.7. Example linearity check for NAS-3X before deployment.

SMHI use four phosphate standards to calibrate the MicroLab and aim to do this before every deployment. SMHI has not yet deployed the MicroLab. The tests performed are done in the lab. Before a test can start SMHI does a linear calibration with known PO4 standards. The constants from the calibration are put into the software of the MicroLab (Fig. 3.1.6). To date, the MicroLab has been bench tested at SMHI. The unknown samples tested have also been run on a regular nutrient analyzer (IO Analytics) for validation.

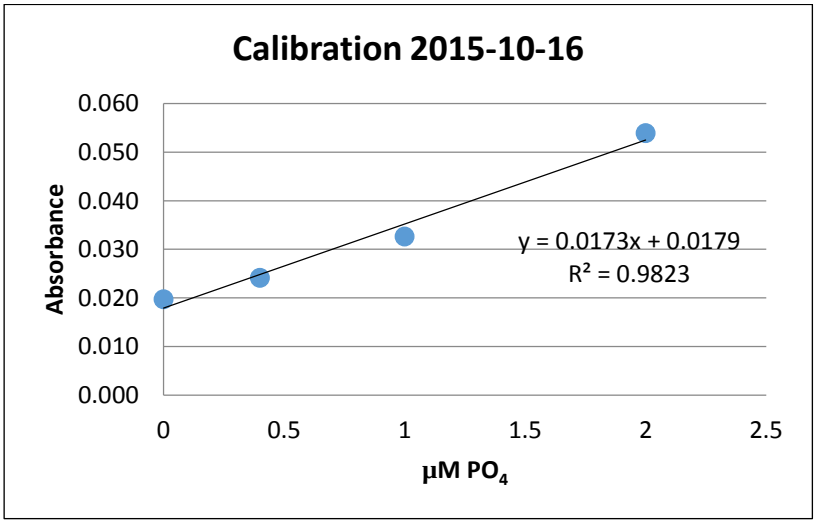


Figure 3.1.8. Example calibration plot of MicroLab from SMHI.

For calibration of the CHEMINI, seawater is directly pumped from the environment, through a coarse filter, and supplied to the CHEMINI analyzer located on the land based, buoy or underwater structure. In situ calibrations are performed during every deployment, with a frequency dependant on the parameter. For iron determination on the seabed observatory in situ calibration was carried out once a day (four replicates at noon for the 25 µmol L^{-1} iron (III) standard) to correct for possible instrument drift. The precision of the standard concentration of 25 µmol L^{-1} Fe(III) analyzed daily in situ was 1.07% (n = 522, 3 replicates per day), illustrating the satisfactory analytical performance of the CHEMINI and validating the in situ iron concentrations obtained. An experimental design taking into account the variations of environmental parameters will be developed soon following the

COFRAC acreditation (Comité FRAncais d'Accréditation). An estimation of the global unncertity (T°C, pressure, flowrate, replicates) will be calculated.

3.1.1.5. Data issues

The raw NAS data are processed using the NutrientDATA software to generate a csv file which can be manipulated by the user. Cefas have identified the following issues as the most common cause of poor quality data:

1. Rotary valve malfunction - the sample is not injected into the colorimeter
2. Bubbles trapped in the colorimeter – interference with sample absorption measurement
3. Tubes becoming disconnected to/from the colorimeter - the sample is not injected into the colorimeter
4. Colorimeter light source failure – poor light absorption measurement

Cefas use an experienced operator to quality control the NAS data. The NAS data are compared with results from in-situ bag samples which are collected on the SmartBuoy using a discrete water sampler and with discrete samples collected using a CTD rosette before and after every deployment. Both these sample types are analyzed for inorganic nutrients using a continuous flow analyser in the laboratory (Kirkwood 1996). The on board nitrate standard is analyzed pre and post deployment to check for stability.

Cefas use the TOxN data from the NAS for the assessment of eutrophication under OSPAR (Foden et al. 2010; Gowen et al. 2008) and for optimising the design of monitoring programmes (Heffernan et al. 2010) and for other research programmes. SMHI would like to use the MicroLab for eutrophication monitoring and research programmes but it is not currently operational.

Cefas deployed an ISUS uv nitrate sensor (Sea-Bird Scientific, see section 3.1.3 for principles of operation) in November 2008 to January 2009 as a trial deployment on the Warp SmartBuoy (Thames estuary). There was also a NAS deployed at the same time and a comparison of the data is shown in Figure 3.1.9. The ISUS is an optical sensor and therefore the two sensors operate in a very different way.

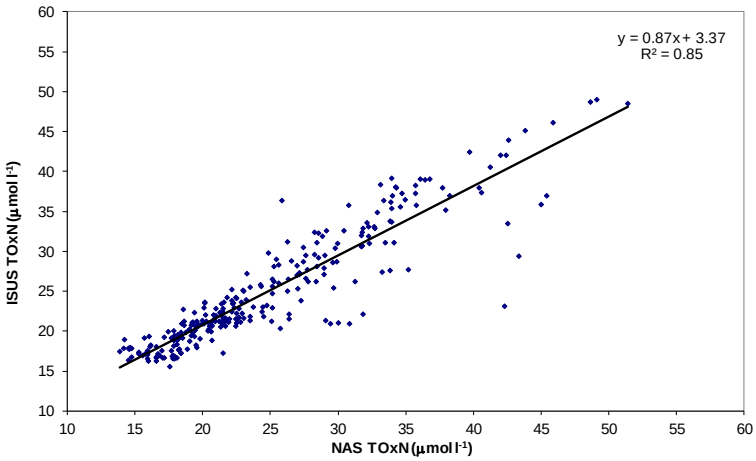


Figure 3.1.9. Comparison of NAS TOxN with ISUS TOxN in the Thames estuary in November 2008 to January 2009.

SMHI are in a development stage therefore they have not yet had any problems with data handling or quality control.

Ifremer have identified the following as the main causes for instrument malfunction of the CHEMINI:

1. Clogging
2. Hydraulic connection
3. Reagent precipitation
4. Tubing becoming disconnected
5. Failure of electronic cables
6. Saturation of the memory

3.1.1.6. Links with other WPs and with other EU initiatives

Links need to be made from this WP to task 5.5 (Enhancement of Quality Control procedures for sensor based biochemical data). Experience from JERICO-NEXT partners in operating and quality control of data from nutrient sensors must be used in the development of best practice for the quality control of biochemical data.

The EU FP7 Programme 'Common Sense' (http://www.commonsenseproject.eu/) has developed a range of sensors for different applications, one of which is a eutrophication sensor. It is a modular, wet chemistry system designed for in situ analysis of nitrate, nitrite and phosphate with a detection limit of 0.05 mmol l^{-1} for each analyte. The sensor is at TRL 7.

3.1.1.7. Summary

The in situ chemical nutrient analysers give data directly comparable with traditional laboratory based analysers as both use wet chemical methods. The sensors operated by JERICO-NEXT partners require an experienced operator for servicing and set up in the laboratory prior to deployment at sea.
The majority of in situ chemical nutrient analysers have been developed at research institutes and are not yet commercially available. There is a lack of in situ chemical nutrient analysers which are currently commercially available, limiting the number of of in situ nutrient measurements made and, hence, there is a considerable requirement for inexpensive, easy to use nutrient sensors. A greater selection of commercially available systems would allow more in situ nutrient measurements to be made.

3.1.1.8. References

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3.1.2. Portable flow injection nutrient sensors

3.1.2.1. Description of the sensors

HZG uses chemical analyser from the company SYSTEA (Italy) connected to FerryBox systems. The Micromac C is a microprocessor controlled analyser for automatic monitoring different water constituents such as nutrients or dissolved iron, which can be measured colorimetrically. The systems are based on an analytical technology developed by SYSTEA named LFA (Loop Flow Analysis; Azzaro & Galetta 2005). The sample as well as the calibrant and water for dilution are pumped by a peristaltic pump through the loop consisting of a reaction chamber, colorimeter etc. which are placed into the loop by switching different valves. The analysers at HZG are designed to measure sequentially nitrate/nitrite, silicate, ammonia and o-phosphate. For all parameters the traditional laboratory methods for measuring nutrients in seawater (Grasshoff 2009) are applied. For the determination of nitrate, the Micromac C uses a photochemical reduction by UV-radiation instead of the traditional copperized cadmium column, for the reduction of nitrate to nitrite. The pH of the sample is adjusted to pH=7.7 by adding a solution of TRIS buffer (Tris-Hydroxymethylaminomethane adjusted to pH=7.7 with concentrated hydrochloric acid) and DTPA (Diethylene triamine pentaacetic acid) and the sample will be subsequently irradiated by an UV-lamp. As the sequential analysis of four parameters takes about one hour, this device is preferably used for stationary measurements but it is less suitable for underway measurement due to the poor time resolution.

Figure 3.1.10. (a) SYSTEA Micromac-C; (b) SYSTEA µMAC-1000.

For underway measurements the µMAC-1000 device is used either in mono or sequential multi-parametric configuration. Each device is programmed for one or at least two parameters using the same chemical methods as described for the Micromac C device except for ammonia. Ammonia is determined by the reaction of ammonia with orthophthaldialdehyde (OPA) and measuring the intensity of the fluorescence signal (Kérouel and Aminot 1997).
Both systems can be programmed for different measurement cycles, which can be either run autonomously or will be remotely activated by certain commands via a serial interface (RS-232). The results are stored internally or can be sent to the connected computer via the serial interface as well.

3.1.2.2. Current modes of deployment

The multi-parameter system Micromac-C is only operated in stationary FerryBoxes such as the station at the mouth of the Elbe River in Cuxhaven. The analyser is connected to the water loop of the FerryBox and gets filtered water via a cross-flow filter, as the sample water is very turbid. The FerryBox computer controls the nutrient analyser and receives the measured nutrient concentrations as well. Fig xx3 shows as an example a time series of salinity and nutrients (NO_3 and o-PO_4) measured in the estuary of the Elbe River for one month.

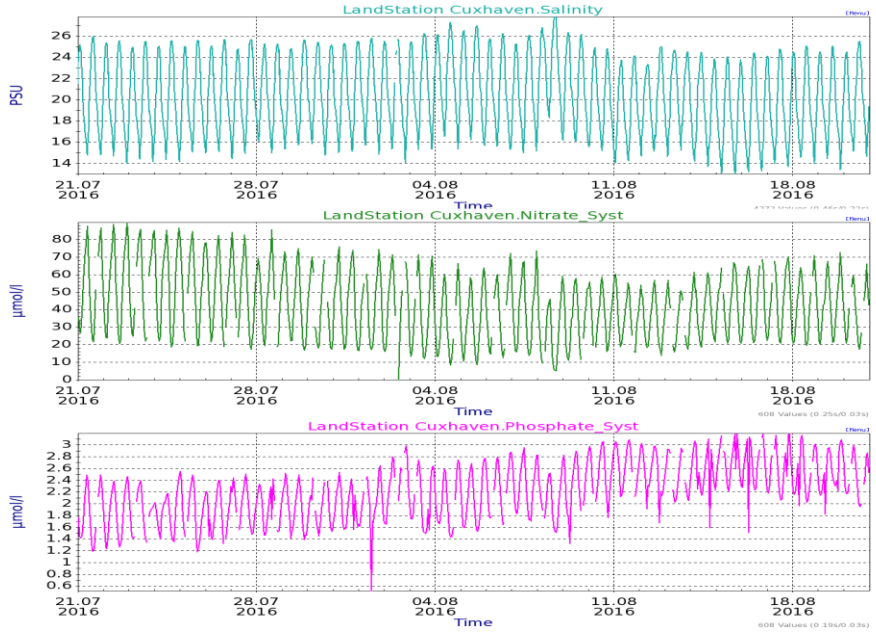

Figure 3.1.11. Example of salinity and nutrient data measured with SYSTEA Micromac-C in the estuary of the Elbe River at Cuxhaven in August 2016.

The µMAC-1000 devices were applied in underway FerryBox systems operated on different routes in the North Sea. The devices are connected and controlled by the FerryBox computer. The complete control and data acquisition software is programmed in LabView. Again, the sample water is filtered by a cross-flow filter. For the analysis of nitrate, the µMAC-1000 uses a copperized Cd-column for reduction of nitrate to nitrite. Figure 3.1.12 shows an example of nitrate data together with salinity and chlorophyll-a fluorescence data along a transect from Immingham (GB) to Cuxhaven (DE). These data indicate between 4°E and 6°E a distinct decrease in salinity together with anomalous high nitrate values at that season and an algal bloom caused by an exceptional drift of riverine water masses from the Rhine River into this area (Petersen et al, 2011).

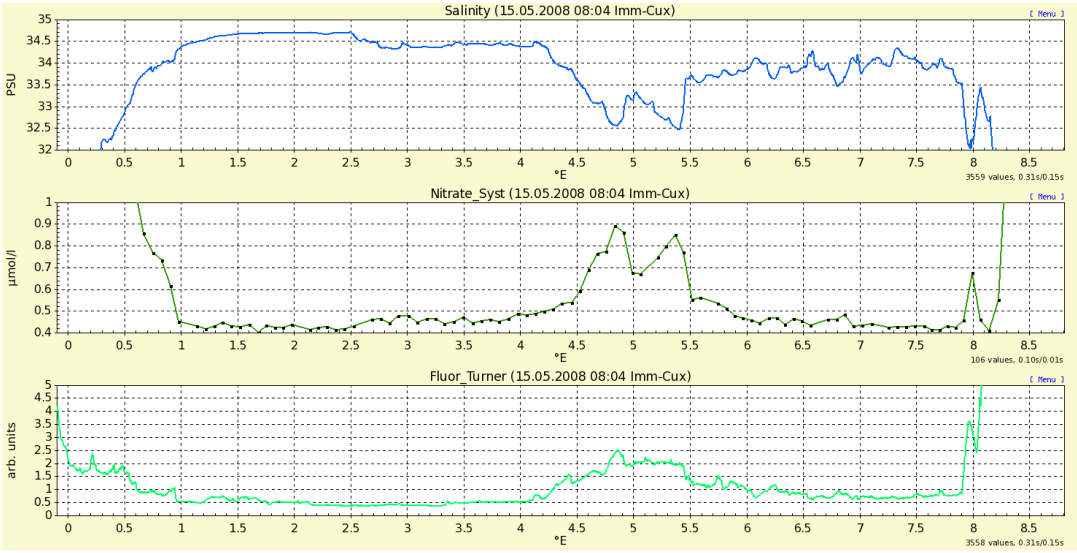

Figure 3.1.12. Salinity, nitrate and chlorophyll-a fluorescence measured along a transect from Immingham (GB) to Cuxhaven (DE) in May 2008.

3.1.2.3. Reliability of the sensor and technological difficulties

The example in Figure 3.1.12 shows the advantages of continuous recordings of nutrient data along a certain transect with a much higher chance to detect such short-term events. However, in case of the µMAC-1000 device it turned out that the failure rates of this instrument were quite high and additionally required a high level of input from an experienced user. The main issues were blocked or not properly working valves resulting in the small hoses disconnecting from the connector due to overpressure and subsequent flooding of the device. A further problem was that the electronic section was not separated from the wet section with the tiny hoses and valves which sometimes caused additional failures in the electronic section. Due to the high failure rates and high personnel efforts the operation of these nutrient analysers was discontinued on underway FerryBoxes after several years.
A much better performance was obtained with the Micromac-C even the device can be only used in stationary systems due to the size and time resolution. This system requires much less maintenance (but anyway an experienced user) and can be autonomously operated for a long time. As the electronics and hydraulics are completely separated, even in case of an accident, such as a leakage, the electronics remains intact.

HZG experience of the Systea Micomac:

Precision	5-10%
Accuracy	Depends strongly on calibration procedure and how often it has been carried out
Reliability	Micromac C: 80% µMac-1000: 50%

3.1.2.4. Sensor calibration

SYSTEA recommends a one-point calibration in the appropriate concentration range. As especially for nitrate the concentrations in the open sea and in the estuaries (e.g. estuary from the Elbe River and the Humber River) differs by more than one magnitude the sample has to be diluted at higher concentrations before starting the analysis. The devices are programmed in such a way that if the extinction values exceed a certain value the analyser takes a sample again which will be then diluted by a certain amount of Milli-Q water. Thus, in case of nitrate the analyser has to be calibrated for different concentration ranges. Normally the analyser is calibrated in the lab against a certain standard in Milli-Q water. Fig. 3.1.13 shows a linearity test for standards prepared in solutions of different salinity. The figure reveals a slight dependency of the calibration on salinity probably caused by varying reduction rates at different salinities. This small influence of salinity on the measurements is normally neglected as the water samples have quite different salinities along a certain track.

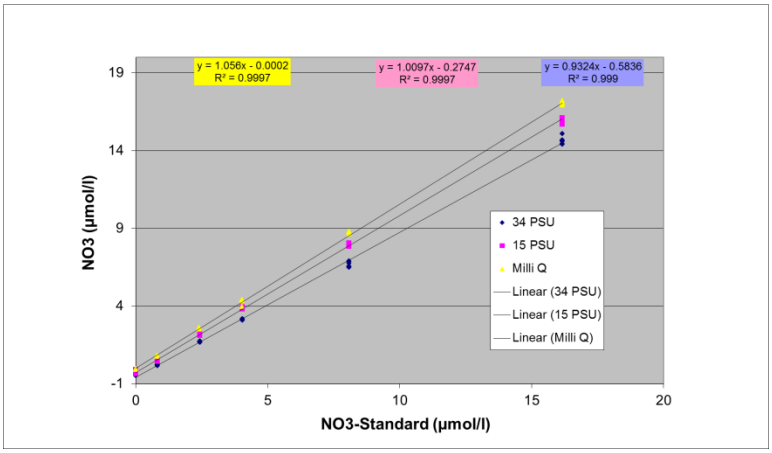

Figure 3.1.13. Linearity check of nitrate calibration at different salinities.

For quality control, bottle samples are taken by a cooled automatic water sampler on a regular basis. These samples are analyzed in the lab with an autoanalyser (continuous flow analyser Technicon AA3) for comparison with field data. Fig. 3.1.14 shows as an example a comparison of o-phosphate measured by the SYSTEA µMAC-1000 connected to the FerryBox and discrete bottle samples measured in the lab.

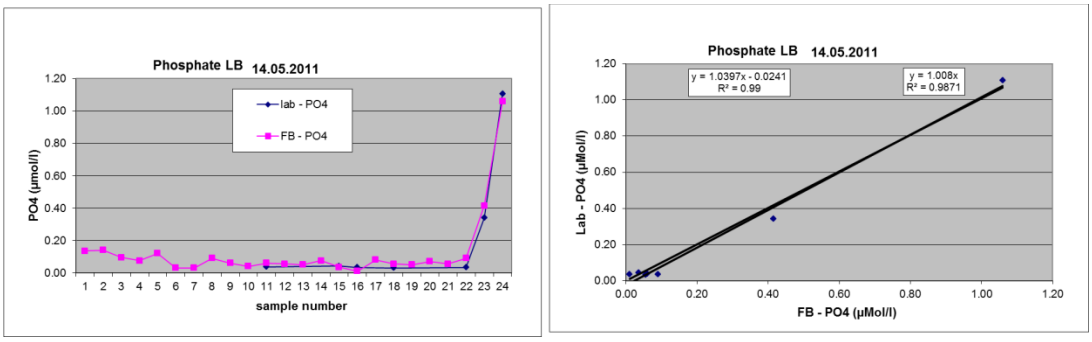

Figure 3.1.14. Comparison of bottle samples analyzed in the lab with FerryBox data (Lysbris May 2011).

3.1.2.5. Data issues

The nutrient data sampled by the FerryBox system are delivered together with all other data to the COSYNA database in real-time (every 10min) from the fixed stations and in near-real-time from the underway FerryBoxes when the ship arrives in the next harbour.

The following main issues were identified for poor data quality or missing data:

1. Drift of the baseline due to changing reagent blanks resulting in wrong measurements at very low concentrations.
2. Blocked or broken valves causing overpressure within the hydraulics with subsequent disconnection of tubes from the connector.
3. Instability of reagents at higher temperatures (e.g. in the engine room of a vessel) requiring more frequent (e.g. fortnightly maintenance intervals)

Biofouling is normally not an issue as the pH of the water samples taken by the analyser are modified by addition of acids or bases to prevent the development of biofilms. However, the formation of coatings of the dyes on the windows of the cuvette changes the optical properties. These coatings reduce the sensitivity of the sensor and can only be removed by strong acids or bases.

3.1.2.6. Links with other WPs and with other EU initiatives

- EU project SenseOcean (www.senseocean.eu)
- EU project SCHeMA (www.schema-ocean.eu/)

3.1.2.7. References

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3.1.3. Biosensors

Whilst not currently deployed by JERICO partners, biosensors have been included in this report as an additional sensor type for future consideration. Unisense have developed a nitrate/nitrite and a nitrite biosensor (http://www.unisense.com/NOx). The sensor consists of an electrochemical N_2O transducer with a biochamber attached. The biochamber contains active denitrifying bacteria which reduce the NO_x^- to N_2O. The N_2O passes through a silicone membrane to the transducer where it is detected. This biosensor is designed for a wide range of applications from waste water and seawater to mud and soil extracts. Cefas trialled an early version of this biosensor in a flow through configuration on the research vessel in 2006 and in discrete sample mode in 2007. Good results were obtained on discrete samples compared to samples analyzed in the laboratory using flow injection analysis. Given the biological nature of these sensors, environmental factors must be carefully controlled to maintain sensor performance (e.g. temperature, nutrient conditions of bacteria, composition of the solution). As with all sensors, the biosensors require careful calibration but they do not require chemical reagents.

3.1.4. Water samplers

In addition to nutrient sensors there are in situ water samplers that collect discrete water samples which are collected into sample bags. The sample bags are spiked before deployment with an appropriate preservative depending on what the sample is to be analyzed for. If sample bags are spiked with mercuric chloride, samples can be analyzed in the laboratory after recovery for inorganic and organic nutrient analysis. There are several commercially available water samplers including:

1. McLane remote access sampler (http://mclanelabs.com/remote-access-sampler-2/). The sampler can collect up to 48 samples of either 100ml or 500ml and is rated to a depth of 5500m.

2. Cefas Technology Ltd (CTL) water sampler (https://www.cefastechnology.co.uk/products/water-sampler/) described in Stern et al. (2015). This can collect up to 16 samples of a volume programmed by the user and is rated to 20m depth.

3. Envirotech LLC Aquamonitor. This is no longer commercially available as the company no longer exists. It can collect 50 samples up to 1000ml volume. There is a shallow water version (100m) and a deep water version (2500m).

All the above samplers require a considerable amount of space and power and would not be suitable for a buoyancy glider. The most compact sampler is the one from CTL which has been used on the Continuous Plankton Recorder (Stern et al. 2015) and on a surface Wave Glider (https://www.cefas.co.uk/news/cefas-scientists-complete-world-s-first-on-demand-autonomous-marine-water-sampling/).

Cefas routinely deploy the CTL and Envirotech water samplers on the SmartBuoy moorings for determination of dissolved inorganic nitrogen and silicate and dissolved organic nitrogen (Johnson et al. 2013; Suratman et al. 2010). Some bags are spiked with Lugols Iodine instead of mercuric chloride for the determination of phytoplankton species composition and abundance (Greenwood et al. 2011). Afbi routinely deploy the McLane RAS on their station 38A mooring in the Irish Sea for the determination of inorganic nutrients and phytoplankton species composition and abundance (Gowen and Stewart 2005).

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3.2. OPTICAL SENSORS FOR BIOLOGICAL PARAMETERS

3.2.1. LED Fluorometry

3.2.1.1 Description of the LED fluorometers

Fluorescence is a sensitive way to perform various in situ measurements (ACT 2005). Nowadays, field fluorometers for various applications are typically having LED as an excitation source and photodiode as a detector. By selecting appropriate LEDs and filters, excitation and emission wavebands may be matched with the fluorescence properties of different compounds (Fig. 3.2.1.). The modern field fluorometers are relatively small-sized and inexpensive. In this document, we describe the LED fluorometers for detection of Chlorophyll *a* (Chla), phycobilins and coloured dissolved organic matter (CDOM).

Chla is present in all photosynthetic organisms, and has a central role in photosynthesis. The most widespread method to estimate phytoplankton biomass is to measure the concentration of Chla. Laboratory methods for measuring chla concentration are reliable, but have high cost, require water sampling, and only limited number of observations can be obtained. In situ methods, mainly fluorometry, may provide large amounts of (online) data cost efficiently and at the relevant time/spatial scales of biological processes (Lorenzen 1966), but provides only a proxy of the real Chla concentration. Chla fluorescence from living cells provides only a semi-quantitative estimate of Chla concentration because 1) in the cell part of the Chla is located in the non-fluorescing photosystem I and 2) cell photochemical status (physiology) affects the magnitude of fluorescence yield. Chla fluorescence is typically measured using blue (440-470 nm) LED for excitation and detection of emission at around 680 nm (Babin 2008,Zeng & Li 2015).

Phycobilins are photosynthetic pigments found especially in cyanobacteria, cryptophytes,red algae and in a few dinoflagellates and ciliate *Mesodinium rubrum* (Seppälä et al 2007). Their abundance can be used to track differently coloured groups, typically phycobilins are considered as indicators of cyanobacteria (Zamyadi et al 2016). The principles of phycobilin fluorescence are similar to Chla fluorescence, as the fluorescence intensity is a proxy of pigment concentration. Wavebands used for the detection of different phycobilins, i.e. red phycoerythrin and blue-green phycocyanin, vary. In addition there are differences in the spectral properties among the phycoerythrins, depending on the structures of the phycoerythrins and reflecting if species have been adapted to clear oceanic or coastal waters. In contrast to Chla, the maximum excitation and emission bands are very close to each other in phycobilins making their detection challenging. For phycoerytrin rich phytoplankton in coastal waters these maxima are around 570nm / 575 nm and for phycocyanin around 630 nm / 640 nm. This leads to sensitivity-specificity dilemma, due to overlapping spectra, especially for phycocyanin (Seppälä et al 2007). Moving the wavebands away from the actual maxima will decrease the amount of spectral overlap but also decreases the sensitivity. Sensitivity can be increased using wider optical bands but as a consequence of this fluorescence from other pigments (Chla) starts overlapping. As there are no standardized wavebands for phycobilin fluorometers, the sensitivity and specificity of fluorometers found in the market vary.

CDOM fluorescence, fDOM, is used to estimate the amount of dissolved organic matter (DOM) and carbon (DOC) (Fellman et al 2010). The relationship between fDOM, absorption of CDOM and DOC varies for different components in two ways. First, excitation-emission matrix for different compound groups differs considerable, challenging their detection using one waveband LED fluorometry. Secondly, DOC specific fluorescence yield varies between components. For these reasons DOC-fDOM relationships are instrument and location specific. Wavebands for CDOM fluorometers vary considerably.

Fluorometers available from different manufacturers vary considerably in size, shape, materials, wavebands, available accessories, and thus price. It is very hard to compare the sensitivity of instruments, without actually performing tests, as there are no commonly agreed reference materials for different applications. Some of the most common instruments, with associated waveband information, are listed in table 3.2.1.

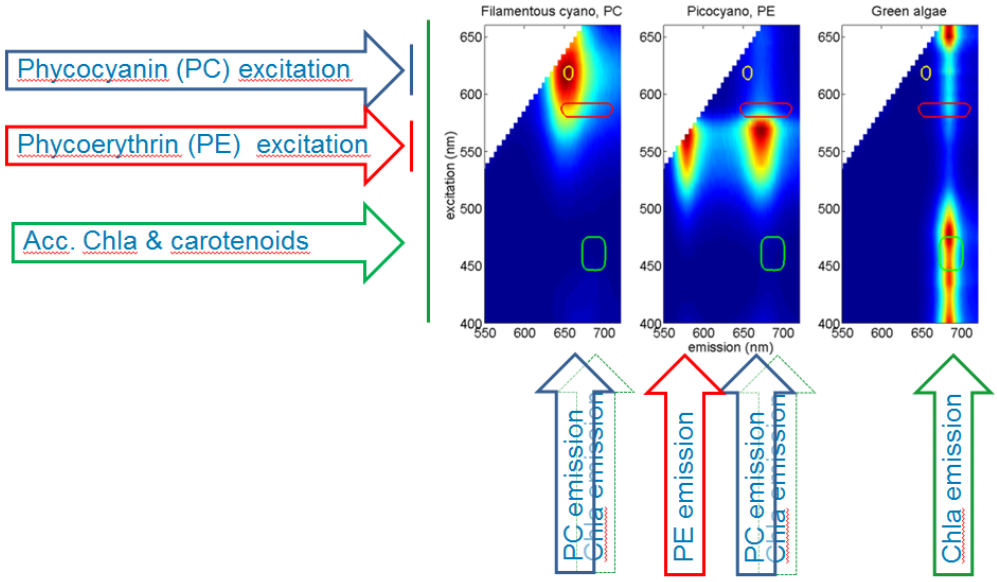

Figure 3.2.1. Visualisation of different wavebands for detection of chlorophyll a (Chla), phycoerythrin (PE) and phycocyanin (PC). Fluorescence excitation emission matrix has been presented for three differently pigmented phytoplankton type, phycocyanin rich cyanobacteria (left), phycoerythrin rich cyanobacteria (middle) and green algae (right). Cyanobacteria show very low fluorescence from Chla, while the green algae do not contain any phycobilin pigments. The arrows, and associated lines, show the excitation bands (left from the figures) and emission bands (below the figures).

Table 3.2.1. Some examples of LED fluorometers and the excitation/emission wavebands. The list is far from complete, as most of the manufacturers do not give the waveband information in their webpages.

Chla Sensor	**Manufacturer**	**Excitation/emission wavelengths**
ECO FL	WETLabs	470/695 nm
C3/C6P	Turner Designs	460/696nm or <635/>695nm
MicroFlu-chl	Trios	470/685 nm
Phycoerythrin Sensor		
Eco FL	WETLabs	518/595 nm
C3/C6P	Turner Designs	525/>590nm
Phycocyanin Sensor		
Eco FL	WETLabs	630/680 nm
C3/C6P	Turner Designs	590/>645nm
MicroFlu-blue	Trios	620/655 nm
fDOM Sensor		
ECO FL	WETLabs	370/460 nm
C3/C6P	Turner Designs	365/470 nm
MicroFlu-cdom	Trios	370/460 nm

3.2.1.2 Current modes of deployment

LED fluorometers can be used in principally in all oceanographic platforms. The diversity of LED fluorometers is very large (Figure 3.2.2.), depending on the parameter to be measured, sensitivity of the instrument and technical solutions. Some sensors are designed to be included in larger sensor packages or CTDs. Commonly

they have depth ratings of 60-600m. Some fluorometers are very small, the smallest with a weight of 100g, and show very low power consumption <1W, with typical input voltage 5-25 V. Many manufacturers provide chambers for flow-through applications and connectivity to third party data loggers. Fluorescence is sensitive for biofouling, and various preventive methods have been demonstrated, including wipers, copper components, nanocoating, pressurized air release and flushing with acid or detergents.

Figure. 3.2.2. Examples of LED fluorometers from different manufacturers (left), during calibration exercise (middle) and installed on flow through system (right).

3.2.1.3 Reliability of the sensor and technological difficulties

Fluorescence detection is based on relatively simple technology, and for this purpose the LED fluorometers have high TRL (8-9). The main issues with different sensors are related to the long or short term stability of the instruments, due to biofouling, condensation of water inside or deteriorating of the optics. As the LED fluorometers are single channel instruments, resolving blanks, biofouling, drift or other interferences requires discrete sampling, additional measures or good knowledge of the system.

Despite the biofouling prevention methods applied, manual cleaning of the sensors are required. This needs to be done with care in order not to scratch the optical window, especially for those instruments having non-glass optics. Sometimes even the wiper attached to the sensors may scratch or otherwise damage the outer optical parts very badly. For some sensors condensation of water has been noted, especially when used in flow-through applications when the sensor body is warm and water flowing by the optical window is cold. This issue is typically seen as an abnormal increase in fluorescence values during winter months.

Comparison of the sensors, measuring the same parameter, is sometimes challenging as the instruments from different manufacturers have different optical setups. It is not possible to rank the instruments, based on their performance, but one has to acknowledge that they integrate the fluorescence signal form slightly different wavelength ranges. To overcome such issues, one may select using only one type of fluorometer for a given area/study. Especially phycocyanin fluorometers, depending on the wavebands used, are vulnerable to overlapping signals from Chla and phycoerythrin (Seppälä et al 2007).

Temperature affects fluorescence intensity, and some instruments provide temperature compensation. For algae this is typically not needed, as temperature is just one part affecting physiology and may be overridden by other effects. Temperature dependency of fDOM needs to be checked for each instrument type and using typical CDOM from the study site (Ryder et al 2012).

It is important not mixing the technological difficulty and technological limitation when working with phytoplankton fluorescence. Fluorometers are most often providing accurate description of fluorescence intensity, but the interpretation of this signal as concentration of pigments or cell numbers is not straightforward due to photobiological processes in living cells affecting the fluorescence yield.

3.2.1.4 Sensor calibration

Primary calibration of LED fluorometers has not been agreed and the results are most often in relative units. The most often suggested methods for calibration use Chla in organic solvent, fluorescein, rhodamine, or algae cultures, but all with their limitations (Earp et al 2011). For Chla fluorometers many manufacturers provide the results in units of µg Chl L^{-1}, based on the calibration carried out with phytoplankton cultures. This provides the first-hand proxy of concentrations, but is not a traceable calibration, and cannot be repeated at different times. For cyanobacteria, some manufacturers even tend to make the "calibration" using cell counts, ignoring totally the fact that cells come in different sizes and with different pigment content. fDOM sensors may be an exception as typically the fluorescence is presented as quinine sulphate or perylene equivalents. Some manufacturers seem to understand the consequences of this problem and some initiatives towards traceable calibration will be hopefully taken. For some sensors there are solid secondary standards available. These may be used to track the performance of single instrument but not to perform actual calibration. One aim of JERICO-NEXT tasks 2.4 and 2.5 is to promote traceable and harmonized calibration of LED fluorometers.

3.2.1.5 Data issues

Interpretation of fluorescence data may be challenging and it is typically considered as a semi-quantitative proxy of concentrations, helping to identify timing, location and relative magnitude of events, not exact biomass and also assist in sampling and prewarning. Without commonly agreed methods for calibration, the data from different operators (or different years/instruments) remain unconnected. The fluorescence data flowing into different databases stay incomparable as long as there is no traceable calibration.

Validation with field samples is an important step in analyzing fluorescence data. The most common methods rely on linear regression, but this tend to fail, e.g. for Chla, when the changes in the phytoplankton physiology are "larger" than changes in biomass (example day-night shifts in non-photochemical quenching). Then, alternative methods for validation should be sought to make use of very sensitive fluorescence measurements.

In high irradiance conditions, phytoplankton protects their photosystems from bleaching through nonphotochemical quenching processes (Milligan et al. 2012, Müller et al. 2001). The consequence of this is suppression of fluorescence quantum yield, i.e. decrease of Chla fluorescence vs. concentration. This is often observed in depth profiles as a decreased signal in chlorophyll fluorescence in the upper layer during daytime compared to night time. A similar effect may be observed in day-night cycles from a Chla fluorometer mounted near the sea surface on an oceanographic buoy. Nighttime Chla-fluorescence is in general higher than daytime chla-fluoresence, sometimes by a factor of two to three. Another reason for mismatch between chlorophyll fluorescence and Chla concentration is due to differences in pigmentation between taxonomic phytoplankton groups.

3.2.1.6 Links with other WPs and with other EU initiatives

In Jerico-NEXT project LED fluorometers are used on several different instrument platforms. The inter comparison and relation between LED fluorescence records and other optical automated methods are studied in WP3.1 and in WP4 JRAP#1, especially for phycobilin fluorescence. Fluorescence of Chla and phycobilins are used as indicators of biological activity in JRAP#5 when studying carbon fluxes. Calibration of fluorometers will be upon touched in WP2, task 2.5. The JERICO-NEXT community seek additional funding opportunities to move further with calibration issues, together with relevant industry partners.

3.2.1.7 Summary

LED fluorometers are used in many different platforms recording the abundance of phytoplankton biomass, amount of cyanobacteria and fDOM. Despite the long history of such measurements, there is no commonly agreed methods for primary calibration of fluorometers yielding inconsistent datasets. This hampers the use of

data as the records from different sites/studies are disconneted. Lack of proper calibration also make large-scale studies of variability in Chl *a* fluorescence vs. concentration impossible (which would be the starting point for more accurate field validation).

3.2.1.8 References

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3.2.2. Spectral fluorescence

3.2.2.1. Description of the spectral fluorometers

Spectral fluorescence reflects the pigmentation of phytoplankton cells and is a tool to obtain chemotaxonomic information on the living phytoplankton community (e.g. MacIntyre et al 2010). In the living phytoplankton cells, mainly the pigments associated in the Photosystem II of the cells contribute to the fluorescence signals (Babin 2008). In Photosystem II the antenna pigments (carotenoids, accessory chlorophylls) absorb light at their specific wavelengths and transfer it further to Chlorophyll a (Chla) and towards reaction center, which is the site for photochemical reactions. The energy transfer from the accessory antenna-pigments towards Chla is very efficient and from these pigments no energy is lost by fluorescence. Contrary, considerable part of the energy (0.5-5 %) obtained by Chla is lost as fluorescence, with emission band centred at 682 nm, and the intensity depends mainly on the photochemical status of the cells (Babin 2008). In addition to Chla, also phycobilin pigments, found in certain taxonomic phytoplankton groups, show autofluorescence.

Fluorescence excitation spectra, measured at the Chla emission waveband, reveal the spectral shape of the light absorption by accessory pigments. As the major taxonomic groups differ in their pigmentation, and these pigments have considerable different spectral properties, there are also consistent differences between excitation spectra of different taxonomic pigment groups (Fig. 3.2.3). These groups can be characterised as follows: photosystem II antenna of green algae (Chlorophyta) contain mainly Chla and Chlb, antenna of brown algae (e.g. Dinophyta and Bacillariophyta) contain mainly Chla, Chlc and carotenoids (e.g. fucoxathin, peridinin), antenna of cryptomonads (Cryptophyta) contain Chla, Chlc, carotenoids and typically one form of phycobilin pigment. For Cyanobacteria (Cyanophyta) Chla emission is very low as they contain only very low amount of Chla in Photosystem II, and the excitation spectra show only excitation peaks for Chla and phycobilins but it is also influenced by overlapping phycobilin fluorescence.

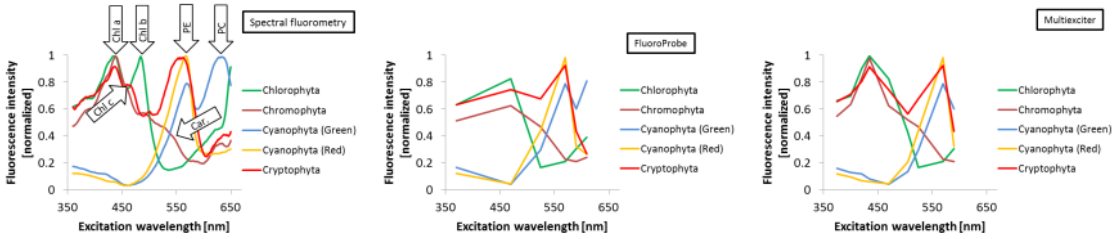

Figure 3.2.3. Example of spectral fingerprints, measured for different taxonomic pigments groups, using laboratory spectral fluorometry (left) and as simulated for wavebands for two commercial field fluorometers, FluoroProbe (middle) and Multiexciter (right).

In late 1970's it was suggested that spectral fluorescence could be used as a tool to get rapid information on phytoplankton taxonomy, at the level of pigment groups (Yentsch and Yentsch 1979). High resolution spectral fluorescence could be measured using laboratory spectrofluorometers, but they are not easily adaptable for field work. The commonly used field fluorometers for this application use LEDs as an excitation source and the number of wavebands is reduced typically from 6 to 9 bands. Within JericoNext community two brands are used, FluoroProbe or AlgaeOnlineAnayser from bbe Moeldanke and Multiexciter from JFE Advantech.

Multiexciter (JFE Advantech Co, Ltd, Japan) is a 9-wavelength LED fluorometer (375, 400, 420, 430, 470, 505, 525, 570 & 590 nm), with additional capacity to measure turbidity, temperature and depth. FluoroProbe (bbe Moldaenke GmbH, Germany) is a LED fluorometer with 6 wavelengths (370, 470, 525, 570, 590 & 610 nm), and it has additionally capacity to measure light transmission, temperature and depth (Fig. 3.2.4). Both instruments may be equipped with a mechanic wiper to prevent biofouling. Instruments provide data output as raw fluorescence data and as µg Chla L^{-1} for those taxonomic groups included in the spectral library of the software. User is able to amend or replace components in the library. Details of the software used to calculate

concentrations are well described in the user manuals (even though the algorithms used to retrieve phytoplankton groups are not detailed; Beutler et al, 2002), as well as which are the steps required for the user if changes in the original fingerprints are required. AlgaeOnlineAnalyzer, also from bbe Moldaenke, is another spectrofluorometer for phytoplankton studies, with slightly different wavebands (370, 430, 470, 525, 590 and 610). It has high sensitivity as it uses photomultiplier detection and is designed for online monitoring in flowthrough applications.

3.2.2.2. Current modes of deployment

Multiexiter may be purchased as “logger type” version for prolonged moorings without cabling, or as “cable type” for online applications. There are different versions for different depth ranges (0-50 m to 0-500 m). Instrument has a power consumption approx. 900 mW in continuous mode and has a weight 1.6 – 1.9 kg. Housing is made of titanium and the optical window is made of epoxy-acrylic resin. Instrument is designed for profiling or mooring applications. It comes with a black bucket, which may be used e.g. in reference and calibration measurements. There is no flow-through chamber available from the manufacturer, limiting the use of the instrument in ferrybox systems. The aim of SYKE is to design a flowthrough chamber for Multiexciter during the JericoNext project and carry out tests of the instrument functionality in flowthrough system. There are not many publications where Multiexciter data has been used, using a search (“instrument name” “fluorescence” “phytoplankton”) in Google Scholar 2013 onwards, only 6 references can be found.

FluoroProbe can be used both in logging mode, with internal data storage, or in online mode when connected to PC. Depending on the model, it may be operated from depth range 0-100 to range 0-1000 m. FluoroProbe weight approx. 6.4 kg. Protective housing may be added, to decrease the effect of ambient light on measurements, as well as flow-through cylinders for continuous measuring and recording. The housing is either V4A steel or carbon fibre. Flow-through chamber for continuous measuring and recording, calibration chamber and benchtop workstation for cuvettes are available. With Google scholar search, as above, tens of references can be found.
A typical deployment of spectral fluorometry is carried out parallel to other online biological observations and validation using either microscopy or HPLC analysis of discrete samples.

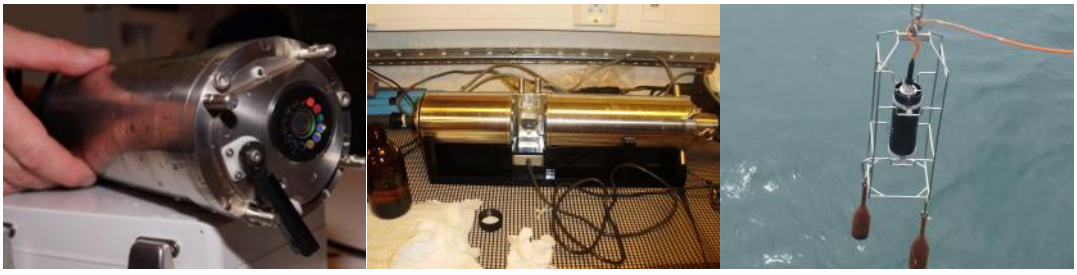

Figure. 3.2.4. Commercial spectral fluorometers: Multiexciter (left), FluoroProbe in lab measurement mode using Workstation (middle) and FluoroProbe in profiling mode (right).

3.2.2.3. Reliability of the sensor and technological difficulties

In measuring fluorescence intensities the spectral fluorometers are robust, and thus have high TLR (8-9), though the traceable calibration is lacking (see below). If we consider that the primary use of spectral fluorescence is taxonomic separation of phytoplankton pigmentary groups, then it is difficult to assess the TRL, as this step includes user decisions for data-analysis.

The use of spectral fluorescence for taxonomic separation is based on the principles of spectroscopy that fluorescence spectra is a sum of spectra of each component present and fluorescence of each component is

linearly related to its concentration. Spectral fluorometry of phytoplankton relies on Chla specific spectral fluorescence response (fluorescence intensity measured per µg Chla L^{-1}) measured for different taxonomic groups. To obtain these fluorescence spectra of the components (taxonomic groups), fingerprints, measurements from pure phytoplankton cultures are used. After obtaining the spectral fluorescence signal of the sample, non-negative least squares technique is typically the preferred method to estimate contributions of different taxonomic groups, in terms of µg Chla L^{-1} per group.

The use of fingerprints may be challenging, as optimally one 1) would need to include the fingerprints from the most represented taxonomic phytoplankton pigment groups found in the study area, 2) should not include fingerprints for those groups that are not found in the study area, 3) would need to include CDOM spectra of the study area as a separate fingerprint and 4) would not be allowed to use co-varying fingerprints. Any violation of these challenges will lead to biased results (Seppälä & Olli 2008, Houliez et al 2012, Escoffier et al 2014). The additional difficulties may arise as the obtained stable fingerprints for cultures do not really represent variability found in the nature, as 5) diversity in pigmentation, and thus spectra, between species within each pigment group is large, 6) for each species the pigment ratios, thus spectra, are adjusted as a response environmental factors and 7) variability in Chla-specific fluorescence intensity is high due to cell physiology (Seppälä & Olli 2008).

Despite the long list of limitations above, the technique may be used to track the distribution of phytoplankton taxonomic groups, or, more generally, the changes in phytoplankton community. This cannot be done at the taxonomic level acquired by microscopic-analysis or HPLC-pigments, but at very high spatiotemporal resolution, opening opportunities allow to analyze the effects of physical and chemical forcing factors on phytoplankton community structure. Spectral fluorescence data may be used to observe trends and locations in phytoplankton events, especially when combined with other information with higher precision in taxonomy. Spectral profiles may also be used as tools for finding out different phytoplankton communities, to be sampled with additional techniques. When used alone, without supporting or validation data, results from spectral fluorometry need to be handled with care.

3.2.2.4. Sensor calibration

Multiexciter is factory-calibrated at 570 nm using Rhodamine solution, while no such calibration is available for FluoroProbe. For FluoroProbe it is stated that instrument is calibrated using algae cultures, meaning that reference spectra for certain species are measured in standardized conditions. These calibrations, however, do not provide traceability and therefore raw spectral results, or estimated taxonomic phytoplankton composition, are not comparable between instruments or between data collected before and after factory calibration. This effectively prevents collection of consistent and comparable data sets and sharing the fingerprints between instruments or years of operations.

There is an urgent need to provide examples how the traceable calibration would allow combining datasets from various sources and to disseminate the results to users and manufacturers. The simplest method to get traceable results with FluoroProbe might be using the benchtop workstation and determine instrument/time-dependent factors using pure Chla in organic solvent, with a stable fluorescence signal. Multiexciter, with epoxy-acrylic optical window, is not compatible with organic solvent and this approach is not valid but other type of reference material, like Rhodamine, may be used. The challenge, using Chla or Rhodamine, is however that the fluorescence signal may be very low for some LEDs thus preventing the efficient recording of their intensity variations.

3.2.2.5. Data issues

The primary data from spectral fluorometers is simply the fluorescence intensity, with relative units. Without traceable calibration the data from different sensors remain unconnected and cannot be effectively pooled for

applications. If the traceable calibration could be settled, fluorescence may be represented in equivalents to standard fluorophores.

The taxonomic information from spectral fluorometers, using linear spectral decomposition as provided by software, is largely affected by selected fingerprints. For example, changing one of them will change the concentration of all the components. It needs to be stressed that the fluorescence spectra, i.e. fingerprint, is not constant even for single species, but it varies due to growth conditions (light, nutrition, growth phase). Thus, selection of fingerprints for spectral analysis is quite incidental and may result in biased information. At minimum, whenever taxonomic data from spectral fluorometry is reported and stored, the reference to the spectral library used should be given in metadata. As the fingerprint spectra are not stable, and cannot be interchanged between instruments due to lack of traceable calibration, also the taxonomic data from different instruments cannot be considered directly comparable.

Raw data may be analyzed with multivariate methods (e.g. PCA, PLS) with at least three different aims; 1) to derive the major independent spectral components in a given dataset and using these as alternatives for fingerprints of laboratory cultures, 2) to estimate concentration of some major components, which are known to have independent spectra, when validation samples are available and 3) in analysing spatial structures or temporal changes in phytoplankton communities (Seppälä & Olli 2008, Alexander et al. 2012, Harrison et al. 2016).

3.2.2.6. Links with other WPs and with other EU initiatives

Within JericoNEXT project we explore the different statistical methods in analysing the spectral fluorescence data using existing datasets for Multixciter and FluoroProbe (WP3.1). We aim to compare the performance of different chemometric methods and the simple classical least squares method provided by instrument software, in analysing chla concentration, taxonomic composition and the amount of CDOM. In addition we use the method in WP4.1 (JRAP#1), aiming to understand in which conditions (location, season, bloom type) the spectral fluorometry could provide additional and useful information of phytoplankton community, when compared to traditional single waveband fluorometers and to other online technologies like flowcytometry, as started to be performed within the INTERREG DYMAPHY Project (www.dymaphy.eu; Houliez et al. 2012, Lizon et al. 2015). We also try to take first steps in defining the data structures and needs of metadata, when spectral fluorescence results will be stored in the international databases (WP5). In addition to activities in JERICO-NEXT, we follow the development of new sensors, e.g. the Matrix-Flu of Trios GmbH within the EU project NEXOS. We also aim to directly disseminate our findings with instrument manufacturers.

3.2.2.7. Summary

Spectral fluorometry is a tool to obtain taxonomic information on phytoplankton community at the level of pigment groups or to analyse spatial structures in phytoplankton communities. Despite the advertisements by companies, it should be considered as a replacement of microscopy or other more detailed taxonomic techniques. The most important issue, towards wider and more reliable use of spectral fluorescence results, is to get an agreement how to provide traceable calibration for different instrument. Without this the results with different instruments, or between years, remain unconnected. Obviously this requires close cooperation between manufacturers, biologists, metrologist and data scientists.

3.2.2.8. References

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3.2.3. Fluorescence induction

3.2.3.1. Description of the instruments for measuring variable fluorescence

The traditional methods in measuring phytoplankton primary productions rely on measuring gas exchange (rate of change in O_2) or fixation of carbon (rate of ^{14}C or ^{13}C incorporation). The methods require manual sample manipulations and long incubation and therefore they are not cost-efficient. Fluorescence induction methods have been proposed as sensitive, fast and automated alternatives in measuring primary production (Kromkamp and Forster 2003, Suggett et al 2010). Fluorescence induction measurements result in several parameters describing the state of photophysiology of the phytoplankton cells. Fluorescence intensity measured for living cells depends on the rate of light absorption and quantum yield of fluorescence. Photochemistry competes efficiently for the light energy and if all reaction centers are open, minimum fluorescence (F_0) is measured. If, in turn, all reaction centres are closed, maximum fluorescence (F_M) is measured. When cells are in actinic light, e.g. at natural light levels, the steady state fluorescence (F) is measured. The mostly used indicator for the health of cells is photosystem II photochemical efficiency factor which is calculated as $(F_M-F_0)/F_M$ (Kolber et al 1998).

There are two main technques in measuring variable fluorescence: single turnover technique like Fast Repetition Rate Fluorometry (FRRF) and multiple turnover technique like Pulse Amplitude Modulation (PAM) fluorometry. In FRRF short (microseconds) intense light flashes cumulatively causes single closure of PSII reaction centres. From the fluorescence induction curve it is possible to fit different parameters, like functional absorption cross-section of PSII and connectivity parameter (Kolber et al 1998). FRRF requires very bright flashes and stringent control system, making also the instrument rather expensive. Typically there is no space for several light sources in FRRF, but the most recent versions have several excitation bands. PAM uses relatively long (milliseconds) saturating light pulses that produces several photosynthetic events (turnovers). In principle, the system can be build up at relatively low cost, but adding different features for measurements beyond F and F_M is possible.

For FRRF technique, there are two main types of instruments used in JERICO-NEXT community, FastOcean (Chelsea Technologies Group Ltd, UK) and FFL-40 (Photon System Instrument, Czech Republic) (Fig. 3.2.5) FastOcean has three excitation bands at 450, 530 and 624 nm and PMT detector, while FFL-40 has two bands at 458 and 593 nm and photodiode detector (Houliez et al 2017). Both instruments allow measurement of rapid light curves, using different levels of actinic light illuminating the sample. For PAM techniques, PhytoPAM (Heinz Walz GmbH, Germany) is used in JERICO-NEXT (Fig. 3.2.6). It is has several wavebands and using the technique similar to spectral fluorometry aims in resolving fluorescence parameters for various phytoplankton pigment groups. The new version of PhytoPAM allows also determination of functional absorption cross section of PSII. Other PAM instruments are also available (e.g. from Photon System Instrument, Czech Republic; Turner Designs, U.S.) and additional techniques measuring phytoplankton productivity with fluorescence include FIRe - Fluorescence Induction and Relaxation System (Satlantic, U.S.) and Profiling Natural Fluorescence radiometer (Biospherical Instruments Inc., U.S)

3.2.3.2. Current modes of deployment

FastOcean may be connected to profiling systems and moorings. It has a depth rating down to 600m. The whole profiling system weighs 20 kg (in air) and has approx. 5 W power consumption. It can be operated through cable connection but also autonomously using batteries and internal data logging. The sensor could be connected to Act2 system making it operational for laboratory bench applications but also for flow-through applications. With Act2 fluorescence light curves can be measured and a solenoid system is available for switching the sample during operation. FastOcean system is operated by several JericoNext partners.

FFL-40 is designed for flowthrough applications. It is self-contained system, and it includes a PC with web server having possibility for remote controlling. It is equipped with a measurement chamber and secondary chamber with actinic light. The secondary chamber is temperature regulated by sea water flow through an outer. The sample can be circulated between two chambers, to prevent settling and temperature raise during measurements. Within JericoNext community, FFL-40 is operated by SYKE.

Figure 3.2.5. Commercial FRR fluorometers: FastOcean (left) and FFL-40 (right)

Phyto-Pam is used by some JERICO-Next partners to study photochemical efficiency and photosynthetic parameters of the phytoplankton community along an environmental gradient (Houliez et al., 2012). Phyto-PAM (Walz) is designed as a laboratory instrument but may be used also in field applications. The Phyto-PAM allows to determine the content of active chlorophyll in natural surface waters down to 0.1 µg Chl/l and is used to assess the photosynthetic performance and light-adaptation status of the various types of phytoplankton with the multiple turnover technique (MT). It allows theoretically to differentiate between differently pigmented groups of micro-algae as green algae, brown algae (diatoms and dinoflagellates) and cyanobacteria. Fluorescence is excited alternatingly at high repetition rates by µsec saturation pulses of 470, 520, 645 and 665 nm light originating from light emitting diodes (LED). A miniature photomultiplier detector serves for extremely sensitive fluorescence detection. A Spherical Micro Quantum Sensor US-SQS is included to measure the photosynthetically active radiation (PAR) within the two types of cuvettes to calibrate the light list of the instrument. The modular version has two separate LED-array cones for measuring light and actinic illumination (Fig. 3.2.6), featuring a 10 x 10 mm cuvette.

A new Phyto-PAM system version with different emitter-detector units and more compact for field study is now available and allows to estimate a functional absorption cross section of PSII for different wavelengths (Schreiber et al 2012). With the new system, the photosystem II photochemical efficiency and photosynthetic parameters are not expressed by phytoplankton groups but by wavelength used. This is a more accurate methods than the first Phyto-PAM version for which physiological properties cannot be estimated by phytoplankton groups according to Houliez (PhD thesis, University of Lille 1, 2012).

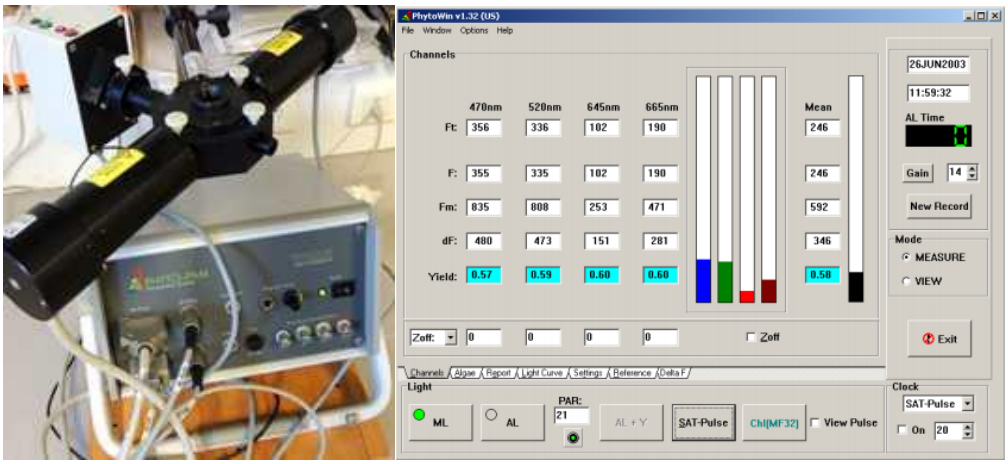

Figure 3.2.6. Commercial Phyto PAM I (Walz).

3.2.3.3. Reliability of the sensor and technological difficulties

The basic technology of FRRF and PAM for aquatic applications is mature (and high TRL of 9) but the accessories for flow-through systems are rather recent and with lower TRL. However, there is a large diversity in products from different manufacturers, in measuring principle, derived products, technical solutions and therefore also in price and reliability. While some instruments only provide indication of phytoplankton health by measuring photosystem II photochemical efficiency factor, some instruments aim to differentiate photosynthetic parameters for various taxonomic groups, using several wavebands and reference spectra or retrieve primary production rates, after careful optical characterization of the instrument.

FRRF instruments used within JERICO-NEXT community are recently designed for flow-through applications, and various difficulties have been settled (as explored within the PROTOOL FP7 project). Sequential sampling has been established, protocols for fluorescence light curves can be easily programmed and new possibilities to

use additional LEDs to excite various phytoplankton groups have emerged. But there is still work ahead; systems for keeping sample temperature at the natural level need to be elaborated, there is a need to agree on basic protocols used for light curves and we need to study further what is the added value of the new wavebands. The hardware for flow-through applications is not yet fully tested in real conditions, and experiences will be gathered during JERICO-NEXT and communicated to manufacturers.

When profiling with FRRF, near the surface data may be affected by the red photons from sunlight, limiting the use of data from upper layers in sunny days (Raateoja et al 2004a). Another drawback has been the inability of FRRFs with blue LED to measure variable fluorescence of cyanobacteria (Raateoja et al 2004b). To overcome this, additional wavebands have been suggested targeting cyanobacteria (Simis et al 2012) and such instruments have made available (Houliez 2017), but the added value of new wavebands need to be yet confirmed.

Using the variable fluorescence measured at different LEDs in estimations of photosynthetic parameters differentiated into groups strongly depends on suitable reference spectra (fingerprints). The shape of reference spectra varies between different species belonging to the same algal group but also for a given species in response to different environmental conditions. The use of reference spectra that do not correspond to the taxonomic composition and physiological status of algal groups within mixed assemblages results in significant errors in photosynthetic parameters estimations. The ability of the Phyto-PAM to differentiate the photosynthetic parameters of *P. globosa* from those of Diatom in mixed assemblages is not only dependent on the reference spectra used but also on the species concentration (Houliez 2012, PhD Thesis).

3.2.3.4. Sensor calibration

Fluorescence induction technique is based on analyzing and modelling the fluorescence increase from F_0 to F_M. To get data fitting to work properly, the light intensities and duration of pulses need to be known. This is obviously the task of manufacturer, as most of the users do not have capacity for such operations.

Blank correction is an important step in areas of high dissolved organic materials or scattering particles and low concentration of phytoplankton. This should be done using 0.2-0.4µm filtered sample water. Unfortunately in most systems the blank correction means that all the data need to be refitted after blank subtraction. Instrument response function may be determined using Chla or Rhodamine, to correct for nonlinearities in instrument response. For FastOcean, the details are included in sensor manual and in Suggett et al (2006). Details of instrument cross-talk calibration for FFL-40 are given in Houliez et al (2017). Additional calibration factor, Ka, for FRRF could be determined according to Oxborough et al (2012) and Silsbe et al (2015), which provide new possibilities in estimating concentration of functional photosystem II reaction centers, one of the major unknowns in the equations predicting primary production using fluorescence data. For PhytoPAM, the importance of light calibration is also valid and the user may perform light measurements using micro quantum sensor. The new version (PhytoPAM-II compact) also allows transfer of reference spectra between instruments.

3.2.3.5. Data issues

Variable fluorescence data is challenging. First level data includes fluorescence induction curves, where fluorescence is measured at µs-scale, including some hundreds of raw fluorescence data per curve and model output of fluorescence kinetic parameters. In second level, time series of fluorescence induction curves are measured stepwise at different light levels. Finally model will be fitted to the light curve data to obtain photosynthetic parameters (Figure 3.2.7).

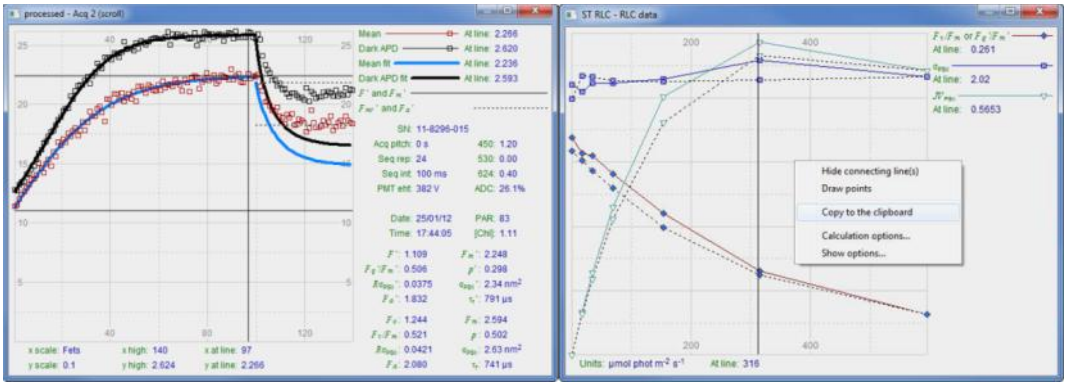

Figure 3.2.7. Examples of FRRF data. Left, example of fluorescence induction curve and right, example of fluorescence light curve.

While measuring the basic fluorescence parameters is relatively straightforward, the actual issue is that, and here the manufacturers may have different opinion, the final product from variable fluorescence measurements is not yet replacing traditional primary production estimates. Fluorescence induction methods are able to provide estimates on electron transport rate, and with some assumptions this may be converted to rates of oxygen evolution or carbon fixation, but still there are large uncertainties in this conversion (Lawrenz et al 2013).

Regarding biofouling, the instruments have the same issues as any other optical instruments. Periodic cleaning and reading of blanks is obligatory for getting reliable data.

3.2.3.6. Links with other WPs and with other EU initiatives

Within the JERICO-NEXT project (WP3.1) we aim at estimating conversion factors between electron transport rate determined with FRRF and carbon fixation rate. Our aim is to analyse the significance of diel cycles and seasonal succession on the level conversion factors for the Baltic Sea. We also use the whole array of fluorescence induction methods during JRAP#1 and JRAP#5 studies, in different parts of the European coastal seas.
Experiences gathered during JERICO-NEXT need to be communicated to manufacturers, including especially issues of additional wavebands and optimal fluorescence – light curve protocols. Moreover, the methodology for determination of calibration factor, Ka, for FRRF needs to be evaluated.

3.2.3.7. Summary

Fluorescence induction methods provide information on the health of the phytoplankton populations and, depending on the instrumentation and measuring protocol, may provide estimates of electron transport rate, photosynthetic parameters and eventually estimates of primary production rate. Information is extremely valuable when assessing the ecological status of the sea areas. Due to the diversity of technological variants, harmonization of operations is very difficult. New systems suitable for flow-through studies have emerged recently and they are used within JERICO-NEXT community in various coastal seas. It would be extremely important to communicate the best practices among users and connect with manufacturers.

3.2.3.8. References

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3.2.4. Spectral absorption

3.2.4.1. Description of the Hyperspectral Absorption Sensor

Absorption properties of water constituents are important for validation of ocean colour data and in determination of different water quality parameters. In natural waters, spectral light absorption is influenced by abundance and quality of phytoplankton, non-phytoplankton particles, coloured dissolved organic matter and coloured dissolved inorganic compounds. Measuring light absorption in situ is challenging as the concentrations are typically low and long pathlength is needed for reliable detection at all wavelengths, and secondly, light absorption needs to be separated from scattering, which is not possible using standard spectrophotometry. There are some technical solutions available to measure light absorption of natural waters in situ using either reflective and non-reflective tubings to resolve absorption and scattering or integrated cavity sphere to increase the pathlength and minimise the effect of scattering. New commercial sensor using the latter method has emerged recently, but there exist no long-term experience with the sensors yet. However, Jerico-Next community has experience of non-commercial integrated cavity technology, as presented here.

The Hyperspectral Absorption Sensor (HyAbS) is a custom-made sensor developed within the EU 7th framework programme “NeXOS” and basically a modified and advanced version of the manual or semi-automated PSICAM (point-source integrating cavity absorption meter, Röttgers et al. 2005). The aim was to build an integrating cavity device, which can be used stand-alone and fully automated on platforms and aboard ships of opportunity in combination with other automated instruments (e.g. FerryBox, Petersen et al. 2011). Hence, the integrating cavity setup had to be integrated into a software operated valve and pump system, which is necessary to provide the required liquids for automated reference and calibration measurements, a prerequisite for unattended long-term operations.

The central part of the HyAbS is the integrating cavity, which allows sensitive measurements due to a long optical path length and eliminates errors introduced by light scattering by particles. It is made from teflon and is modified with water inlet and outlets, enabling a continuous operation in flow-through mode (Fig. 3.2.8). The water enters the cavity by an inlet at the top left. The outlet at the top right allows potentially occurring air bubbles to leave, while the one at the bottom serves as an outlet for larger and heavier particles, which otherwise might accumulate in the cavity. Sample water and other necessary liquids are pumped into the cavity by a membrane pump (Flojet, Xylem, USA), guided by a system of tubes and solenoid valves (Bürkert Fluid Control Systems, Germany). The temperature of the liquid is measured by a PT1000 temperature sensor (Honeywell, USA), which is integrated into the tube system directly at the entrance of the cavity. Light is provided by a 150-W IT 3900 lamp (Illumination Technologies, USA). The light intensity within the cavity is measured in the wavelength range of 400 to 710 nm with an Avaspec-ULS2048XL UV/VIS-spectrometer (Avantes, Netherlands). All components are controlled by custom-made software based on LabVIEW running on a conventional notebook. The original calibration procedure using dye was modified and measurements are now performed using a solid standard, automatically controlled by a motor (see below).

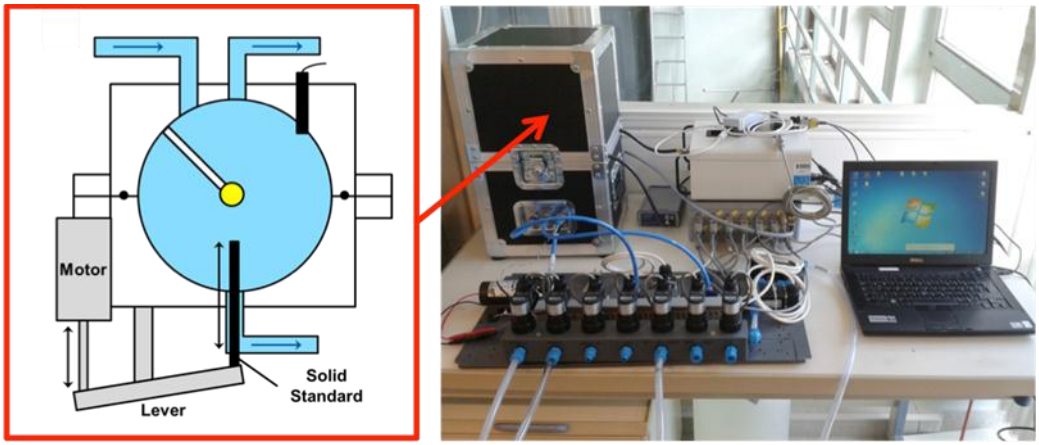

Figure 3.2.8. Lab set-up of the HyAbs and detailed technical drawing of the cavity including spectrometer (left) and single point lightsource and water in- and outlet (right).

Basically, the measurement procedure of the HyAbS follows the one of a manually operated PSICAM device (Röttgers et al. 2005). Afterwards, the transmission between the sample (seawater) and the reference (purified water) can be measured and used together with reflectivity, temperature, and salinity to calculate the absorption coefficient of the water constituents. The user is able to set up a schedule for the automated operation of the HyAbS by arranging custom program blocks. Thereby, the time and course of measurements can be defined, e.g. when calibrations are performed and when the instrument is measuring sample water. In a future version the complete measurement procedure will be remotely controlled by a FerryBox for instance.

3.2.4.2. Current modes of deployment

In the course of the HyAbS development, also field tests have been conducted to obtain information on the long-term performance of the device under realistic conditions. Two research cruises have been conducted, one aboard the R/V “Sonne” in the North Sea in September 2014, and another one aboard the R/V “Heincke” in July 2015 along the Norwegian Coast. For direct comparisons, discrete absorption measurements were performed using a conventional PSICAM. The HyAbS was continuously in operation during the whole time of both cruises (6 days and 19 days, respectively). During the first cruise, it was operated in semi-automated mode (valves and pump were manually switched to do the reference and calibration measurements), but on the second cruise, the system worked completely autonomous. No major technical problems occurred and the HyAbS provided stable and reliable measurements (for more details see Wollschläger et al. 2016).

Another test cruise has been conducted in the Baltic Sea in June 2016 after refurbishing the system from a liquid to a solid standard calibration procedure (see below). This set-up allows a complete automatic running mode over long time scales, since no liquid dye or bleach is necessary and the calibration measurements are performed by a software controlled motor. The HyAbS can be directly connected to the ships water system or any other pumped sensor system. During this cruise the HyAbS was connected to the water supply of a FerryBox and successfully tested in an automatic mode under supervision. Additionally, the FerryBox can provide the necessary salinity values for real-time computation of the absorption coefficients.

3.2.4.3. Reliability of the sensor and technological difficulties

The use of an integrating cavity device in long-term flow-through operation holds its own challenges regarding stability of the measurements, potential cavity contamination, automated handling, and data processing. Furthermore, the necessary control software has to be able to control not only the measurement unit itself, but also the valves and pumps of the flow-through environment, providing sample and reference fluids. Although

the above-mentioned test cruises provided stable and reliable results and the automatic operation of the sensor was tested successfully, there are still some improvements to be accomplished, including:

- Optimization of algorithms and expand phytoplankton species database to improve data quality.
- Implement real-time absorption spectra calculation since data are post-processed so far.
- Improve software – hardware (i.e. motor and spectrometer) communication for long-term operations.
- Sufficient supply of purified water for reference measurements.
- Integrate sensor in a compact rack for transport and safety during cruises.

The technology readiness level (TRL) is currently 'level 5' as the prototype was tested in the intended environment. Two cruises within the framework of the EU NeXOS project for validation and demonstration in May and July 2017 in the North Sea after finishing the above improvements it is expected to increase the TR-level to a successful tested prototype (TRL 6).

3.2.4.4. Sensor calibration

The basic principle of absorption measurement using the HyAbS is similar to the conventional measurement of absorption in a spectrophotometer: Light passes a volume of liquid on a defined path length, and from the ratio between the light loss measured after passing the sample (e.g. seawater) and the loss after passing a reference (usually purified water), the transmission of the sample can be calculated. However, instead of being constant like in a photometer, the optical path length within an integrating cavity is a function of its reflectivity. Therefore, the reflectivity of the integrating cavity has to be determined by a calibration measurement, which previously has been a time consuming and tedious process using nigrosin dye and purified water (MilliQ-Standard, electrical resistance >18.2 MΩ). Additionally, the cavity had to be bleached for 15 min with NaOCl solution (0.2 %) afterwards, followed by rinsing with purified water.

One of the major modifications and achievements is the replacement of the nigrosin solution with a solid standard during the calibration. This solid standard is basically a black plastic stick, which can be moved into the cavity automatically via a motor. The solid standard calibration improved the system in several ways: (i) Space requirements are reduced, because the containers for the calibration liquid as well as for the bleach are no longer necessary. (ii) The operation period can be prolonged, since the system is now independent of a regular dye supply. (iii) Calibrations can be performed easily at every reference measurement, and time otherwise lost due to the need for cavity bleaching can be used for measurements. Since the reflectivity can vary over time due to e.g. particles sticking at the cavity walls, it is important to repeat the calibration from time to time to obtain accurate absorption coefficient measurements. In summary, quantity and quality of the data was substantially enhanced and calibration procedures can be performed automatically.

However, due to the continuous sampling principle, reference measurements cannot be conducted according to each sample measurement, as it would be the case when using a manually operated PSICAM. Instead, they have to be performed in larger time intervals. For the time in between, reference values are linearly interpolated based on these measurements. Calibration of the HyAbS has to be performed at least once per day, but to account for reflectivity changes due to cavity contamination it should be performed as often as possible, especially in particle-laden (coastal) waters. Like the reference measurements, also the values obtained for reflectivity are interpolated linearly to obtain variables for absorption coefficient calculation in between the calibrations. Furthermore, the calculations of the absorption coefficient have to be corrected for the influence of temperature and salinity on the absorption of the water itself.

3.2.4.5. Data issues

The data produced from the HyAbS is threefold (Fig. 3.2.9). The primary data provided by the HyAbS are absorption coefficient spectra in the range of the visible light (400-710 nm) with a resolution of 2 nm. These

spectra are the combined absorption spectra of the dissolved fraction (CDOM), and the particulate fraction (including phytoplankton and its pigments). From these absorption spectra, secondary parameters are derived: Since the pigment absorption coefficient in the red chlorophyll-*a* absorption peak (a_{pigm} 676 nm) is linearly correlated with the chl-*a* concentration present in the water this value can be used as a proxy for phytoplankton biomass. It can be derived from the total absorption coefficient of the constituents at this wavelength (a_{p+cdom} 676 nm) by assuming the influence of CDOM as negligible in the red spectral region ($a_{p+cdom} = a_p$) and subtracting the coefficient measured at 700 nm to account for the absorption of the non-pigmented matter in the sample:

$$a_{pigm}\ 676\ nm = a_{p+cdom}\ 676\ nm - a_{p+cdom}700\ nm$$

Similarly, the particle absorption coefficient at 700 nm (a_p 700 nm) can be used as a proxy for total suspended matter (TSM, Fig. 3.2.9). In the current version of the HyAbS software both parameters are directly derived from the absorption spectrum using the appropriate equations.

Additionally, the hyperspectral absorption data provides an indicator for detecting differences in the phytoplankton community composition. Generally, the shape of absorption spectra is largely influenced by the phytoplankton pigments present in the investigated water sample. Thereby, it is possible to differentiate groups present by characteristic features visible in the hyperspectral absorption coefficient data (Fig. 3.2.9). A common procedure to enhance these features is the calculation of the fourth derivative of the spectra. In order to provide additionally also taxonomically relevant information, the degree of similarity of the measured spectrum can be compared to the most likely spectrum of a reference database.

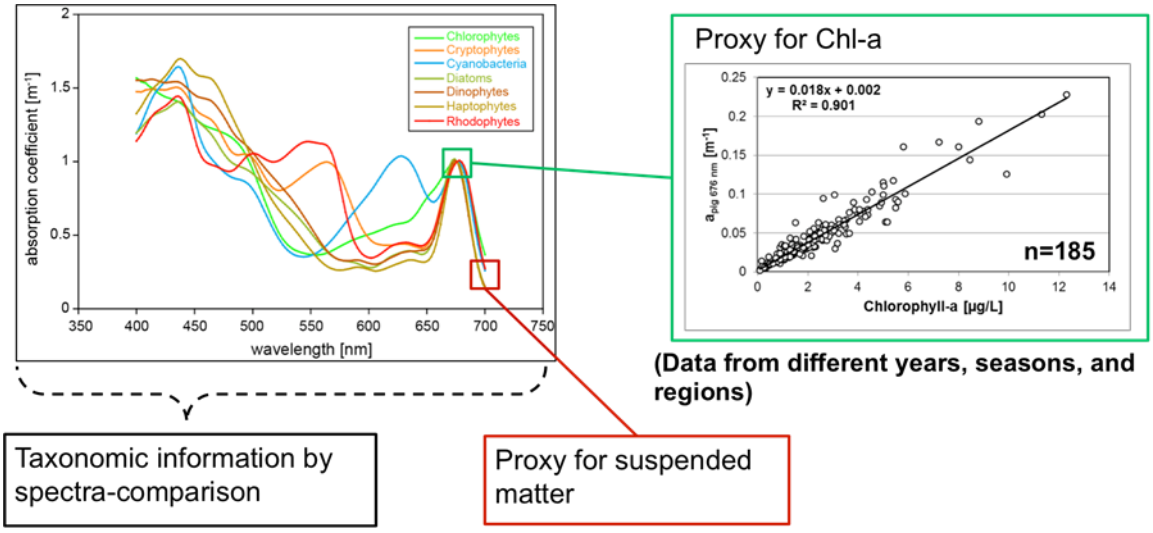

Figure 3.2.9. Data output from the HyAbS. Colored lines indicate measured absorption spectra of different phytoplankton groups. Green box: Proxy of chlorophyll-a as a measure of phytoplankton biomass. The black line indicates the correlation between HyAbs absorption coefficient measurements (y-Axis) and HPLC measurements of Chlorophyll-a (x-Axis).

3.2.4.6. Links with other WPs and with other EU initiatives

A strong and fundamental link exists to the EU-project NeXOS in which framework most of the basic modification from the previous PSICAM version and refurbishment have been conducted. Two cruises for validation and demonstration will be done in May and July 2017. Additionally, further sensor development and the HyAbS integration into the FerryBox will be performed in the work package 3.1. of the JERICO-NEXT

project. Finally, there is also a potential link to work package 3.4. including the detection of GeoHABs due to the HyAbS capability to detect different phytoplankton species. New commercial OSCAR sensor will be evaluated in tasks 3.1 and in JRAP#1.

3.2.4.7. Summary

Spectral absorption measurements with the HyAbS provide reliable proxies of phytoplankton biomass, suspended particles in the water column and the phytoplankton species distribution. Using a solid standard calibration procedure instead of a liquid dye allows a fully automatization of the measurement. This allows connecting the sensor to FerryBox systems on ships of opportunity without supervision and obtain data in real-time.

3.2.4.8. References

Petersen, W., Schroeder, F., Bockelmann, F.D., 2011. FerryBox - Application of continuous water quality observations along transects in the North Sea. Ocean Dynamics 61 (10), 1541-1554.

Röttgers, R., Schönfeld, W., Kipp, P.R., Doerffer, R., 2005. Practical test of a point-source integrating cavity absorption meter: the performance of different collector assemblies. Applied Optics 44 (26), 5549-5560.

Wollschläger, J., Voß, D., Zielinski, O., Petersen W. (2016) In situ observations of biological and environmental parameters by means of optics – Development of next ocean sensors with special focus on an integrating cavity approach. IEEE Journal of Ocean Engineering 99: 1-10.

3.2.5. Spectral reflectance

3.2.5.1. Description of the radiance and irradiance sensors

Hyperspectral radiance and irradiance sensors can be used as a method to estimate as reflectance above water to be used e.g. to validate level 2 satellite products. For this purpose,

- an irradiance sensor is mounted towards zenith providing the total (diffuse and direct) downwelling light Ed
- a radiance sensor looking towards water provides upwelling light from the sea and sea surface, L t
- another radiance sensor pointing towards the sky provides information for correcting sky contributions from the upwelling component, Ls

Both radiance sensors are mounted with the same azimuth angle (φ) in order to provide measurements from associated areas on sea surface and sky. Azimuth angle φ relative to sun should be close to 135°, and θ relative to zenith close to 40° in order to minimize sun glint and shadowing effects (Mobley, 1999) (Fig. 3.2.10). Upwelling measurements must be corrected for sky reflection on sea surface leading to the water leaving radiance

$$L_w^{\uparrow} = L^{\uparrow} - R(\theta) L^{\downarrow}$$

Where R is the Fresnel coefficient, a function of solar and sensor zenith angles as well as wind at sea surface. More elaborate coefficients can be used instead, such as the POLREF coefficient, which also include the effects of light polarization on aerosols and sea surface. Marine reflectance is determined by normalizing with sky irradiance measurements

$$\rho_w^{\uparrow} = \pi L_w^{\uparrow} / E_0^{\downarrow}(\theta_s)$$

This result can be used in satellite validation techniques. However, as for remote sensing, many other parameters such as TSM or Chl-a can be derived from these measurements using the adequate algorithms.

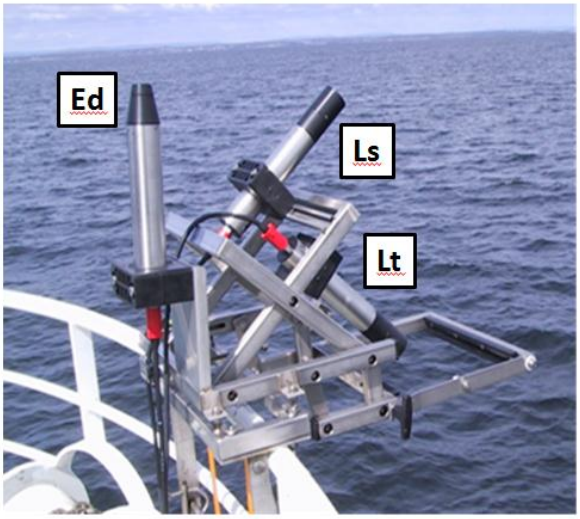

Figure 3.2.10. Typical setup of the three sensors involved in the measurents.

Sensors typically used for shipborne applications are three RAMSES sensors (TriOS Optical Sensors, Germany), which have 3.3-nm spectral resolution. Such systems are either passively looking from ships bow or are automatically rotated follow Sun (Simis and Olsson 2013). In the AERONET-Ocean Colour network, installed on fixed platforms CE-318 autonomous sun photometer is used to track sun and perform all measurements. These SeaPRISM systems have fixed wavebands at 412, 443, 488, 531, 551 and 667 nm (Zibordi et al 2009).

3.2.5.2. Current modes of deployment

The sensors must be installed on the platform as close as possible to the configuration above (Fig. 3.2.10). The measurements and installation can be on a research vessel, fixed platforms and on moving ship like in connection with a FerryBox installation. For moving platforms, a constant azimuth angle relative to sun is not always easy to achieve. Therefore, heading measurements are required. For smaller platforms zenith angles may also change and altitude measurements must be provided. Wind measurements are also needed.

Installed sensors should not point towards a shadow, a structure or sea surface disturbances. On moving platforms, sensors should point outside the generated bow wave. Similar disturbances may occur in rivers or strong current areas. Installation examples on board moving vessels are shown in Figure 3.2.11. Several sets of sensors are used on some vessels in order to increase requirement occurrences of relative azimuth and zenith angles.

3.2.5.3. Reliability of the sensor and technological difficulties

The sensors shown in the example above are less reliable at the edges of the measured spectrum. However, the largest difficulties in such measurements are to achieve ideal conditions, such as weather and sea conditions, sensor angles relative to sun, and of course the correct choice of the Fresnel Coefficient.

3.2.5.4. Sensor calibration

Sensors are calibrated on an optical table with a NIST traceable lamp. Example of radiance and irradiance sensor calibrations are shown in figure 3.2.12. Temperature and current (lamp intensity) must be very stable during the calibration process. This is usually performed in a recognized laboratory. For some sensors, the

need of calibration can be monitored with a field control lamp providing a known spectrum. However, this is also temperature dependent and should be performed in a controlled environment rather than in the field.

3.2.5.5. Data issues

The amount of data can be quite large as sensors may have several hundreds wavelength channel each. Quality control in order to remove inappropriate measurements should include:

- Measurements performed in low or to high (sun glint) light conditions
- Measurements performed in shadow
- Measurements performed in variable conditions (sky or sea condition, platform)

3.2.5.6. Links with other WPs and with other EU initiatives

These types of measurements are used in the EU-project HighROC and in the ESA validation activities connected to the Sentinel 2 and 3 validation. NIVA are developing tool in an ESA project called VAMP and its follow up.

Figure 3.2.11. Left, top to bottom: (1) irradiance sensor on top of mast installed on ship (C), (2) downward looking radiance sensors below antenna dome on ship (B), upward looking radiance sensors on ship (B) and upward looking sensors on ship (C). Right, ship installations. Top to bottom: (A) 1 pair of radiance sensors is placed on the back and irradiance sensor in the mast. (B) 2 pairs of radiance sensors, one looking forward and the other backward. The downward looking sensors are placed below the antenna dome. (C) 2 pairs of radiance sensors, one looking on starboard and the other on port side. The downward looking sensors are placed just below the bridge all other instruments being installed on the monkey deck.

Figure 3.2.12. Example of the optical table used for irradiance sensor calibration.

3.2.5.7. Summary

Light measurements above water can be used to determine some biogeochemical parameters at the sea surface. This involves measurements from different instruments and sensors oriented in specific directions towards the sky and sea surface. Measurements must be corrected for reflection at sea surface and polarization effects of light within the atmosphere. The final parameters of interest are derived from special algorithms applied on the corrected measurements.

3.2.5.8. References

Mobley, C.D. (1999) Estimation of the remote sensing reflectance from above–water methods. Appl. Optics, 7442-7455.

Simis, S.G.H., Olsson, J. (2013) Unattended processing of shipborne hyperspectral reflectance measurements. Remote Sensing of Environment 135: 202–212

Zibordi, G. Holben, B., Slutsker, I., Giles, D., D'Alimonte, D., Mélin, F., Berthon J-F., Vandemark, D., Feng, H., Schuster, G., Fabbri, B.E., Kaitala, S. & Seppälä, J. (2009) AERONET-OC: a network for the validation of Ocean Color primary radiometric products. Journal of Atmospherical and Oceanic Technology, 26: 1634-1651

3.2.6. Turbidity and scattering

3.2.6.1. Description of the scattering and turbidity sensors

Turbidity or measurements of scattering gives an indication of the amounts of particles in the water and is a good proxy for the total suspended material (TSM). TSM in open water include phytoplankton and particles due to runoff from land and re-suspension of bottom sediments. Turbidity sensors are often one of the core sensors in FerryBox installations.

The measuring principle should follow the ISO standard EN-ISO 7027:1999 as close as possible. This standard is a Nephelometric laboratory method based on a 90° (+/-2.5°) scattering at 860 nm (+/- 10-15 nm) wavelength detection. The recommended turbidity range with this principle is 0-40 FNU (Formazin Nephelometric Units), but linearity up to 100 FNU are found (Sørensen, unpubl.). Some sensor on the marked are using another wavelength e.g. in the blue part of the spectrum, which can be influenced by high absorption of dissolved organic material. Older turbidity laboratory methods used tungsten lamps where the blue part of the spectrum was dominating.

Widely used measurement unit for turbidity is the Formazin Turbidity Unit (FTU) and Nephelometric Turbidity Units (NTU), but they are in practice equivalent if the same measurement principle and calibration are used. The introduction of the ISO standard 7027:1999 the unit changed to be FNU (Formazin Nephelometric Units) since one introduced the LED measuring wavelength at 860 nm. Using the new standard with NIR LED reduce the influence of high absorbing water (colour), but the it is not a large problem in open sea water.

The sensors used among the JERICO-NEXT partners are summarised in the table 3.2.2 below. The models are slightly different in size and some have a wiper as a standard, which is recommended (Fig. 3.2.13). Alternative cleaning is also used by applying high-pressure air to the sensor head, regular acid washing or other biofouling methods.

Sensor	Manufacturer	Measurement principle
ECO FLNTU	WETLabs	light scattering 700 nm
Turbidity sensor	SeaPoint Sensor Inc	light scattering 880 nm
Turbidity sensor	Polymetron sensor (out of production)	light scattering 880 nm
Scufa II	Turner design (USA)	light scattering (blue)
CUS31-W2A	Endress & Hauser (Germany)	light scattering (red)
Cyclops	Turner design (USA)	light scattering 850 nm
Turbidity Xchange Sensor	AML Oceanographic Instruments	light scattering 880 nm

Table 3.2.2. Overview of the most common sensors used within the JeriCO partners.

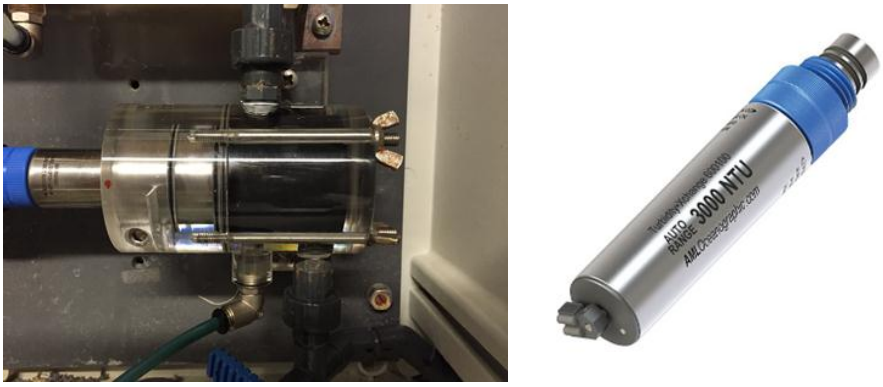

Figure 3.2.13: AML turbidity sensor without wiper in a black cuvette with air pressure cleaning (left) and a sensor with a wiper (right). The sensor with a wiper in a black cuvette is now standard at NIVAs FerryBox installations.

Other related measuring principle used for water clarity or studies of the inherent optical properties (IOP) of the sea is attenuation (transmissiometers). This principle gives the beam attenuation (*c*) at different wavelength like in the Wetlabs ac9-instrument that measures both scattering (*b*) and absorption (*a*) at 9 wavelengths. The backscattering coefficient (*bb*) is more related to the turbidity and several small *bb*-meters like Wetlabs ECO BB (BB3) are available, but the specifications are different with a different measurements angles (124°) and different wavelengths (e.g. 470, 532 or 650 nm) than the recommendation in the ISO-standard. A model Wetlabs ECO NTU is using a wavelength of 700 nm, which is closer to the recommended wavelength and the model ECO FLNTU combining Chla fluorescence and scattering are in used by many Jerico-Next partners (TBC).

3.2.6.2. Current modes of deployment

The turbidity sensors can be used on all platforms like FerryBox, fixed platform, buoys and gliders and they have low power consumption, are small and easy to use. They are normally standard sensors also on CTD used for traditional research cruises. On FerryBox they are mounted in the water stream together with the other optical sensors and work fine as long as air bubbles are prevented to reach the sensor, using a debubbling unit if needed. When used in a flow through systems with cuvettes, back scattering and reflection from surfaces must be prevented. It is recommended to use a sensor head with a wiper to prevent biofouling and also remove micro air bubbles that can attach to the sensor head.

3.2.6.3. Reliability of the sensor and technological difficulties

All the sensors described in the table above have been on the marked for some time and are on a Technological Readiness Level, TRL 9. They are robust, easy to install and maintain in an operational environment. They are widely used for monitoring of water quality, dredging operation and as proxies for calculation of TSM for e.g. remote sensing product validation. The biofouling should be taken care of for long time installation and a wiper are recommended (Fig. 3.2.14)

Figure 3.2.14. Scufa II turbidity sensor installed on a FerryBox (left)with visible biofouling in the flow through system and a FLNTU sensor with an antifouling copper wiper (right) recovered after 8 months of operation in fixed station (E1M3A-Cretan Sea).

3.2.6.4. Sensor calibration

One should follow the calibration operation recommendation from the manufacturer and the calibration should be based on the ISO-standard using the Formazin Turbidity Standard which can be purchased in 4000 FTU concentration, but can also be prepared from chemicals in your own lab (see ISO-standard). We recommend to use the small HACH 2100P instrument or similar to check prepared standards and to compare with the in situ sensors. This gives the best traceability for long term control of the sensors. Be careful with the measuring cuvettes in the manual laboratory methods and clean for humidity outside the cuvette. Be aware of large floating particles (zooplankton) that give errors in the readings. An example of calibration setup and results are shown in Figure 3.2.15

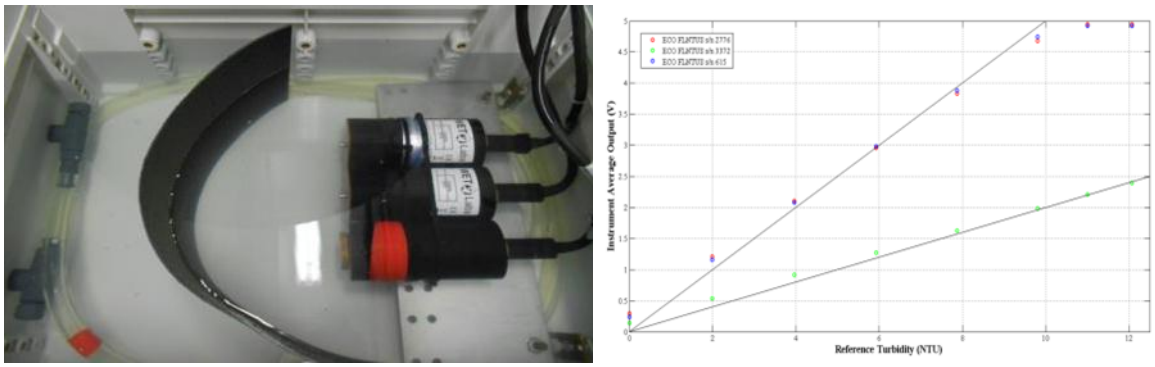

Figure 3.2.15. Wetlabs FLNTU turbidity laboratory linearity validation set up (left) and data using different concentrations of Formazin Turbidity Standard (right).

3.2.6.5. Data issues

If the sensor is used as described in protocols the data quality should be high. One should be aware of interference from air bubbles in flow through systems since small bubbles will easily cause scattering giving wrong values or at least spikes of turbidity. Use the QC-routines of spike test, frozen values and other relevant tests. If no wiper or cleaning are used biofouling will after a while cause wrong data.

If the turbidity is to be used for calculating TSM certain tests to establish the Turbidity/TSM relation should be performed. One often reports this ratio to be close to 1, but this must be controlled since deviations from this general conversion factor are found.

Metadata information of the sensor specifications of e.g. wavelength and scattering angles are important to report since this influence the optical signal (turbidity) from different particles types. Use standard Formazin solution to calibrate the sensor.

3.2.6.6. Links with other WPs and with other EU initiatives

The sensor is standard on most FerryBox installations and will give additional variable to understand the particle load to the area where the JARPs need particle data. The sensor data are used in the satellite EU-project like e.g. HighROC, JMP-EUNOSAT as a proxy for TSM.

3.2.6.7. Summary

Turbidity sensors are commonly used in several installations and represent a core sensor in e.g a FerryBox installation. The maintenance procedures and calibration are straight forward and the data should be of high quality if the recommendations are followed.

3.2.7. Pulse shape-recording (scanning) automated flow cytometry

3.2.7.1. Description of the automated flow cytometry

The principle of the flow cytometer (FCM) analysis is based on three interoperating systems: Optical, Fluidics and Electronics. In most of the cases, manufacturers built their machines based on a two-fluidic system (sheath fluid, which drives the sample through a capillary section) coupled to an optical system (Photomultiplier, PMT) for particle detection after light deviation at the intersection between a laser beam (of a certain wavelength and power) and the particles passing one by one (single-cells as well as colonies). In the last decades, three automated cytometers were implemented to monitor aquatic ecosystems at high frequency and *in situ*: the Seaflow (Swalwell et al. 2011), the Imaging FlowCytobot (IFCB) (Olson and Sosik 2007) and the Cytobuoy FCMs (Dubelaar et al. 1999). Amongst them the pulse shape-recording cytometer (PSR FCM) from the Cytobuoy® company (also known as Scanning Flow Cytometer-SFCM), is the only one to our knowledge which offers the possibility of automatically recording the optical pulse shape of every particle (integrating the whole set of the corresponding optical features) passing through a laser beam.

Within the PSR FCM, the sample is collected by a peristaltic pump and injected into the fluidics part. Then, the sample and the sheath fluid (which corresponds to 0.4µm then 0.1µm-filtered seawater continuously running and recycled inside the flow cytometer) meet themselves and the flow of particle becomes separated within a laminar single row.

An optical system is equipped by one or two lasers to discriminate phytoplankton pigments with different power to be considered (15 to 75 mV). The laser configuration depends on what species dominate in studied area and what is the target of investigation.

Laser-particle intersection gives five variables for each particle (single-cells or colonies): two of them result on light deviation (Forward scatter, FWS for size; and Sideward scatter, SWS for the shape), and three result on light emission (red, orange and yellow fluorescence, respectively called FLR for Chlorophyll *a,* FLO for phycocyanin and phycoerythrin and FLY for pheopigments) (Fig. 3.2.16). An extra variable gives information about how particles are curved: the curvature. Beside signal length (“Length”), maximum height (“Maximum”), average height (“Average”) and time integrated signal height (“Total”), several others derivative signal characteristics are computed/extracted from each variable such as “Inertia”, “Fill factor”, “Center of Gravity”,

"Asymmetry" and "Number of Cells". The PSR CFM is designed to analyze phytoplankton community from picoplankton to microphytoplankton with a size range from one to eight hundred micrometers (Dubelaar and Gerritzen 2000).

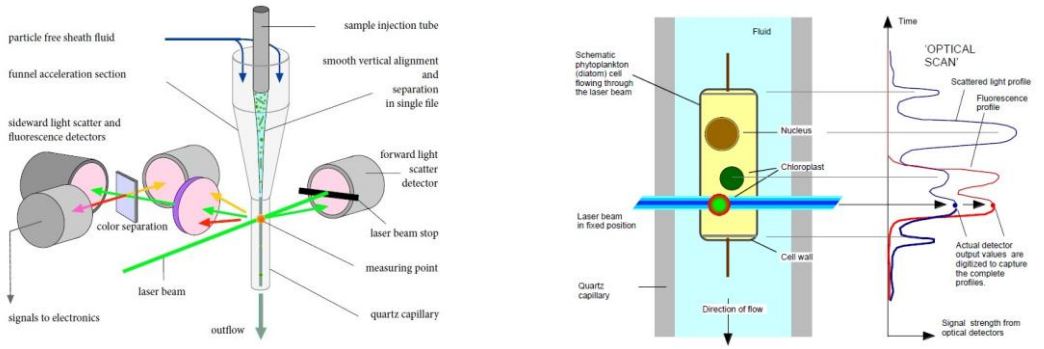

Figure 3.2.16. Principle of (left) analysis and (right) scanning of Cytobuoy Flow cytometer (Dubelaar and Jonker 2000, http://www.cytobuoy.com/).

3.2.7.2. Current modes of deployment

Three versions of PSR FCM are on the market. The CytoSense (benchtop) is designed for lab experimentation, analysis of discrete samples or continuous measurement from fixed depths through a pumping system, onboard research vessels or fixed platforms. The CytoSub differs from the CytoSense by a special hull resistant to depth, which makes it submersible and operational up to two hundred meters' depth with two different pressure modules for the hull (one for shallow water, up to twenty meters and another for deep waters, up to two hundred meters). The CytoSub is also used for submersible data recording (Thyssen et al. 2008) which is developed in the next part. The PSR FCMs can be specially optimized for buoy implementation: the CytoSense can be installed in a box (e.g. EOL Buoy in the Bay of Villefranche, Thyssen et al. 2014) and/or a dedicated system can be implemented, consisting in a buoy powered by solar panels with the CytoSub inside (becoming a CytoBuoy configuration). Thanks to the three possible configurations, the PSR flow cytometers can be implemented in different ways (Fig. 3.2.17), as a benchtop system (CytoSense); for continuous measuring onboard Research Vessels or Ships of opportunity (CytoSense and, for profiles, CytoSub), on dry platforms (CytoSense or CytoSub), on wet platforms (CytoSub) and Buoy (CytoBuoy).

The benchtop version has been deployed in several surveys (Rutten et al. 2005, Guiselin 2010, Bergkvist et al. 2012, Bonato *et al.* 2016, Breton et al. 2017). "Ships of opportunity" or research vessels are implemented with the CytoSense, which is then connected to the vessel's subsurface water inflow system for thermosalinographs temperature and salinity sensors or to a dedicated inflow system (Thyssen et al. 2009, Bonato *et al.* 2015, Thyssen et al. 2015). PSR flow cytometers have been used on fixed platforms (Pomati et al. 2011, Dugenne et al. 2014) and buoys (Thyssen *et al.* 2014). Thyssen *et al.* (2008) used their CytoSub in two different configurations: as continuous recorder on ship and as submersible profiler. A CytoBuoy has been used on a coastal buoy (Pereira and Ebecken 2011)

Figure 3.2.17. Different implementations of PSR Flow Cytometers. From left to right: DPHYMA cruise (DYMAPHY project, Eastern English Channel, April 2012) (Bonato, et al., 2015), EOL buoy (Bay of Villefranche sur mer)*(Thyssen, et al., 2014), CytoSub in Monterey Bay (Rines, 2010), Lake Lugano (Switzerland) (Pomati et al., 2011).*

3.2.7.3. Reliability of the sensor and technological difficulties

Specially designed for automated measurements and for phytoplankton autofluorescence detection the PSR FCM needs a continuous water and power supply only to work (except for the Cytobuoy device which is powered by solar panel). In order to maximize the amount of cells or colonies analyzed, it is necessary to be able to eliminate the most of non-fluorescent particles, because instrument memory is limited. This can be solved by applying a threshold on red fluorescence (= trigger level) during the recording. This step involving in increasing or decreasing to the red fluorescence sensitivity is necessary but generally takes only few minutes and is only needed for new studied area. Two or more thresholds are also applied to maximize phytoplankton detection (see manuals of operational procedures for applying automated flow cytometry released within the DYMAPHY INTERREG project, www.dymaphy.eu, Rutten et al., 2013; 2014). A low trigger maximizes pico- and nanophytoplankton detection whereas a high trigger is better for microphytoplankton as well as eutrophic waters (Creach et al. 2012) and oligotrophic areas (Thyssen et al. 2008, Thyssen et al. 2014).

For each type of deployment modes, the sheath fluid has to be as close as possible to the sample (i.e. Distilled water for freshwater analysis and filtered seawater for salty analysis), this reduces light scattering at the intersection with the laser beam (see Rutten et al. 2014 for more details). In addition, attention should be made to the accumulation of salty water on tubbing, as it might clogg the filters and cause a blast of tubbing of the cytometer. To prevent this accumulation, the two fluidics systems have to be cleaned regularly (at least twice a month for continuous recording and more for lab) with Milli-Q® water.

Minor issues require a good knowledge of the tool. Flow cytometers are composed of three interoperable systems (fluidics, optical and electronic system) and problems in the fluidics may have consequences on sensors (temperature, pressure) but they could also impact the particle detection (over or underestimation of signals). In addition, some technological limitations were detected within the Imaging in Flow System in some devices. Because of a non-optimal resolution (1.6 pixels per micrometer) of the camera, high quality pictures are difficult to obtain. This limitation should be avoided with the new camera (3.3 pixels per micrometer) that is being built.

3.2.7.4. Sensor calibration

In order to get a good calibration and validation, it is necessary to use calibration beads for laser alignment, size calibration and fluorescence calibration. With a known size for beads, a ratio between real size (Beads manufacturer) and estimated size (Length FWS from Cytometers) is calculated, and then the estimated size-correction factor can be applied to each particle to get accurate cell or colony size which could help discriminating between phytoplankton size-groups (Pico-, Nano- and Microphytoplankton). These beads are fluorescent to get detected by the fluorescence detectors (PMT) and serve to normalize fluorescence from particles.

PSR flow cytometers can work as single tools and can be compared with other instruments in order to calibrate, complete and improve their discrimination of phytoplankton groups, genus, and, in some cases, up to species and lifeforms:

- Pico- and nanoplankton concentration with flow cytometer and/or microscopy. Rutten et al. (2005) compared abundances from optical microscopy and PSR FCM. Strong and correlation was found (R^2 = 0.8). Thyssen et al. (2008) compared conventional cytometer with the CytoSub by using 3.6 µm and 10 µm beads. Significant and strong correlations were found in the two case (R^2 = 0.95 for 3.6 µm beads and R^2 = 0.99 for 10 µm beads with both $p < 0.001$).
- Pigmentary values could be compared between HPLC and PSR FM fluorescence. During the DYPHYMA cruise (DYMAPHY project, Artigas et al. 2015), HPLC and PSR FCM showed a high and significant correlation coefficient ($r = 0.89$; $p < 0.001$). Other comparisons made during this campaign are presented in Table 3.2.3.
- Biomass values by multi-spectral fluorescence analysis (Houliez et al. 2012, Thyssen et al. 2015). E. Houliez compared biomass values estimated by the Fluoroprobe® and cell counts the PSR FCM and correlation is $r = 0.89$; $p < 0.001$).
- Some nano- and microplankton genera and species identification and counts and cell biovolumes by coupled or parallel image inflow analysis, light and scanning electron microscopy, etc (Artigas et al. 2015, Rutten et al 2013).
- A comparison made by Thyssen et al. (2015) between red fluorescence from PSR FCM and Turner fluorometer, HPLC and AOA fluorometer (Fig. 2.3.18) showed a very strong correlations. These results were upper than 0.8 and very significant ($p < 0.01$).

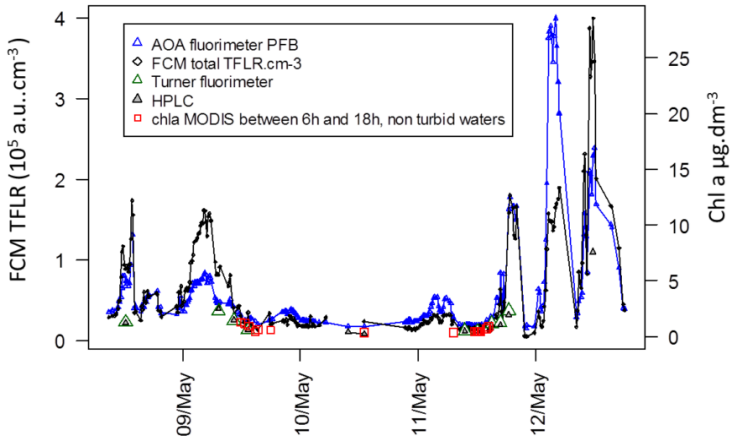

Figure 3.2.18. PSR FCM total FLR compared to Chl a (Thyssen et al. 2015).

Spearman's correlation coefficient	Chla_Extr. fluo $n = 20$	Chla_HPLC $n = 20$	Chla_FluoroProbe $n = 13$	Fluorescence _CTD $n = 17$	Fluorescence _Cytometer $n = 17$	Chla_fluorescence "Optical Probes Package" $n = 16$
Chla_Extraction	1	0.88 ***	0.92 ***	0.77 **	0.89 ***	0.84 ***
Chla_HPLC		1	0.87 **	0.75 **	0.86 ***	0.83 ***
Chla_FluoroProbe			1	0.99 ***	0.92 ***	0.99 ***
Fluorescence_CTD				1	0.79 **	0.98 ***
Fluorescence _Cytometer					1	0.92 ***
Chla_fluorescence "Optical Probes Package"						1

Table 3.2.3. Spearman correlations between sensors during DYPHYMA cruise (Artigas et al. 2015).

Inter-calibration exercises with different PSR FCMs need to be carried out in order to check if data gathered with two different machines can be easily comparable. This is an important issue to be considered because the machines can differ on different parts: lasers (one or two) can be different (460 or 488 or 532 or 561 nm for the first and 445 or 635 or 640 or 660) with different power to be considered (15 to 75 mV). A calibration of each machine is also frequently needed because labs do not trigger on the same level and the same parameters. Furthermore, the sensitivity of the PMTs can be, in the new versions of the machines, modified manually (from 0 to 110) for each parameter (SWS, FLO, FLR, FLY) in the same time. So every scientist should be able to define the best settings for the studied area and period considered. Further information is available within the DYMAPHY project reports (Rutten, 2010 and Rutten, 2014).

Moreover, the size-range considered by this type of flow cytometer is 1 µm up to 800 µm. However, the thickness of the laser beam is 5 µm so size for the picoplankton less than 5 µm is almost overestimated (Guiselin, 2010, Rutten, 2013). And finally, these tools provide information about size, internal composition, fluorescent content, and can provide, if coupled to a imaging inflow system, images which can help discriminating up to genus or sometimes species and can help size calibration of big cells (Dugenne et al. 2014).

3.2.7.5. Data issues

One major problem with the data reliability is the significance of abundance estimation. Clusters composed by smallest particles are relevant, because they are strongly represented, but clusters composed by biggest cells (diatoms and/or dinoflagellates) often contribute less than 1% to the total concentration. In that case, abundance and fluorescence levels calculated from these clusters may not be significant. To overcome this problem, a high trigger level is applied as well as a longer acquisition time, in addition to the low trigger level which allows to detect and count picro- and nanoplankton cells (Rutten et al. 2014, Thyssen et al. 2008).

To get proccessed data, Cytoclus software is used to plot a combination of variables (SWS, FWS, FLO, FLR, FLY) and parameters (features) based on optical section for each particles (Length, Total, Maximum, Mean, Inertia, Centre of gravity, Fill factor, Asymetry, Number of humps, TOF). Clusters are then drawn with shape-similar particles with similar fluorescence too. Results can be exported per cluster or per cell. Working by cluster is usefull for monitoring phytoplankton dynamics and following the composition whereas working at the single cell level is helpfull to understand functionnal diversity in each cluster and maybe to discriminate groups in a cluster by post-analysis (Krause et al. 2014).

Although manual analysis of samples through the use of CytoClus software remains the most widely used method, there are today automated or semi-automated recognition tools/approaches allowing to identify phytoplankton groups, thanks to their optical profiles (Caillault et al., 2009; Hébert et al., 2015; Poisson-Caillault et al., 2015) or some extracted characteristics (Malkassian et al., 2011). These approaches are based on machine learning methods for which some algorithms have been optimized during the DYMAPHY project and included into a commercially available software (EasyClus, Rutten, 2010) as well as a free “R” toolbox (DYMAPHY, Caillault et al., 2015). Different categories of classification methods are implemented in this tool, which can be applied according to the quantity of a priori knowledge used in the classification process.

Moreover, in terms of colonial organisms, it is possible to identify but not yet count individual cells of colonies with the CytoSense system. Even though the colonies substantially contribute to annual productivity, the set of biomass estimators are essentially calibrated in terms of cells per volume unit. Today, the flow cytometer coupled with CytoClus software is able to distinguish chain-forming diatoms since the repetitiveness of chains provides distinctive pulse shapes. But in the case of more complex colonial forms, it is necessary to resort to advanced automatic methods. In the LOG-CNRS lab, work is conducted to address this problem of (semi-) automated enumeration and classification of colonies (which includes the haptophyte *Phaeocystis globosa* and chain-forming diatoms that are the dominant members of the phytoplankton community in the English Channel) in terms of how cells of differing chain lengths affect the pulse shape observed with the CytoSense.

3.2.7.6. Links with other WPs and with other EU initiatives

Within the JERICO-Next project, partners and international experts discussed during two Workshops in Wimereux (June 2016) and in Gothenburg (September 2016) about the state of the art and previous work and results gathered during recent European projects as JERICO (FP7), DYMPAHY (Interreg IVA "2 Seas"), Protocol (FP7)„etc. Strategy for inter comparing the different techniques and devices, and for improving both their implementation and data analysis, were discussed, which represents the basis of ongoing WP3.1 for building an automated platform for phytoplankton analysis based on optical automated methods.

Links are also made with JRAP #1 (WP4.1), which four main questions are considered: (1) to get closer to resolve natural variability in plankton dynamics, (2) to improve the understanding of algal blooms (and HAB), (3) to clarify how JERICO-next can be linked to the MSFD, (4) to develop an observation platform and other deployment modes. Current work is also being performed on the definition of a common database which is include in the task 5.1.

3.2.7.7. Summary

For high frequency acquisition of phytoplankton *in vivo* and *in situ* data, three automated flow cytometers are currently applied: the Imaging FlowCytobot (McLane®), the Seaflow (Armbrust Lab) and the CytoSense/Sub/Buoy (CytoBuoy®). Amongst the three machines, Cytobuoy® flow cytometers are the only machines allowing a pulse shape recording of optical profiles per particle, making it possible to rely on a more complete recording to discriminate amongst phytoplankton groups. Moreover, they are designed to analyse from pico- to microphytoplankton cells (wide size-range from 1 to 800 µm). Three different configurations allow a variety of implementations as: benchtop analysis on discrete samples or continuous analysis from a pumping water system on board research vessels, ships of opportunity and/or dry platforms (CytoSense configuration), a submersible device (CytoSub configuration) for fixed or mooring platforms as well as for underwater profiling and a third one autonomous system for moorings (CytoBuoy configuration). These tools provide information about size, internal composition, fluorescent content, and can provide, if coupled to a imaging inflow system, images which can help discriminating up to genus or sometimes species and can help size calibration of big cells (Dugenne et al. 2014). The wide range of analyzed particles and the different possible deployments make it a powerful instrument to monitor aquatic ecosystems at the single cell/colony level. From previous work, it was underlined that improvements need to be made for both inter comparison of results from one device to the other, as well as for data analysis, in order to make standardized operational procedures to be settled down and data analysis semi-automated (WP3.1). In order to increase the reliability of data, PSR FCM can be compared with results gathered with other sensors: images and size with imaging inflow systems (coupled or not to the machine), microscopes, pigmentary groups composition by HPLC analysis or addressed by multi-spectral fluorometers. This work is still in progress in the WP 3.1, and implementations are currently performed within JRAP#1 (WP4.1), and data are processed in order to be included into a cytometer module in databases (WP5.2) within the JERICO-Next project.

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3.3. SENSORS FOR VARIABLES OF THE MARINE CARBONATE SYSTEM

3.3.1. pCO_2 sensors

3.3.1.1. Description of pCO_2 sensors/systems

GO 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 2L/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 Pelltier 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.

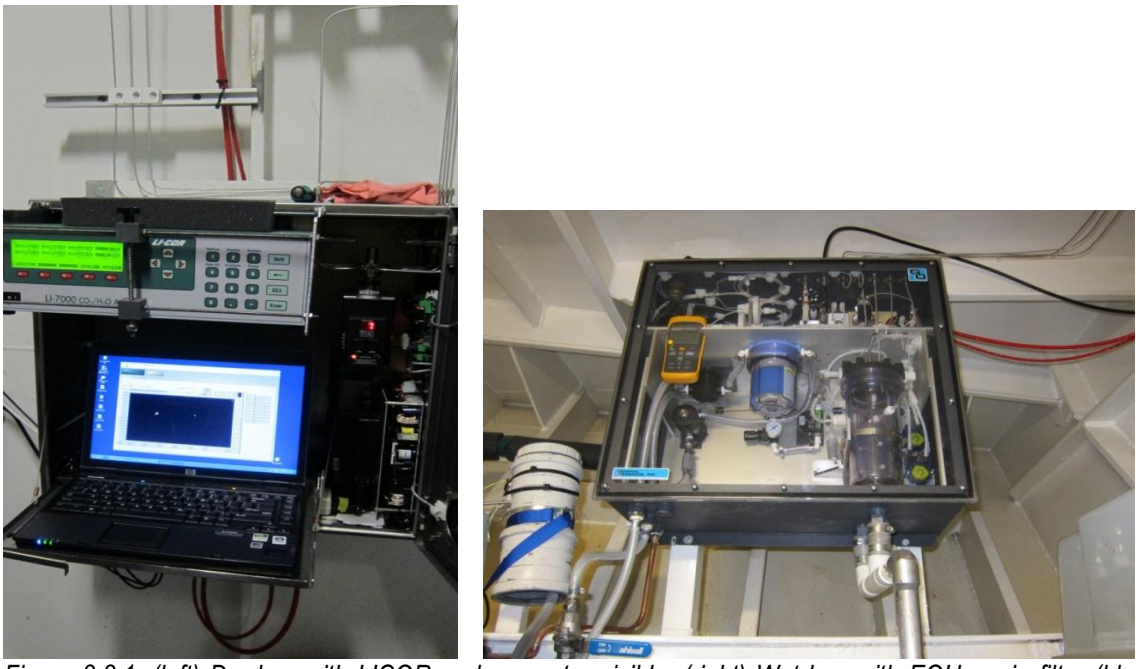

Figure 3.3.1. (left) Dry box with LICOR and computer visible. (right) Wet box with EQU, main filter (blue cap) and temperature measurement (yellow).

Franatech 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 a high temperature (>600 deg C), solid state detector.

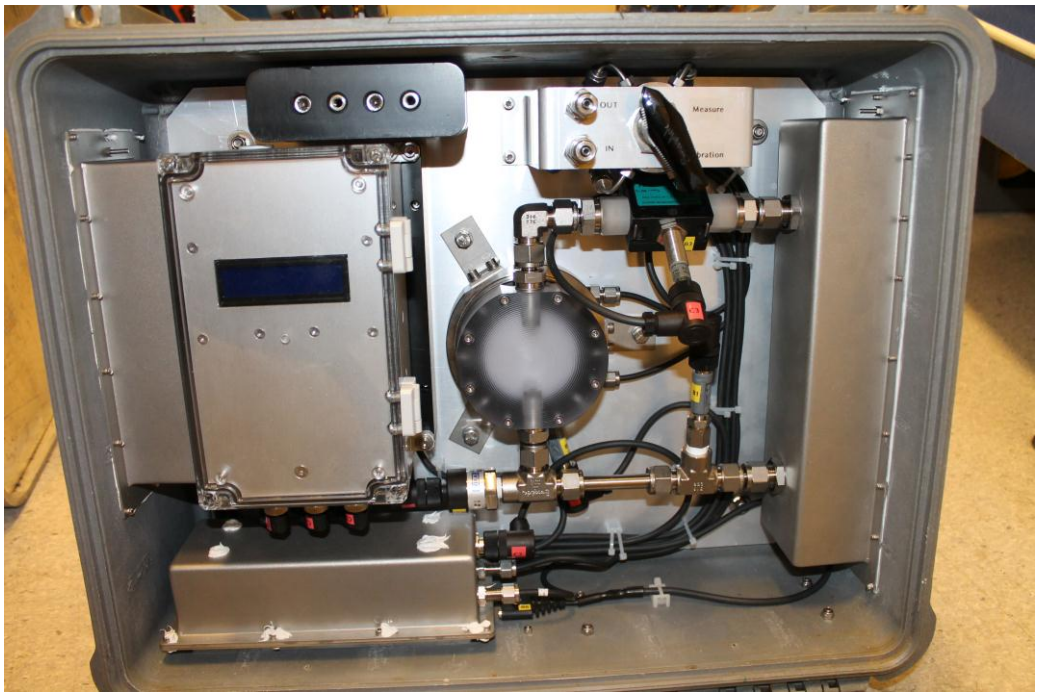

Figure 3.3.2. An example of a Franatech pCO_2 sensor system. The leftmost part contains the electronics, datalogger and display. The circular object in the middle is the equilibration chamber. And the detector lies along the bottom of the case. Water inlet and outlet on the right side of the unit with water pressure and water detector mounted. On the top the gas interface for calibration.

SuperCO_2 system:

SuperCO_2 (Sunburst Sensors LLC) has a principle of equilibrating a gas stream to match the partial pressure of the dissolved CO_2 in a liquid stream, measuring the gas in parts per million (ppm) using a Licor 840A. It takes periodic atmospheric readings and checks the Licor using gas standards. Originally it was equipped with the membrane equilibrator with a single pass equilibration scheme.

Figure 3.3.3. SuperCO_2 measuring system at Utö Atmospheric and Marine research station. (A) instrument, (B) equilibrator and valves, (C) calibration gases and (D) container for cleaning agent.

Contros HydroC CO_2 system:

- The Contros HydroC 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 24 V).
- Dissolved gas diffuses from the water through a thin film hydrophobic composite membrane into an internal headspace where all the partial pressures equilibrate. The gas concentration is measured by non-dispersive infrared spectrometry (NDIR) within a 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; all the data are saved on an internal data logger and/or transmitted by cable.
- A constant flux of water on the membrane is guaranteed by a SBE 5T pump, equipped with an antifouling copper cap and controlled by the sensor's software.
- The sensor hosts an internal CO_2 scrubber to measure the system blank (air with zero CO_2 concentration) that is useful for baseline drift corrections.
- The typical measuring cycle consists of a "warm up" phase, where the NIDR reaches a temperature of 40°C, a measurement of the system blank, "zeroing", the "flushing", when the sensor reaches the equilibrium with the sample and then the measurement.
- The sensor is also available with the "Arctic version" that can operate with a temperature range of -2°C to +15°C,

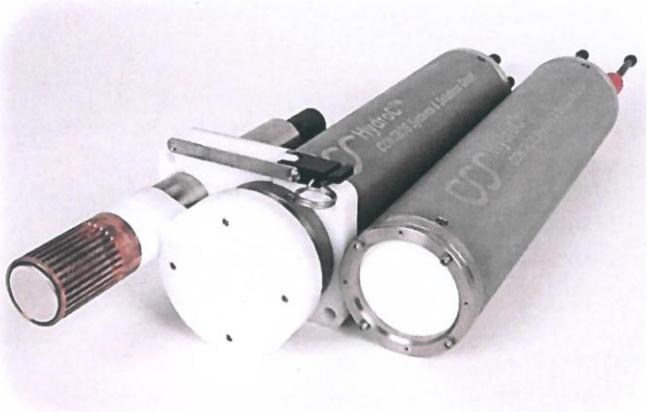

Figure 3.3.4. Picture of the Contros HydroC CO_2 equipped with the protection for the membrane and the pump with the antifouling inlet (on the left) and only with the membrane (on the right).

3.3.1.2. Current modes of deployment

GO system:

- Due to its size and power needs, the GO pCO_2 system is primarily used on FerryBox operations.

Franatech system:

- Because of the power and pump requirements, at this time this system is operated with FerryBox installations on passenger and container ships. The system can also be used with shore-based installations.

SuperCO_2 system:

- The system is operated in flow-through mode using shore-based installation, but could be installed on FerryBox systems as well.

Contros HyrdroC CO_2 system:

- According to the manufactor, the Contros system can be used both on profiling systems and mooring frames, however, the power requirements are high and, without an external source of power, frequent changes of battery are needed.
- It has been successfully used for short term (1-2 weeks) high frequency measurement of pCO_2 variability in bottom waters in coastal areas (Saderne et al, 2014), integrated in a "flow through" system on board of oceanographic ships, mainly for testing purposes (Fietzek, et al 2014), and integrated in a profiling system in an Arctic environment (Meire et al., 2015).
- Here we present an installation for long-term monitoring purpose, in a Mediterranean coastal area (Gulf of Trieste, Northern Adriatic Sea). The Contros HydroC CO_2 is moored to an elastic beacon at 3 m depth over a 25 m depth seafloor, 7 nm form the coast (Fig. 3.3.5.). Power is provided by external batteries charged by solar panels and the instrument is programmed to perform a 20 min measurement every 6 hours.

Figure 3.3.5. Scheme of the PALOMA elastic beacon hosting the Contros sensor (left), the sensor installed at 3 m depth (instrument on the left), near a multiparametric CTD probe (right).

3.3.1.3. Reliability of the sensor and technological difficulties

GO system:

- TRL level 7
- The GO system is quite complex and therefore needs a lot of knowledge to operate.
- The system is also very sensitive to disturbance to the water flow in to the EQU. If the water flow is not correct, the results from the measurements are corrupted.
- In this setup, there has been a lot of problem getting the water flow correct. The pump has cut several times, unfortunately mostly due to staffing problems.
- General Oceanics has not overseen the installation and it has therefore been difficult to discuss some problems. They instead offered a training course in their facilities in Miami. Since the water flow is such an important part of the measurement, it would have been good if they had looked at the piping.

Franatech system:

- TRL 8
- The Franatech pCO2 sensor is in operation on three different FerryBox lines. One limitation is that the calibration procedure is currently carried out in the lab, and therefore the frequency of calibration can be improved. As mentioned in 3.3.1.3., a shipboard calibration unit is being developed.
- A difficulty with this system is the reliance on a relatively constant flow of seawater through the equilibration chamber. The sensor system is therefore dependent on the seawater pump system to be operational. Several iterations of matching a pump in terms of speed and robustness have been carried out and from 2017 a new pump will be in operation on all ships.
- Another issue with the system is the equilibrator membrane. While its short-term operation is predictable, the build-up of mineral and bio-fouling has potentially unconstrained effects on the efficiency of equilibration between the wet and gas phases. While collecting discrete “control” samples for carbonate chemistry analysis can help, the effect of the change in equilibrator membrane behaviour must be improved. One recent improvement has been to reduce the volume of the equilibrator which improves the rate of equilibration between the wet and gas phases, and therefore somewhat reduces the reliance on the membrane. This new version is in operation from 2017.

SuperCO$_2$ system:

- TRL 8-9
- Issues with the original membrane equilibrator have been noted. Despite the cleaning cycle (H_20_2; 6 h interval), during extended measuring periods the membrane clogged. As an alternative a showerhead equilibrator (SHEQ) was purchased. In this model ~1000 mL/min of air is recirculated through the equilibrator while water is sprinkled through the air so that the air equilibrates with the water. A small volume of air is diverted from the loop to the Licor 840A for measurement and is replaced by 'make-up' air from a low CO_2 source. Technically the system works but response time of showerhead is longer than for membrane, but this is considered to be ok for fixed platform.

Contros HydroC CO_2 system:

- TRL 9
- The Contros HydroC CO_2 is a fully commercial system that, according to our experience, has proven to be able to collect good quality data for 2-4 months of unattended deployment in the Mediterranean coastal environment described in the previous section.
- A difficulty faced during the installation was to provide enough power to the instrument. This point has to be carefully planned also taking in consideration the length of the cable between the instrument and the batteries.

3.3.1.4. Sensor calibration

GO system:

- An 8-port 16-position Valco multiposition valve selects the gas being analyzed by the LICOR. Five standard gases are placed a deck above the GO instrument and is analyzed repeatedly during the automatic measurements of CO_2.

Franatech system:

- The detector is calibrated periodically with three CO_2 gas standards prepared by NOAA-ESRL when the system is brought back to the lab. Linearity has been observed between ~200-1000 ppm pCO_2. As with other NDIR detectors, there is some drift over time. Discrete seawater samples are also collected and analyzed for total dissolved inorganic carbon and total alkalinity for calculating pCO_2 and comparing to sensor data.
- A shipboard gas calibration system is being designed.

SuperCO2 system:

- The system is calibrated using reference gases with high quality made in-house at FMI. Validation and intercomparison with in water measures is missing still

Contros HydroC CO_2 system:

- 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.
- Discrete water samples were also collected and analyzed for pH_T and total alkalinity for calculating pCO_2 and comparing with sensor data, however a good comparison is difficult when the water column is stratified with variable physical and chemical properties.

3.3.1.5. Data issues

GO system:

- After analysing the mole fraction of CO_2 it has to be recalculated and corrected since it is dependent up on both salinity and temperature. This has to be done in separate software.

Franatech system:

- The detector measures the mole fraction of CO_2 and is together recorded with various measurements from the Franatech system (chamber water T, chamber air T, seawater flow rate, pressure at various points in the system, etc.) into data files that are stored in the unit's datalogger, and then transferred to a local computer. This data is then combined with FerryBox-measured sea surface temperature and salinity. A Matlab script using calculations presented in Dickson et al. (2007) and Pierrot et al. (2012) are then used to calculate fCO_2 and correct fCO_2 from the chamber seawater temperature to the in situ temperature. And, as described in section 3.3.1.3., the use of "control" samples aid in quality control of collected data.

Contros system:

- If the copper antifouling protections are used both on the pump and over the membrane, they are able to prevent the formation of crusting fouling and the signal has typically a small drift (2-4 µatm/month in summer and almost no drift during winter), according to our experience.
- The instrument provides the data both as pCO_2 values and as xCO_2 or raw data, along with all the other values (relative humidity, temperature etc.) needed to calculate pCO_2 with external software.
- The software does not compensate automatically for the drift, using the blank values recorded during the measurements, but the manufacturer provides a "drift correction datasheet" that can be implemented on external software and allows a post processing of the data to correct it.

3.3.1.6. Links with other WPs and with other EU initiatives

- WP3: No links as of yet
- WP4: data to be used in JRAP 1 and JRAP 5
- WP5: No links as of yet
- The Franatech pCO_2 system will be used to collect data for the recently funded H2020 INTAROS: Integrated Arctic Observation System project

3.3.1.7. Summary

- Carbon dioxide is a key environmental variable in both the ocean and atmosphere. Global production of CO_2 by volcanic sources, and more recently in geological time, fossil fuel burning, exert strong controls on climate change. Also of importance, is understanding the natural oceanic production and consumption of CO_2 via photosynthesis and respiration, respectively, as well as the anthropogenic CO_2 input from the aforementioned fossil fuel burning and the consequential process of ocean acidification. The sensor systems currently used for measuring ocean CO_2 all rely on an equilibration component (wet-dry membrane equilibration, or showerhead aerosol equilibration) and an infrared detection component. All four sensor systems have relatively high power requirements (ship power or batteries) and are therefore limited in their deployment to ships and fixed platforms. The sensor systems also require calibration gases which can also limit deployment and performance, as well as auxiliary data including seawater temperature and salinity (as with other types of carbonate system sensor systems). A limitation that is common to three of the four systems is a constant flow of seawater to the sensor system. Drift appears to be an important factor that needs to be addressed in the near future.

3.3.1.8. References

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3.3.2. pH sensors

3.3.2.1. Description of pH sensors/systems

NIVA pH system:

- The NIVA pH sensor system makes high-resolution pH detection spectrophotometrically using absorbance ratios at wavelengths corresponding to peaks of the speciation of a suitable indicator (e.g., thymol blue or m-cresol purple). Absorbance spectroscopy is fast, accurate, and inherently involves the necessary stoichiometry for definitions of quantities being measured. The pH detection is performed in the visible spectrum (400nm-700nm) using a technique that has been in use for over three decades. The pH sensor here miniaturizes the laboratory based technique, while still delivering high precision and accuracy measurements (Reggiani et al., 2016).
-

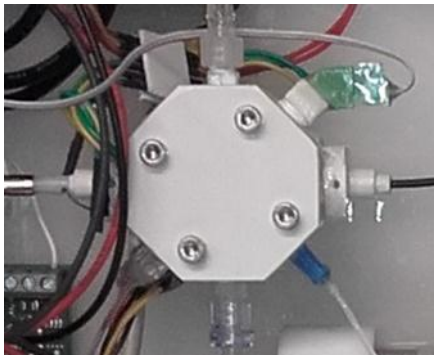

Figure 3.3.6. Close-up of the flow-through cuvette used for continuous in-line, high-resolution absorbance spectroscopy (courtesy of NeXOS project).

AFT-pH system:

- AFT-pH (Sunburst Sensors LLC) is used by SYKE. It is a flow-through instrument that subsamples from internal water reservoir of approx. 1 L. It provides spectrophotometric pH measurement using m-cresol purple dye. pH is measured on the total hydrogen ion scale. There is a need for salinity correction. This sensor is the first AFT-pH instrument modified as low saline version and m-cresol purple dye newly validated using Tris at salinities 2, 6, and 10.

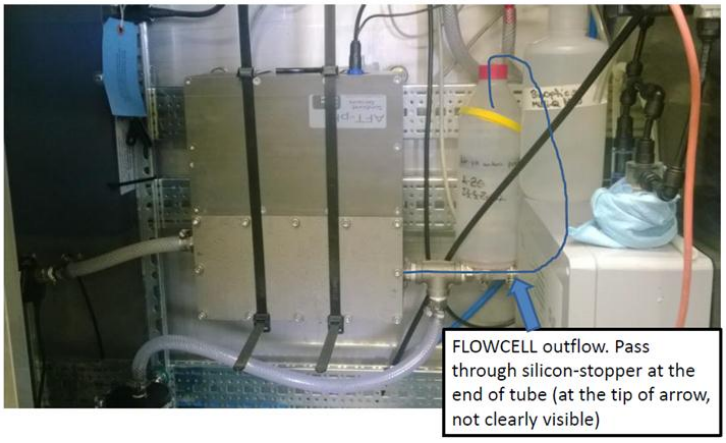

Figure 3.3.7. AFT-pH measuring system at Utö Atmospheric and Marine research station, connected to flow-through system.

3.3.2.2. Current modes of deployment

NIVA pH system:

- The NIVA pH sensor system is primarily intended for surface water operations, where most pH detection systems have been thoroughly and successfully tested. It is limited to power-assisted deployments due to the power consumption of the system's spectrophotometer. It is also limited in size due to limits on how small a high-resolution spectrophotometer can be, but there is likely to be improvements with miniaturized photonic devices within the next couple of years. The system is currently in use with FerryBox systems, and soon to be deployed on fixed buoys, but future work on reducing power consumption and good sampling strategies could support deployment on autonomous vehicles like wave-gliders.

AFT-pH system:

- AFT-pH is use at Utö Atmospheric and Marine research station in flow-through mode. The system may also be applied to FerryBoxes.

3.3.2.3. Reliability of the sensor and technological difficulties

NIVA pH system:

- TRL 9: the system is operational and in use. The primary difficulties are reliability of an auxiliary pump system to divert the flow seawater from a FerryBox system into the pH sensor system, and (bio)fouling of the spectrophotometer lenses that affect absorption spectra. Some extra attention is required during maintenance check-ups.

AFT-pH system:

- TRL 8-9: AFT-pH in SYKE is the first one designed for use in brackish water. It has not yet been validated in full. The current software calculates an incorrect pH due to the pKa equation being optimized for high salinity, and the data need to be post-processed, matching each data point with correct salinity information, coming from another instrument. This still requires implementation of the correction algorithm within the data collection system in future. There was also a technical problem that after analysis, the dye is purged back to main flow, thus influencing other measurements downstream AFT-pH. A custom made solution was applied to collect post-analysis solution.

3.3.2.4. Sensor calibration

NIVA pH system:

- Sensor validation and calibration is carried out by comparison with analysis of discrete samples and measurement of dissolved inorganic carbon and total alkalinity 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). Over-determination 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 certificate reference material and Tris-buffered seawater (Dickson laboratory, Scripps Institution of Oceanography; Pierrot et al., 2012; DelValls and Dickson, 1998).

AFT-pH system:

- AFT-pH calibration is still pending

3.3.2.5. Data issues

NIVA pH system:

- 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).
- Data obtained are thus validated with carbonate chemistry models available in literature, even if the organic component can contribute to mismatches in crossing data obtained from TA-DIC with pH-[CO_3^{--}] pairings.

3.3.2.6. Links with other WPs and with other EU initiatives

NIVA pH system:

- WP3, part of a combined pH-carbonate sensor new development in JERICO-NEXT
- WP4, to be used in JRAP5
- WP5, no links as of yet
- The NIVA pH system will be used to collect data for the recently funded H2020 INTAROS: Integrated Arctic Observation System project

3.3.2.7. Summary

- One goal of ocean carbon investigations is to characterize ocean processes sensitive to acidification. Among the currently available methods for measuring marine carbonate system variables, spectrophotometric pH detection is desirable because it directly measures the change in hydrogen ion concentration (due to ocean acidification), and it is highly suitable for unattended, continuous monitoring systems at (nearly) in situ conditions. Both systems have been extensively tested, with robustness and reliability under deployment on ships of opportunity and shore stations, delivering a data stream under relatively challenging operating conditions. When combined with other carbonate system data, these sensor systems will provide in-situ, continuous data that will be useful for

assessing seasonal and inter-annual carbonate chemistry variability, and aid in validating models that will better describe ocean acidification processes in coastal environments. Low salinity coastal environments still present a challenge to the pH detection techniques.

3.3.2.8. References

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
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Reggiani, E., A.L. King, M. Norli, P. Jaccard, K. Sorensen, and R.G.J. Bellerby. 2016. FerryBox-assisted monitoring of mixed layer pH in the Norwegian Coastal Current. Journal of Marine Systems, doi: 10.1016/j.jmarsys.2016.03.017.
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3.3.3. Total alkalinity sensors

3.3.3.1. Description of total alkalinity sensors/systems

- The CONTROS HydroFIA®TA is a flow-through analyser for total alkalinity (AT), which uses wet chemistry for total alkalinity 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 during the titration is measured using an indicator dye (bromocresol green (BCG), Breland and Byrne (1993)) and VIS absorption spectrometry (Hamamatsu spectrometer, cuvette, degassing unit & heat exchanger in one unit, specific to this instrument). The HydroFIA®TA requires temperature and salinity inputs for precise determination of AT. Temperature is kept constant at 25°C, but external input of salinity is necessary for AT calculation.
- The instrument has a specified accuracy of ± 25 umol/kg (specified by manufacturer and successfully tested in the lab), and precision of ± 5 µmol/kg (specified by manufacturer). In the laboratory, the precision varied within the more likely range of up to ± 20 µmol/kg.

3.3.3.2. Current modes of deployment

- The instrument is meant to be deployed as a flow-through AT analyser, although it can also be used in the laboratory as a bench-top analyser, using the touch screen.
- The instrument does not operate on batteries, so it needs to have a power source at all times. It needs a 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 (live data, Baud rate 115200, data bits 8, parity, none) data interface or Ethernet (batch download). After considerable improvements, and software updates in 2016, 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. At Cuxhaven, it was set up with external control via the FerryBox software. Fig. 3.3.6 shows the variations of salinity and alkalinity during a short time period in December 2016. The strongly tidally influenced salinity signal is well reflected by variations of AT, with higher AT occurring at high tide.

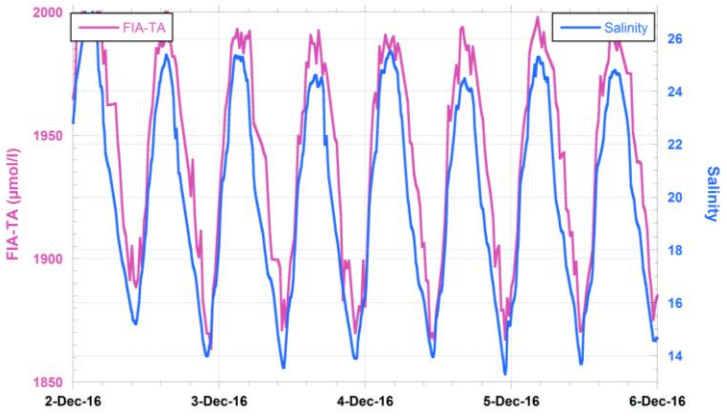

Figure 3.3.8. Salinity and total alkalinity measured at station Cuxhaven.

- In February 2017, the HydroFIA®TA will be deployed aboard the MV Hafnia ferry, travelling between Cuxhaven and Immingham. It is anticipated that this will be a good test for the reliability of AT measurements aboard a moving platform. In addition, the available pCO_2 and pH measurements aboard

the ferry should allow for a reliable characterization of the changes in the carbonate system of the North Sea.

- In spring, 2017 the HydroFIA®TA will be deployed in cooperation with NIVA aboard the MS Color Fantasy, travelling between Kiel and Oslo. The instrument will be simultaneously deployed alongside the NIVA pH sensor, aiming to focus on combining the two measurements, as an example of real-time characterization of the carbonate system. The route will also test the capabilities of both systems in regions with large salinity gradients from 15-35.

3.3.3.3. Reliability of the sensor and technological difficulties

- The HydroFIA®TA was first available in the beginning of 2015, but required a number of design and software improvements, and extensive tests in the HZG laboratory, before collecting reliable AT data. In the laboratory, the HydroFIA®TA was stable over a period of about 1.5 months after considerable improvement in system design in 2015. The TA sensor is at technology readiness level (TRL) 9 (actual technology qualified through successful mission operations) as it is commercially available and has been operationally deployed.
- The recommended salinity range for the BCG indicator dye is 20-35 (Breland and Byrne, 1993), so the instrument is better suited for operation in coastal regions with similar salinity range. In addition, turbid samples require filtration before AT measurements, which may be a limitation for some estuarine applications. Nevertheless, at Cuxhaven, despite the large salinity variations between 14-26 and the high turbidity of up to 200 mg/l suspended matter, the instrument operated well when sampling every 20 min.
- The deployment period of the HydroFIA®TA is primarily limited by reagent volume (currently set at 500 mL of the BCG indicator dye and the dilute HCl). Reagents can be ordered from Kongsberg, or can be made in the lab. Calibration is necessary after replacing the reagents.
- While deployment on the fixed FerryBox at Cuxhaven, Germany was successful, one issue that still needs to be resolved is timing for the external input of salinity. Currently, the salinity is measured at the start of AT measurement cycle, which takes about 6 minutes, and includes system conditioning cycle. On a moving platform, this may be a challenge, and will be tested in the beginning of 2017.

3.3.3.4. Sensor calibration

- 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. In addition, it is recommended that a separate well-characterized total alkalinity reference sample is used as an independent check of the AT stability.
- Sometimes problems with the Hamamatsu spectrometer could interfere with proper calibration of the instrument. That is why it is recommended to use a separate reference sample and test the instrument stability.
- During deployment, it is recommended that check samples are collected for dissolved inorganic carbon (CT) and AT measurements, as an external check of the carbonate system measurements. An example of 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 (Dickson, 2007) was used to calculate pH and pCO_2, and the calculated values were compared to the measured pH and pCO_2 values (see data table below). 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 interfered with the measurements. It is better to fix the samples with $HgCl_2$ upon collection, preferably when a user is riding the ferry.

Bottle number	salinity	temp °C	DIC µmol/kg	HydroFIA®TA µmol/kg	pH Calc	pH Meas (Clark)	pH Meas (ISFET)	pCO2 Calc	pCO2 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

3.3.3.5. Data issues

- It is necessary to filter the water samples before measuring AT with the HydroFIA®TA. This requires the use of a filtration system in line with the instrument (ex. a crossflow filter), or measurement of discrete samples, in which particles have been allowed to settle.
- Fouling has not been observed so far, although it is advised to clean the system with dilute HCl regularly (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. 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 20-30 min between measurements could lead to instrument drift, a problem that has not been resolved yet 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 may be a problem for deployments aboard moving vessels, which usually dock 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 necessary filters to remove questionable data. This issue will be tested in the beginning of 2017 aboard the MV Hafnia.

3.3.3.6. Links with other WPs and with other EU initiatives

- In WP3, Subtask: 3.5.2. the combination of a spectrophotometric pH sensor and total alkalinity sensor will be further developed and intensively tested alongside pCO_2 measurement and bottle samples analyzed in the lab to check the quality of the data.
- In WP4, Task 4.5. (JRAP #5) the new sensor will be applied on FerryBox systems to characterize couplings between phytoplankton abundance, community structure, productivity, biogeochemical C-cycle and C-fluxes.
- In WP5, all alkalinity data recorded in combination with a FerryBox will be freely available in the HZG FerryBox database.

3.3.3.7. Summary

- Total alkalinity is a unique parameter of the carbonate system, which, contrary to the rest of the CO_2 system variables (CT, pCO_2, or pH), does not change during CO_2 gas exchange with the atmosphere. Instead, fluctuations in AT occur on much larger time scales (Yao and Byrne, 1998). The HydroFIA®TA is a state of the art total alkalinity analyser, designed to measure AT in a flow-through system setup, therefore potentially greatly expanding the coverage of AT measurements in surface seawater. The instrument has undergone a number of design, hardware and software improvements since its launch in 2015, and has been successfully tested at a stationary tidally-influenced coastal station in Cuxhaven Germany. In 2017, it will be launched on two moving platforms in the North and Baltic Seas, where it will be robustly tested for its intended purpose of a flow-through analyser of AT, alongside pH and pCO_2 sensors. Careful calibration and checks for instrument drift (especially after leaving the dock) are

necessary to ensure the quality of the TA measurements, but laboratory testing and testing at Cuxhaven have revealed that the instrument is stable for several months, when it is used frequently.

3.3.3.8. References

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3.4. COASTAL PROFILING SYSTEMS

Profiling sensor systems can help to integrate indispensable information on water column characteristics in coastal areas. Two such profiling technologies are quite mature, even if subject to further development, and used within the JERICO network: instrumented coastal profiling floats and fishing vessel-based, net-deployed sensor systems. Besides these technologies, fixed (at the surface or bottom) profilers are also under development in JERICO-NEXT, and are presently in a prototype stage.

Autonomous coastal profiling floats are instruments specifically adapted from conventional open sea profilers to be operational in coastal area. These instruments are designed to generate Lagrangian time series of ocean parameters observation along the water column. 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. Parameters embedded on coastal profiling float are up to now quite limited due to the small size of such floats. Parameters are temperature, salinity, and for some of them, oxygen, fluorescence or turbidity.

Fixed profiling systems are designed to generate Eulerian time series of ocean parameters observation along the water column, allowing to sample parameters across a depth range and to transmit the collected data through satellite or cable communication. We can cite here two types of fixed profiling systems: 1) yo-yo systems, which moves through the water column carrying instruments, allowing to sample ocean parameters across a depth range, and 2) chain of sensors fixed below an anchored buoy or above a bottom tethered device, allowing to sample ocean parameters at fixed and discrete depths.

Yo-yo systems can track along a mooring riser (wire-following profilers), or include a winch that pays out line allowing the profiler to rise through the water column from the seafloor (bottom tethered profilers) or from a subsurface/mid-depth (shallow profilers) or to descend the water column (surface-buoy based profilers) until a fixed depth.

The highly valuable contributions from ships of opportunity (or volunteer observing ships) in oceanography and climate change studies are well established. For example, SOOs (Ship of Opportunity) have received particular attention due to their possibility to feed models with a large amount of data (data assimilation needs sufficient spatial coverage on a regular basis, especially in coastal regions**;** Petersen 2014).
On the other hand, with the aim of improving knowledge of the spatial distribution of fishing effort and catches and assess the state of fisheries resources exploited in the context of an ecosystem approach, in the 2000s, two European institutions (CNR and Ifremer) started to use fishing vessels as SOO. Fishing vessels operate daily and especially in coastal areas (that are usually poorly sampled), thus if well equipped, they can be able to observe both fishery data and oceanographic parameters at a frequency of space and time that cannot be reached by research vessels (unless access to large and sustainable funding). Therefore, the use of monitoring systems installed on fishing vessels is considered one of the new frontiers for operational oceanography (Patti et al. 2016). Table 3.4.1 summarises the coastal profiling systems in use in the JERICO-Next consortium.

System type	CNR-ISMAR	CNRS	HCMR	Ifremer	IMR
Autonomous coastal profilers			✓	✓	
Surface-buoy based profilers		✓			
Bottom tethered profilers	✓			✓	✓
Fishing vessel based systems	✓			✓	

Table 3.4.1. Summary of the coastal profiling systems types deployed by JERICO-NEXT partners.

3.4.1. Autonomous coastal profilers

3.4.1.1. Description of the sensors/systems

Costal profiling floats are vertical untethered profiling systems, easy to set up and to deploy. It behaves like a virtual mooring, for short to long-term observations. It can take measurements at the same location for each profile thanks to the optimized time of ascent and descent through the water column, the short time of transmission at the surface, and its anti-drift capability when grounded on the seabed.

Costal profiling floats provide a standard set of measurements (pressure, temperature and conductivity), as well as a set of technical information. Multidisciplinary sensors can be integrated on this vertical vehicle, which is designed as an open platform. Additional sensors are being currently fitted to measure dissolved oxygen, turbidity and fluorescence.

In standard mode, coastal profiling floats operate autonomously. One of the major features is its IridiumTM satellite bi-directional link: firstly, it offers a fast uplink to transfer data when surfacing after each profile, and secondly, it provides a downlink remote control to reconfigure the mission parameters during operation. For example, users can increase the number of profiles per day and the sensor sampling frequencies when a bloom is detected.

As an example the Ifremer/NKE Arvor-C is an autonomous coastal profiling float that weighs less than 20 kilograms, is 2.1 meters high and has a diameter of 11 centimetres.

The scientific payload, Seabird pumped CTD (Conductivity Temperature Depth) and optionally O2, Fluorescence and turbidity, is located on the upper end cap, as well as a bi-band IridiumTM-Global Positioning System (GPS) antenna (for data transmission, remote control and positioning), and a Bluetooth antenna (for configuration and testing).

An external bladder is fitted on the bottom end cap, to adjust the buoyancy when descending and ascending along the water column, as well as anti-drift claws, to prevent drifting when grounded on the seafloor.

Figure 3.4.1 Arvor-C during the descent.
On the bottom end, its claws prevent drifting when grounded on the seafloor
(Photo credits : Olivier Dugornay – Xavier Caisey (Ifremer)

As an example, the Ifremer/NKE Arvor-C is a coastal profiling float, designed to withstand pressures up to 450 meters depth. It can perform up to 320 profiles when cycling at 200 meters depth. The profile repetition rate can be configured from 1 profile every hour. Its ascending speed reaches 15 to 20 centimeters/second. For instance, a 2-second sampling period provides one single measurement every ~35 centimeters. Data are then averaged into 1-meter high slices to reduce transmission duration.

3.4.1.2. Current modes of deployment

Set up. Before deployment, coastal profiling floats are configured in the laboratory by users (scientists or technical assistants). The main parameters to be set up are the profile repetition rate and the sensor sampling frequencies.

Deployment. Untrained staff can deploy the system, as it only involves removing a magnet in order to power on the float. The coastal profiling floats can then simply be launched at sea from various boats. It sends its GPS location and technical information to the data centre, retrieves remote commands (if any), and then starts its profiles. Boats used for deployment can be from a simple inflatable boat up to a massive oceanographic vessel. Boats of opportunities can be used as well, as leisure sailing boats or cargo boats. A 2 days campaign is enough to recover and deploy floats.

Profiling and Recovery. In standard operation, the coastal profiling floats perform automatic profiles. It remains on the seabed for a given time between each ascent/descent. When the float reaches the surface, it retrieves the commands sent by the user via satellite and adjusts its operation according to the new requested configuration. The float measurements and technical information are sent to the Coriolis data centre via satellite.

When recovery time is decided, the user sends a command to the float so that it stays at the surface. It will then regularly send its GPS position until recovery.

3.4.1.3. Reliability of the sensor and technological difficulties

The main difficulty for such instrument is not the sensor reliability since coastal floats uses conventional on shelves sensors. Above all coastal float technology is a derivative of TRL9 well proven profiling float technology. We can even mention that the limited depth that concerns coastal float is an advantage in term of reliability.

The difficulty that should be mentioned is the vulnerability of the system since it is deployed in coastal area. Then there is risk of trawling which can lead to the destruction of the instrument. In average, the float happens to be trawled once every 2 years. After a trawling, the CTD unit and the antenna need to be replaced.

Another risk is the difficulties in starting the ascent profile when the coastal float is anchored in clay soils, which can lead to delay in surfacing. The coastal profiler is equipped at its bottom with claws that prevent the float to drift, but in case of clay soils it can anchor too firmly.

In situ experiences with such systems, in fact, did not show so much such problems. For example, in July 2009, an Arvor-C Coastal float was deployed in coastal area in the Bay of Biscay, during the first 5 months of deployment, the Arvor-C drifted less than 200 meters per day, north of its deployment position, despite rough wave and tidal conditions. At the same time, a free-drifting profiling float (not anchored on the seabed between profiles) drifted more than 4.3 kilometres per day.

In situ experiences shows that in order to keep instruments in good condition (drift and biofouling), a coastal deployment should not last more than a year for floats equipped with SBE 41CP CTD unit.

Coastal profiling float need to be carefully adjusted in term of buoyancy. Sometime they have trouble to sink and need to be recovered for buoyancy adjustment.

3.4.1.4. Sensor calibration

Embedded sensors as the CTD unit on coastal profiling floats are conventionally calibrated by the manufacturer or by specialized marine metrological laboratory.
SBE 41CP CTD laboratory calibration procedure is well established. IN addition, CTD measurement and samples casts are performed at deployment and recovery. Observed drift is usually minor with respect to natural range of variability of S.

Concerning sensors like O2, fluorescence and turbidity, the sensor calibration is not specific to coastal profiling float. It's more a generic problem.

3.4.1.5. Links with other WPs and with other EU initiatives

In EU JERICO-NEXT WP3, task 3.3, the HCMR is developing the JELAB (Jerico Extended Lagrangian Bio-Geo-profilers) costal float. The objective is to expand the capacities of advanced Argo-type floats (e.g.ProvBio, BioArgo and Arvor-Cm) particularly for coastal applications by extending available payload. HCMR is working on the integration of cameras (mini cameras, possibly lo-light bioluminescence) for water column particle imagery and sea floor imagery.

3.4.1.6. Summary

Costal profiling floats are vertical untethered profiling systems, easy to set up and to deploy. It behaves like a virtual mooring, for short to long-term observations. It can take measurements at the same location for each profile thanks to the optimized time of ascent and descent through the water column, the short time of transmission at the surface, and its anti-drift capability when grounded on the seabed.

Deployment and recovery can be performed from various boats, from a simple inflatable boat up to a massive oceanographic vessel.

With such systems the main risk is the vulnerability linked to the coastal area. For example there is risk of trawling which can lead to the destruction of the instrument.

Coastal profiling floats are regularly deployed in the Bay of Biscay for more than 5 years. Floats are profiling once per day at 12:00 UTC with duration cycle of 1/2 h.

Coastal profiling floats are as well commonly used for short-term deployments in research mode.

3.4.1.7. References

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Coastal Profiling Float, Deployment feedback. Louis Marie (Ifremer), JERICO-NEXT WP2 Workshop Sensors and Systems – 13-14 December – Paris – Session Coastal Profiling systems.

JELAB: Jerico Extended Lagrangian Bio-Geo-profilers. Manolis Ntoumas (HCMR), JERICO-NEXT WP2 Workshop Sensors and Systems – 13-14 December – Paris – Session Coastal Profiling systems.

3.4.2. Surface-buoy based profilers

3.4.2.1. Description of the sensors/systems

Surface-buoy based profilers are conventional fixed buoys that are equipped with a profiling device that goes from the surface buoy down to the bottom anchorage. To illustrate such system that are not so common, we will base this chapter to the EOL (Environment Observable Littoral) buoy system from MOBILIS that was specifically presented during the JERICO-NEXT WP2 Workshop Sensors and Systems on 13th and 14th of December in Paris.

The buoy is fitted with a number of sensors that measure water quality including: conductivity, temperature, depth, pH, dissolved oxygen, chlorophyll A, and turbidity. The buoy is also fitted with a meteorological station. The EOL buoy comprises a 3.6m diameter hull constructed from multiple-section polyethylene floats that are bolted around a central steel structure, with through-hull access for underwater instrumentation and cabling.

The instrument package is automatically lowered from the buoy to the seabed and back at pre-set intervals with data gathered during the lowering and raising operations. All data is transmitted to shore as well as being logged in the on-board data acquisition/telemetry unit. When the instrument package is in its raised position, it is stored within a unique electro-chlorination chamber, which automatically cleans the sensors of any biofouling.

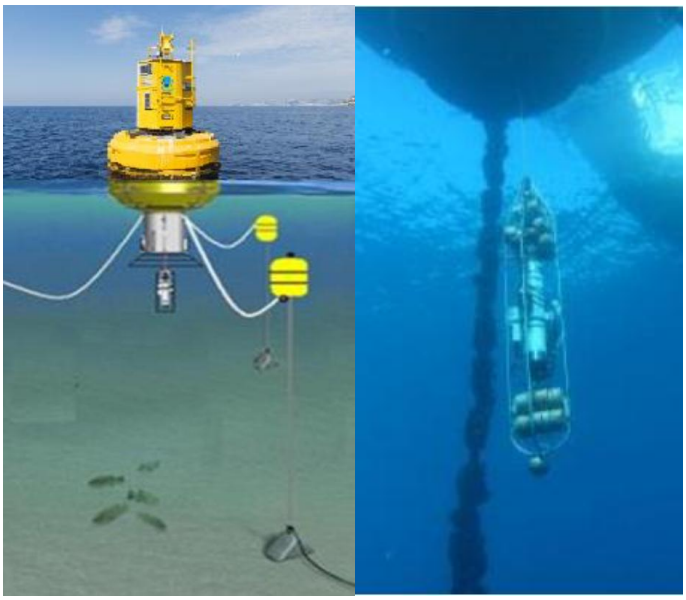


Figure 3.4.2. MOBILIS EOL surface-buoy based profiler.
(Photo credits : MOBILIS)

3.4.2.2. Current modes of deployment

The EOL MOBILIS system weighs 4 tons, has a diameter of 3.6m for a total height (immersed and emerged) of 8 meters and is fitted with 14m^3 of floats. Its wetting is stable with three anchor points that allow the solar panels of the buoy to always face the sun.

The buoy realise continuous profiles several times per 24 hours. Physico-chemical measurements (CTD, Fluorescence, Turbidity) with data below the surface and atmospheric parameters are regularly performed. From 0 to 70 meters depth (Maximum 100m) on the littoral zone, the buoy can be operated autonomously for a time span of 1 year without human intervention.

The data are transmitted from the buoy to the shore by in near real-time by 3/4G or WIFI.

The maintenance for the sensors is a conventional swap of the sensor pack when needed. Multiple runs show that the sensor pack can be left on duty for one year thanks to the dual biofouling protection arrangement described earlier. Consequently the sensors maintenance is mainly driven by the sensor calibration need as recommended by the manufacturers.

3.4.2.3. Reliability of the sensor and technological difficulties

The buoy is operational since 2013 (with more than 1600 profiles realized) without technical interventions except for a replacement of the CTD probe batteries and the calibration of the latter in 2016. A replacement of the anodes was also performed.

The buoy is equipped with an active protection system against biofouling for the sensor pack: 2 protection systems are used at the same time, a preventive one and a curative one. The preventive protection is based on biocide generation by seawater electrolysis in the garage where the sensor pack is parked between profiles. The curative protection is based on brushes placed at the entrance of the garage chamber.

3.4.2.4. Sensor calibration

Sensors as the CTD unit are conventionally calibrated by the manufacturer or by specialized marine metrological laboratory. CTD laboratory calibration procedure is well established. Observed drift is usually minor with respect to natural range of variability of S.

Concerning sensors like O2, fluorescence and turbidity, the sensor calibration is not specific to coastal profiling float. It's more a generic problem.

3.4.2.5. Data issues

Many inter-comparison campaigns have been performed successfully, with external sensors placed close to the buoy or by taking advantage of a SOMLIT (Service d'Observation du Milieu LITtoral) reference point 360m apart from the EOL buoy.

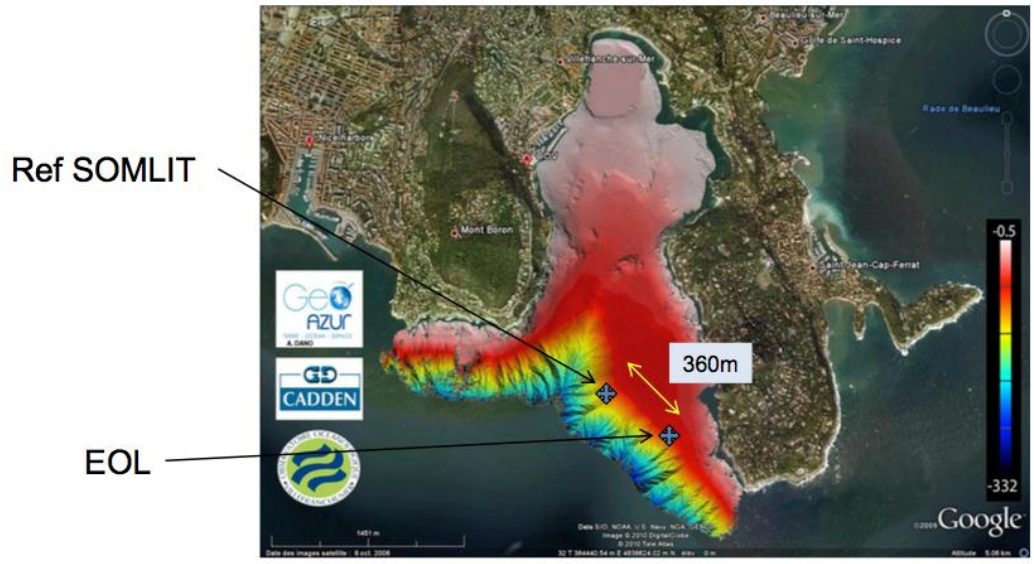


Figure 3.4.3 MOBILIS EOL and SOMLIT reference point.
(Photo credits : GoogleMap)

3.4.2.6. Links with other WPs and with other EU initiatives

In EU JERICO-NEXT WP3, task 3.3, the IMR is developing the YOYO trawl-secured profiling system that is based on a fixe mooring and a profiling device that is as well based on a trawling device.

3.4.2.7. Summary

The EOL Platform with automatic profiles is an autonomous coastal buoy which allows to realize continuous profiles several times per 24 hours, of physico-chemical parameters (CTD – Fluorescence - Turbidity) with data below the surface and atmospheric parameters up to a meteorological station. From 0 to 70 meters depth (Maximum 100m) on the littoral zone, the station is autonomous for a time span of 1 year without human intervention.

The EOL buoy comprises a 3.6m diameter hull constructed from multiple-section polyethylene floats that are bolted around a central steel structure, with through-hull access for underwater instrumentation and cabling. The instrument package is automatically lowered from the buoy to the seabed and back at selected intervals with data gathered during the lowering and raising operations.

The buoy is equipped with an active protection system against biofouling for the sensor pack: 2 protection systems are used at the same time, a preventive one and a curative one. The preventive protection is based on biocide generation by seawater electrolysis in the garage where the sensor pack is parked between profiles. The curative protection is based on brushes placed at the entrance of the garage chamber. It allows keeping clean the sensor pack for a year. Sensor maintenance is mainly driven by internal sensor drift and should be performed according to manufacturers' inputs.

3.4.2.8. References

MOBILIS Web site : http://hydrosphere.co.uk/products-and-services/mobilis-eol-buoy/

Platform with automatic profiles on the coast (EOL), J.M. Grisoni (OOVM), JERICO-NEXT WP2 Workshop Sensors and Systems – 13-14 December – Paris – Session Coastal Profiling systems.

3.4.3. Bottom tethered profilers

3.4.3.1. Description of bottom tethered profilers

Two types of bottom-tethered profilers are considered in this report:

1) active automated profilers (e.g. yo-yo systems), that continuously profile a specified portion of the water column above the seafloor and
2) passive water column profilers (e.g. chains of static sensors) that provide profiles made up measurements from discrete depths above the seafloor.

Yo-yo systems are made of a winch plus a control unit anchored to the seafloor and a profiling unit housing scientific instrumentation (scientific module). The winch pays out line allowing the profiling unit to rise through the water column until fixed depth. After the ascent, the winch recovers the rope taking back the profiling unit in its parking position (see for instance Figure 3.4.2 a). The scientific module can host any type of sensor, provided it is suitable to work in profiling mode (essentially, all the sensors that can be used for profiling from a research vessel). Data transmission can be implemented in various ways: when the profiling unit is at the sea surface, if it is equipped with an antenna, or by interacting with underwater devices by acoustic telemetry (for instance with gliders) or via seafloor cable directly connected to shore or to another fixed underwater device enabled for communication.

Chains of static sensors are subsurface moorings, which contain instruments/sensors fixed at specific depths along the mooring riser. Flotation spheres for subsurface moorings are located below the sea surface and at one or more prefixed depths.

Compared with the chain of sensors, yo-yo systems offer the advantage of a greater vertical resolution, more complex sensor payloads and enable automatic near real time monitoring of multiple ocean properties in the water column. Bottom tethered yo-yo systems can be found on the market, as the Vertical Profiling System (VPS) manufactured and commercialised by InterOcean Systems LLC (http://www.interoceansystems.com/vps.htm), but most devices have been developed by universities and research institutions and remain in the prototype stage (Villagrán et al., 2011; Book et al., 2007; Grandi et al., 2005).

3.4.3.2. Current modes of deployment

YOYO trawl-secured profiling system:
The Institute of Marine Research (IMR, Norway) has developed a YOYO trawl-secured profiling system aiming at collecting daily full profiles of T, S, Chl fluorescence and oxygen, from 300 m depth to the surface, including quasi real time automatic data transmission. The system is composed of a winch anchored to the seafloor and a profiling unit (Fig. 3.4.4), equipped with sensors. The winch releases and then recovers it at regular time intervals, collecting upward and downward profiles.

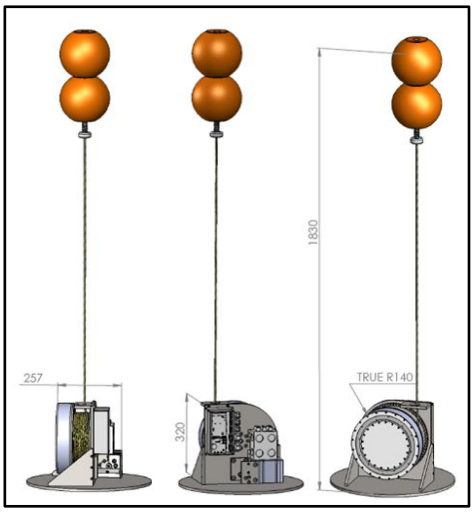
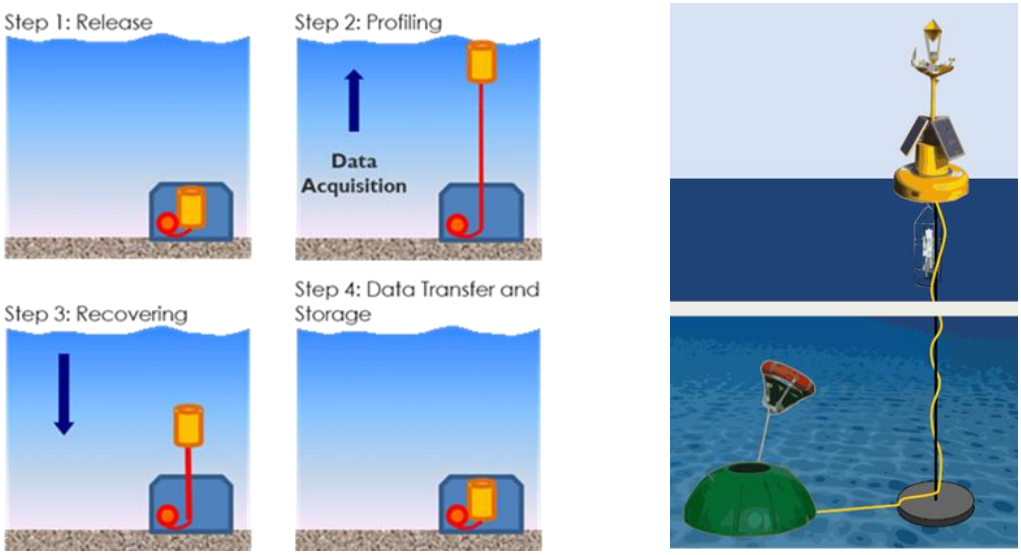


Figure 3.4.4. Scheme of the YOYO trawl-secured profiling system developed by IMR.

BottomUp Profiler (BUP):
The BottomUp Profiler (BUP) is a prototype autonomous coastal profiling system developed by Consorzio Proambiente and the Istituto di Scienze Marine (CNR ISMAR, Italy). It is thought for continuous environmental monitoring of marine-lacustral areas and lakes and is composed of underwater winch, sensors package, underwater batteries pack, communication system and data-logger, contained in a case with low roughness.



(a) **(b)**

Figure 3.4.5. (a) Operational principle of the BUP system; (b) Deployment of the BUP system connected to the elastic beacon E1 of CNR ISMAR.

The system is moored at the sea-bottom and releases the sensors package at defined time intervals for data acquisition. Data are recorded by the sensors during ascent and are transferred to the data logger when the sensor package is recovered inside the case (Fig. 3.4.5 a). It can operate autonomously or integrated to a fixed platform and has been tested for two days in a swimming pool, with good results. The next step will be testing it in the open sea, connecting it to the buoy E1 to control his regular activity and receive data in near real time (Fig. 3.4.5).

MASTODON2D: Mastodon original system equipped with a chain of thermistors at fixed depths
Mastodon2D is an extension of the Mastodon project (Lazure et al., 2015) which provides time series of bottom temperature, with a precision of 0.1°C (a commonly adopted value for coastal observatory systems), with a low cost mooring.
The schematic of the Mastodon system is in Figure 3.4.3, and its core is a near-bottom temperature data logger with ballast and a release device. The release device is innovative and based on «a burn wire » (electrolytic erosion of a copper wire loop under low voltage). To date the release is software-controlled and defined before deployment. Given the uncertainty in marine weather forecasts and ship availability, the temperature probe and its float will remain fastened to the ballast after the release date, to allow recover a few days later, if necessary.

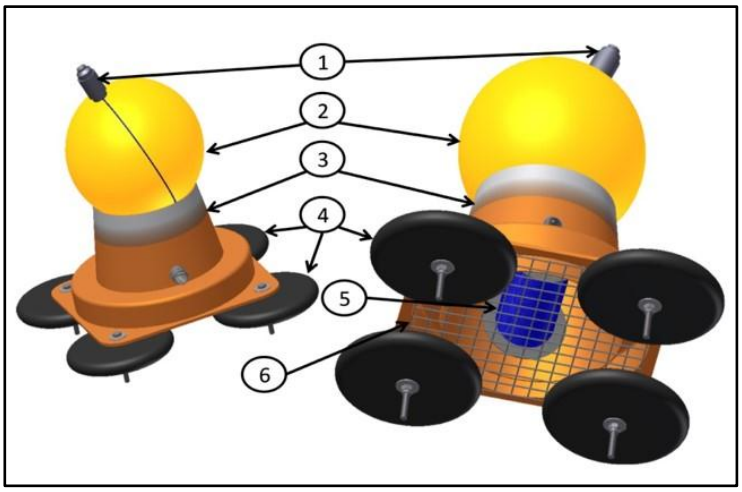


Figure 3.4.6: Scheme of MASTODON developed by IFREMER. 1-electronics, 2- float, 3- traffic cone frame, 4- ballasts, 5- rope spool, 6- wire grate.

The development Mastodon2D consists in adding a vertical line from the bottom to the near surface, equipped at multiple depths with temperature sensors providing data at high spatial resolution (Fig. 3.4.6). A specific electronic card, developed by Ifremer, consists of a low-power microcontroller with a 8MB flash-memory connected to the temperature and pressure sensor. The temperature sensor is a pre-calibrated electronic chip, featuring a 0.008°C resolution and requires no further recalibration. The pressure sensor (precision ≈2cm) measures the location in the water column, correspondent to the temperature. One AAA 1.5-V cell is enough to sample temperature and pressure every minute for one year.

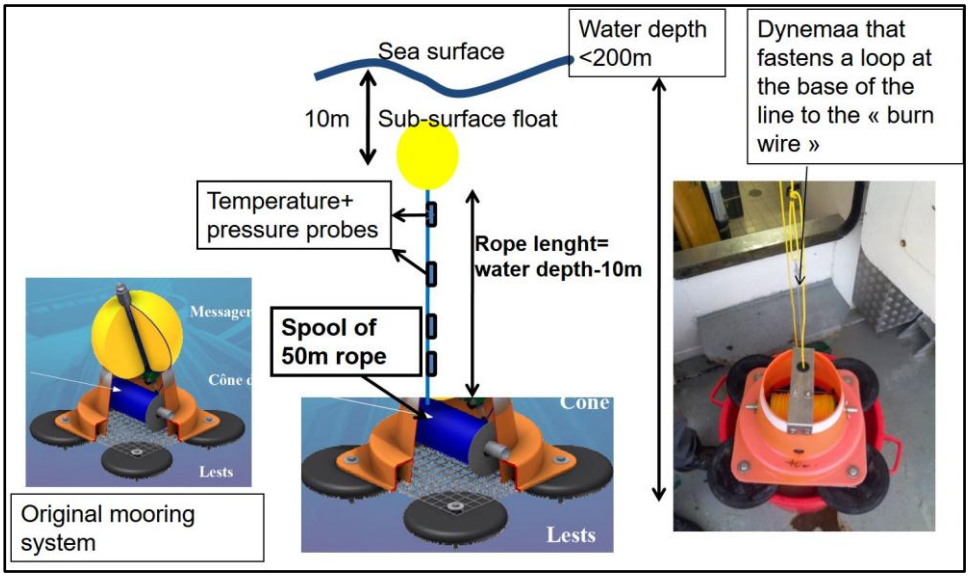
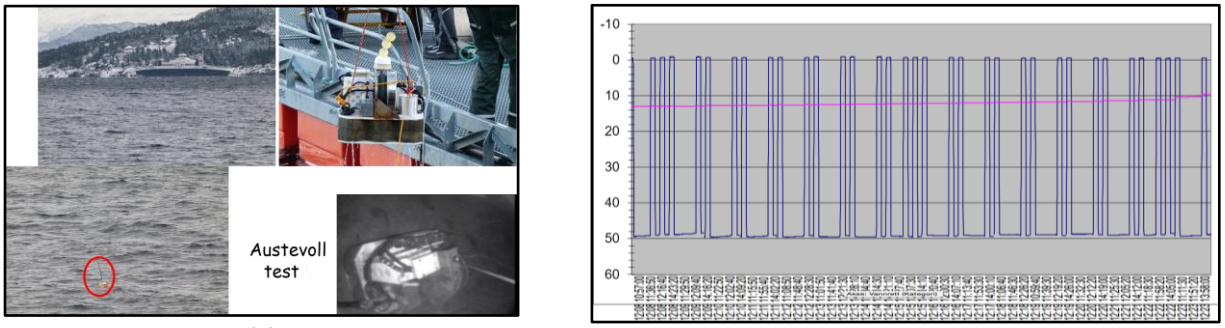


Figure 3.4.7: Rationale for the design of Mastodon2D.

3.4.3.3. Reliability of the sensor and technological difficulties

Generally speaking, yo-yo systems are available on the market, for example the Vertical Profiling System (VPS) manufactured and commercialised by InterOcean Systems LLC (http://www.interoceansystems.com/vps.htm), but most devices have been developed by universities and a few research institutions and remain in the prototype stage (Villagrán et al., 2011; Book et al., 2007; Grandi et al., 2005).
As regards the two yo-yo systems described in the previous section, and referring to the TRL scale adopted by the European Commission in the annex G of the Horizon 2020 – Work Programme 2016-2017:
the mechanical functiong of the BUP system has been tested in air and in a swimming pool, with good results (TRL 4). The next step will be to test it in the open sea, connected to the elastic beacon S1 (Fig. 3.4.5 b);
the YOYO trawl-secured profiling system was successfully tested near the IMR marine station Austevoll (TRL 6). The profiling unit was equipped with an antenna for satellite data transmission (Fig. 3.4.5 a). No technological difficulties were encountered during the test. The next step is to connect the YOYO system to node 1 of the LoVe ocean observatory (Lofoten-Vesterålen), which will provide power supply and will channel the data to shore.



(a) **(b)**

Figure 3.4.8. (a) Deployment of the YOYO trawl-secured profiling system near the IMR marine station Austevoll, (b) recorded depth (15 days test).

Thermistor chains are a mature technology, widely used by the ocean community by decades. The innovation of the Mastodon2D system lies on the electronics (electronic cards equipped with pressure and temperature sensors) and the release device. Both have been demonstrated in operational environment (TRL 7). Moreover, a peculiarity of Mastodon/Mastodon2D is the low cost (400/2000 EUR, without man power). The system was tested in September 2016 by deploying 3 moorings in the Mediterranean Sea at depth of 50,150,150m for few days, equipped with T/P sensors at multiple depths. All mooring were successfully recovered and example of data collected is shown in Fig. 3.4.9.

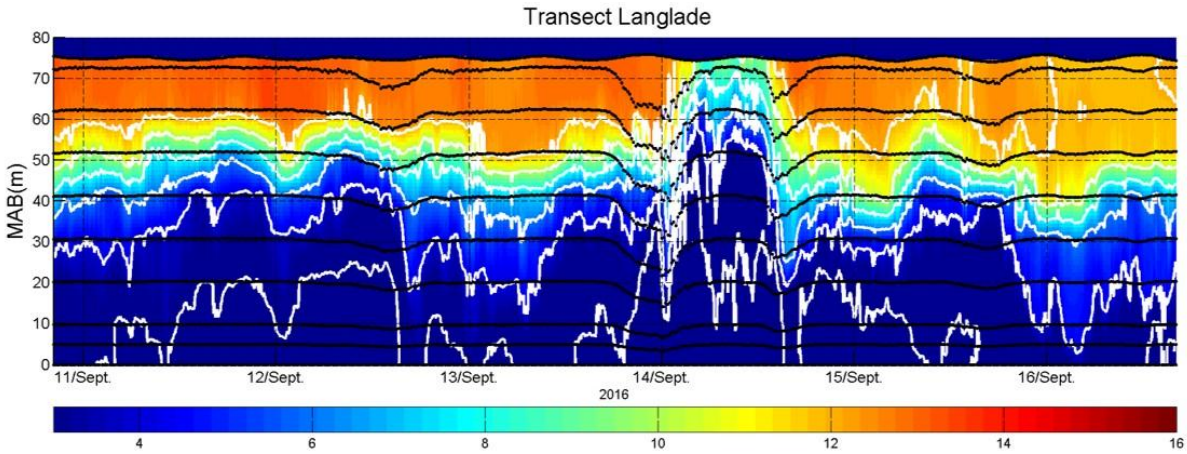


Figure 3.4.9. Sea Temperature evolution in the water column measured by eight T/P sensors on a mooring.

3.4.3.4. Sensor calibration

The calibration issues for sensors used on moored profilers are those usual for the specific sensors with the difficulty that it is not possible to perform frequent calibration since too expensive: the sensors should be removed, replaced by spare sensors, and transported to the laboratory.
Stability of a sensor in the long-term is a peculiar characteristic of the specific sensor. For instance, limiting the argument to the CTD probe temperature (thermistor) and pressure sensors are believed to be very stable over time, but the conductivity sensor is subject to drifts owing to biofouling and other problems. To evaluate and correct the drift in salinity between a laboratory calibration and the next, (bottle-calibrated) CTD station data collected in the site, or in the nearby region, in nearby time periods, can be used as, for instance in Krishfield et al. (2008). Also, several indirect methods have been devised for ARGO floats based on comparison with climatology or shipboard high-resolution CTDs, that can be exported and applied to moored profilers too (Owens and Wong, 2009; Böhme et al., 2005; Wong et al., 2003; Bacon et al., 2001).

3.4.3.5. Data issues

Fouling (biological and otherwise) is the major problem in moored application, especially in coastal areas (Cook, 2010; Delauney et al, 2010). Thermometers are not sensible to fouling, unless in extreme conditions, since the sampling interval is much longer than the time constant of the sensor. Conductivity sensors are very sensitive instead. A very thin coating can change the cell geometry, having a large effect on the conductivity measurement: the conductivity measurement would drift towards lower values due to a decrease in the measurement volume of the conductivity cell. Oxygen sensors become less sensitive when they are fouled, this happens because the membrane permeability is decreased by oil coating, bacterial colonization or other sort of fouling. Optical turbidity sensors emit infrared light and then measure the scatter of light caused by particles in the water; as biological growth on the optics also causes light scatter, an impacted sensor will measure artificially high turbidity values. In general, biofouling can isolate water-quality sensors from the measuring environment and interfere with light transmission, both of which can compromise data. Anti-fouling protection reduces the impact on biofouling on the quality of collected data and allows longer deployment with more accurate data.

Different data quality procedures are required depending on the use of the data. Rather schematically, one can distinguish data that are used in delayed mode (the study of ocean processes, surveys to assess low-frequency variability, building up climatology), from those used in real time (or near real time) for assimilation into ocean circulation models for operational purposes (Pouliquen et al., 2007). These require validation as soon as possible after deployment (ref. to the previous section), and delayed mode quality control.

3.4.3.6. Links with other WPs and with other EU initiatives

Developments of Mastodon2 and YOYO trawl-secured profiling system are undergoing under WP3. Moreover, Mastodon2 is contributing to WP4 JRAP-4. The BUP system is developed under national funds and is not involved in research activities of JERICO-NEXT.

3.4.3.7. Summary

Yo-yo and suspended chains of sensors are well adapted to get frequent profiles of the water column, but have some limitation, especially when deployed in shallow water, regarding: sensitivity of the sensors to fouling, sensitivity of the mechanical parts to wear and to sea aggression (including fishery and vandalism), difficulty to establish the data collection link for the near real time equipped systems.
Nevertheless, Eulerian sampling systems, offer unique advantages: they can be operated in ocean currents, straits, capes, etc. where drifting buoys would be rapidly swept away, give to the ocean circulation model designer the possibility of clearly separate the time and the space components of a water mass motion, operate over the whole water mass column and especially the yo-yo systems offer the advantage of a greater vertical resolution, more complex sensor payloads and enable automatic near real time monitoring.

3.4.3.8. References

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Wong A.P.S., Johnson G.C., Owens W.B. (2003). Delayed-Mode Calibration of Autonomous CTD Profiling Float Salinity Data by Theta-S Climatology. Journal of Atmospheric and Oceanic Technology, 20, 308-318.

3.4.4. Fishing vessel based systems

3.4.4.1. Description of the fishing vessels based systems and the multiparametric probes embedded

In Italy, in the framework of several regional, national and international research projects, the Italian National Research Council (CNR) has been undertaking scientific and technological activities aimed at designing, implementing and operating intelligent and automated systems for the collection of data in support to oceanography, fisheries science and the sustainable exploitation of marine resources since 2003. In that year, in the framework of the EU-FP5 project MFSTEP, some commercial fishing vessels operating in the north and central Adriatic Sea were equipped with an integrated system for collecting data regarding catches, position of the fishing operation, depth and water temperature during the haul, the so called Fishery Observing System (FOS) (Falco et al. 2007, 2011). Thanks to the EU FP7 JERICO project and to other national projects (e.g. SSD-Pesca, RITMARE), the Italian FOS has later been upgraded in FOOS: Fishery & Oceanography Observing System. The FOOS is a modular tool, able to collect more parameter, with more accuracy, and send them to a data center in near real time (Patti et al. 2016; Fig. 3.4.10).
With the same aim, the Institut Français de Recherche pour l'Exploitation de la mer (Ifremer) began coordinating the RECOPESCA project in 2005 (Leblond et al. 2008, Fig. 3.4.11). One of the main result of this project was the development in collaboration with the French company NKE of a new generation of robust sensor systems (recording pressure, temperature and salinity) specifically designed to be mounted on fishing gears and that are self-powered and able to automatically send the data collected, firstly to an onboard receiver and then to the Ifremer central database (Leblond et al. 2010).

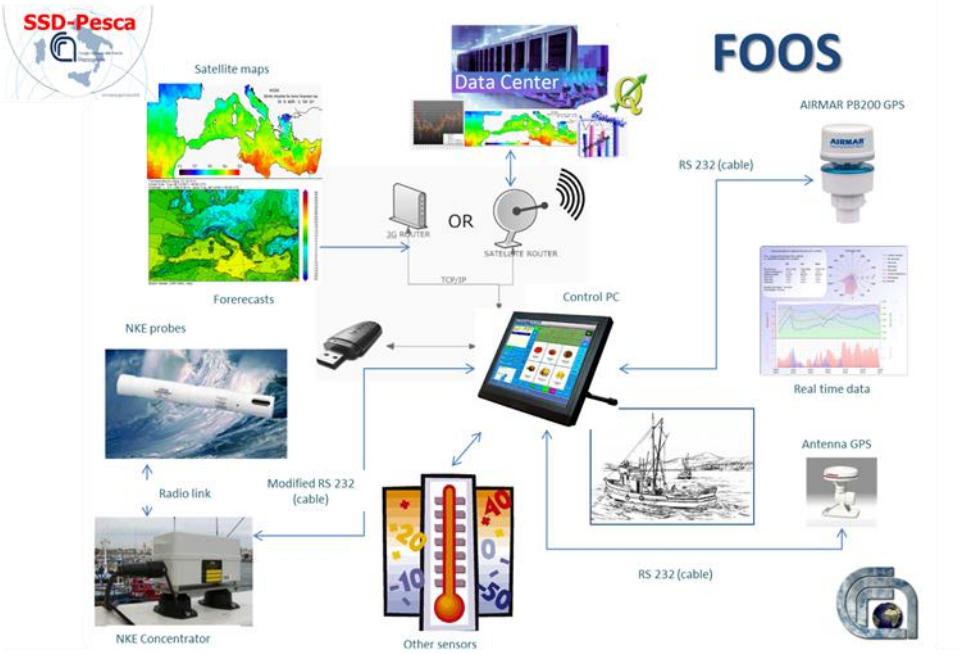

Figure 3.4.10. Scheme of the modular Fishery & Oceanography Observing System (FOOS) developed by CNR.

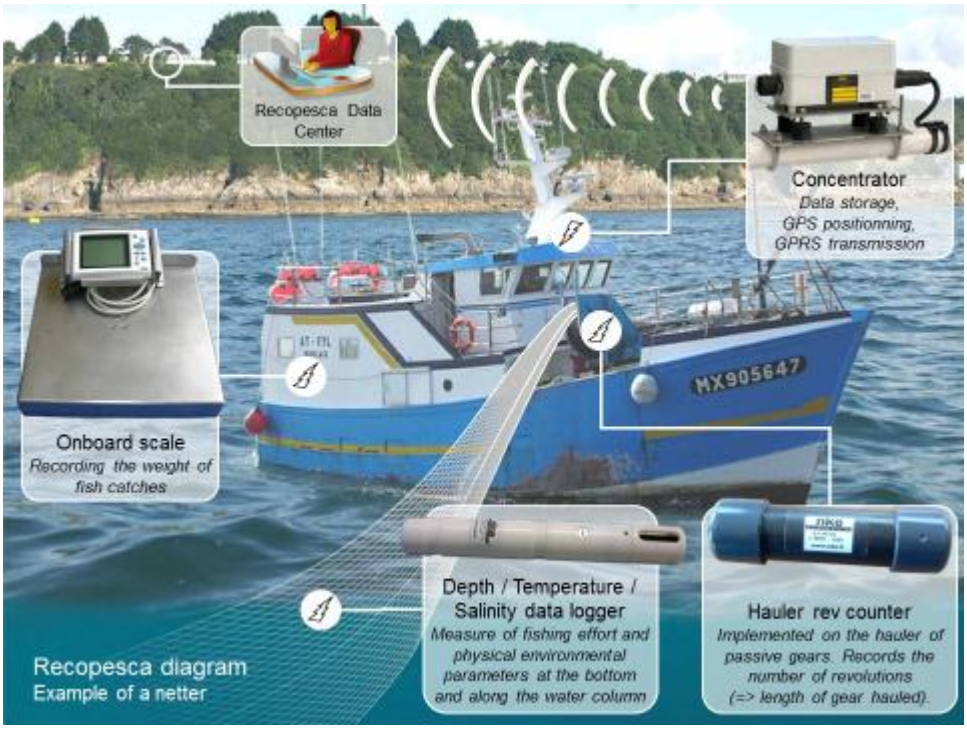

Figure 3.4.11. Scheme of the RECOPESCA system developed by Ifremer.

The FOOS and RECOPESCA share some common characteristics such as:

- the scientific purpose detached by the control purpose intrinsic of other monitoring systems such as the Vessel Monitoring System (VMS) or the Automatic Identification System (*AIS, implemented for marine traffic security),*
- the multidisciplinary approach and the collection of data useful both for fisheries and environmental science,
- the participative approach of the fisherman directly involved in the collection of the data,
- the installation intended to not interfere with the fishing activity,
- the autonomous functioning that requires technical intervention as less as possible,
- the near real time delivery of the collected data to a data center,
- the use of the same commercial oceanographic sensors (produced by NKE Instrumentations, http://www.nke-instrumentation.com) that allows the comparison of the data collected in different areas.

3.4.4.2. Current modes of deployment

While the FOS was initially developed to be installed on pelagic pair trawlers targeting small pelagic fishes, the modular design of the new FOOS allows the possibility to install it on various kind of fishing vessels and thus being employed to monitor different fisheries, targeting different resources and operating in various areas.
Currently the FOOS has been deployed in Italy in two different areas: the Sicilian Channel (where it is mainly installed on deep sea fishing vessels) and the Adriatic Sea. The Institute of Marine Science of Ancona (CNR-ISMAR) implemented from 2013 the AdriFOOS observational system in the Adriatic Sea, by installing the FOOS on 10 commercial fishing boats (7 boats targeting small pelagic fisheries using pelagic pair trawl or purse seine

and 3 bottom trawlers targeting demersal resources), and by building a proper data center which receives daily data sets on GPS tracks, water temperature, salinity, pressure, meteorology, catch amounts, species caught and target species sizes. The core of the FOOS is the electronic logbook equipped with software for the management of communication among the other devices included in the architecture of the system and able to directly show information to the fisherman (Patti et al. 2016; Fig. 3.4.12). In optimal conditions is it possible to obtain information on an average of 300 hauls per boat in a year.

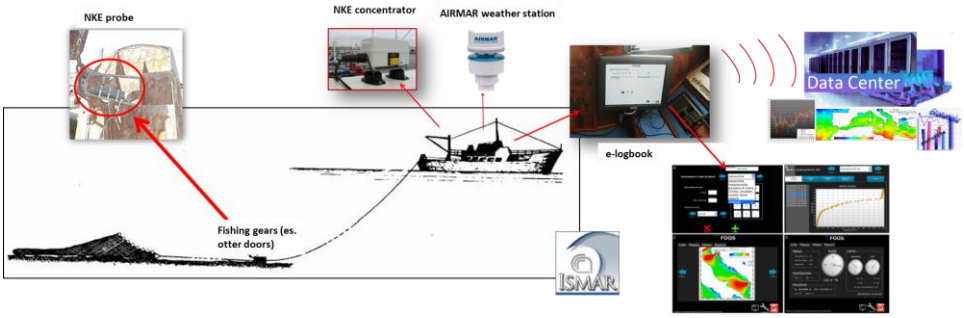

Figure 3.4.12. Scheme of the AdriFOOS deployment, showing the functioning of the e-logbook.

Since 2007, the RECOPESCA system has been widely deployed on an increasing number of voluntary vessels (mainly belonging to small scale fishery operating in coastal areas) after a test period and it is currently installed on 133 different types of vessels, with various lengths and using active or passive gears which represent a cross-section of the diversity of the French fishing fleet (métier, fishing areas, length), on a national scale including overseas territories At the end of 2016 there are 52609 vertical profiles (Temperature, Conductivity and Deep) and trajectories stored in the Coriolis Data Centre (http://www.coriolis.eu.org).

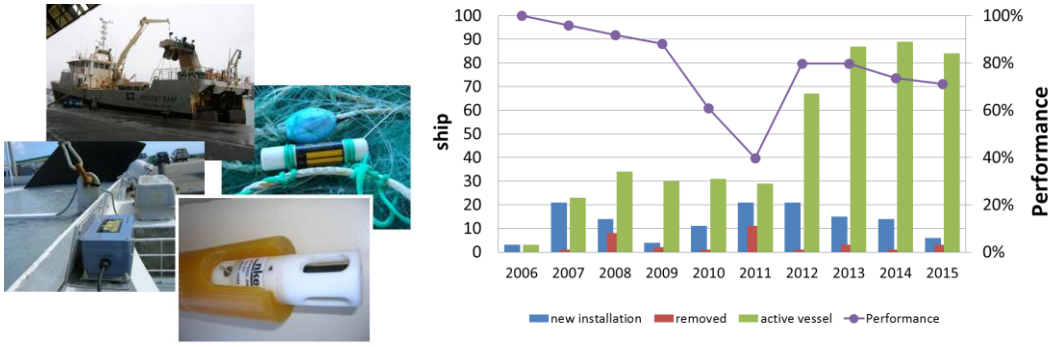

Figure 3.4.13. Example of RECOPESCA deployment and performance graph.

3.4.4.3. Reliability of the sensor and technological difficulties

The TRL of this type of systems may be stated between 8 and 9 as it uses now mature technologies and commercial sensors specifically developed to be mounted on fishing gears, thus robust enough, self-powered, autonomous as much as possible, not interfering too much with the fishing activities and having affordable prices.
Ifremer experienced for example very low levels of NKE probes failure (from 7 to 16%) and a low risk of loss of the probes mounted on the fishing gears (from 12 to 20%). The probes' failures could be permanent if due to physical shocks (they need in fact adequate mechanical protections) or temporary, if caused by firmware bugs, low battery or pressure sensor evolution. The loss of the sensors may be due principally to loss or damage of the gear or misplacement by the fishermen.
Collaboration of fishermen is indeed very important (e.g. periodical maintenance operations on board: washing of the sensors, re-install sensors while changing gears, switch on/off the system).
The transmission of the data to the datacentre in land occurs primarily via GPRS, which allows the vessel to transmit the data when they are close enough to the coast, thus it depends on the provider. Data transmission via satellite communication is possible but costly, thus the choice depends on the needs of real/near real time (e.g. GPRS is ok for the Adriatic Sea, where the typical duration of the fishing activity is 1-2 days).
This kind of system is not yet ready to be installed on the very small boats operating small scale fisheries in the Mediterranean: the FOOS need to be miniaturised.

3.4.4.4. Sensor calibration

The NKE oceanographic probes used in both FOOS and RECOPESCA systems, and thus installed on the fishing gears, are of 2 types: SP2T measuring temperature and pressure and STPS measuring also salinity.
The sensors are calibrated directly in the NKE metrological laboratories, once before sale and then each time they are returned to the company for battery change.
Ifremer usually performs verification of conformity with the metrological requirement (Fig. 3.4.14) before and after the deployment of the instruments (e.g. deployment duration: 6 – 12 months).

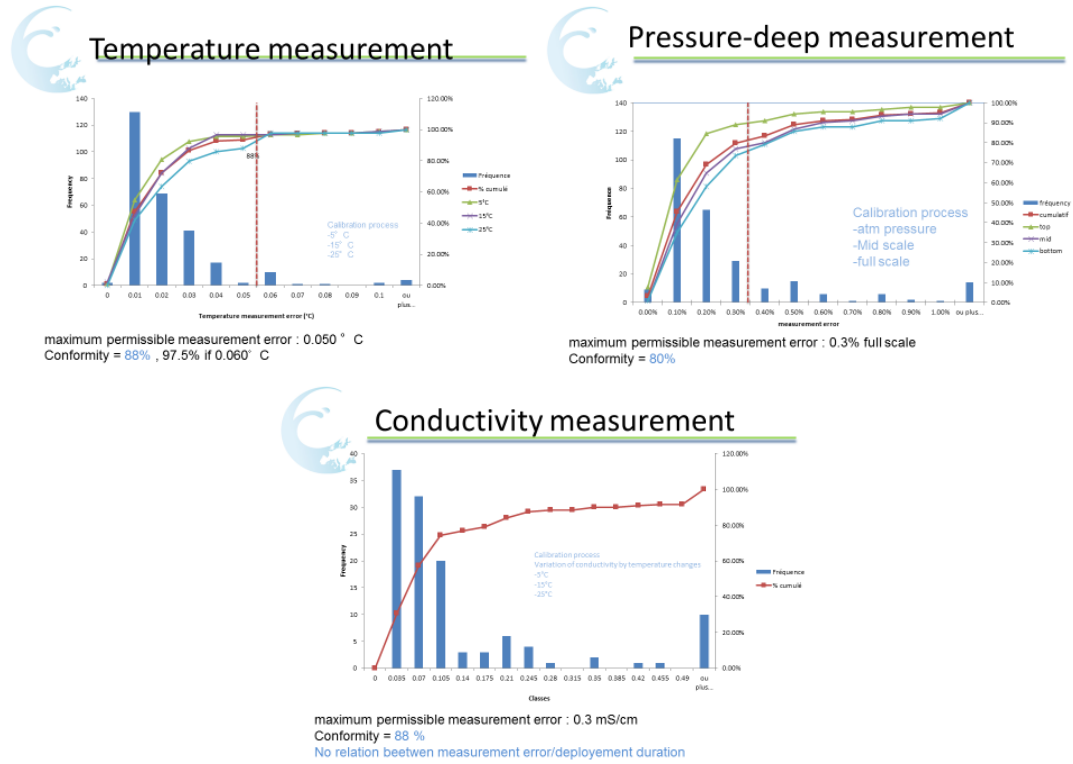

Figure 3.4.14. Verification of conformity with the metrological requirement

Within the JERICO project, CNR-ISMAR tried to assess the accuracy for physical oceanography purposes of both the commercial sensors used in the FOS (Star-Oddi) and in the FOOS (NKE). Thus, in order to establish their accuracy, comparison tests were carried out in field against a calibrated CTD during several surveys at sea. Simulations of data collection during fishing activity were performed in two different ways: by simultaneous profiling of the water column (normal CTD deployment) and by simultaneous dragging on the seabed of the probes and a CTD installed on a sledge (Martinelli et al. 2016). Summarizing the results of these experiments, the data collected by Star-Oddi sensors are useful only considering the data portion where a dwell time at a fixed depth permanence is longer than 50s, while those collected by NKE sensors are much more accurate for both depth and temperature and could be usefully considered for broader oceanographic purposes. The weak point of the NKE sensors is the salinity measurement (Martinelli et al. 2016).

3.4.4.5. Data issues

The evaluation carried out in Martinelli et al. (2016) underlined the optimal conditions for the usage of the considered sensors (comprising the NKE probes) and produced a series of offsets that might be used to enhance the accuracy of the recorded datasets. To make the datasets produced by sensors on fishing gears comparable to traditional oceanographic ones (e.g. CTD transects), these definitely need to be tested to determine the accuracy of the produced datasets. The tests should be performed as much as possible simulating the fishing activities (e.g. use of mechanical protections, speed, mode of deployment…).
From the fishery biology point of view, the data collected through such king of systems already demonstrated their utility (e.g. Carpi et al. 2015). Oceanographic data collected by means of the FOS were already used for the implementation of models (Aydoğdu et al. 2016).
Lamouroux et al. (2016) used a mathematical model to assess the efficiency of the RECOPESCA network and to design draw suggestions regarding possible observation network extensions.

3.4.4.6. Links with other WPs and with other EU initiatives

Data of temperature and (in few cases) salinity measurements acquired by the FOOS, from January 2014 to March 2015, along the fishing tracks and at the various fishing depths were published within the JERICO project (http://www.jerico-ri.eu/previous-project/service-access/targeted-operation-phase/top-2-data-and-maps-from-sensors-on-board-fishing-vessels/adriatic-sea-fishery-and-oceanography-observing-system/). They provide information along the water column (vertical profiles) in different geographical location and at different depths (e.g. horizontal maps) identifying changes in the properties due to physical processes. This dataset will be used within JERICO-NEXT WP4 JRAP#6 to feed Observing System Experiments (OSEs) and Observing System Simulation Experiments (OSSEs) for the Adriatic Sea.
Furthermore, CNR-ISMAR, Ifremer and NKE are all involved in the EU FP7 NEXOS project in which they collaborate for the development, testing and demonstration of new EAF (Ecosystem Approach to Fishery) sensors for fluorescence-Chl a and Dissolved Oxygen to be mounted on the fishing gears and work in the same way of the probes already in use.

3.4.4.7. Summary

The basic idea behind the use of fishing vessels based systems is to acquire data useful for various purposes on a broad spatial and temporal scale with very low costs. The use of a modular dispositive, such as the FOOS or RECOPESCA, installed onboard different kinds of fishing boats, using different gears, targeting different species assemblages and exploiting different areas of the Mediterranean Sea allows obtaining a huge amount of information. At present time water temperatures (especially sea bottom temperatures which are not captured by other tools like the satellites) are the worthiest results obtained from the oceanographic side, but in the near future appropriate and reliable probes able to collect more parameters simultaneously will be available (Martinelli et al. 2014). If the optimal operative conditions are respected and the data are treated properly, the datasets produced daily by these new generation systems are highly valuable from the oceanographic and ecological points of view.

3.4.4.8. References

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3. Conclusions

The harmonization of technologies, methodologies and procedures is a vital step in ensuring efficiency and optimal returns from any kind of instrument, employed on a transnational level such as the JERICO network. This is because such harmonization leads to an intelligent use of resources and information across the network, adds to the consistency of its services and products, and helps to provide uniformed protocols, thereby allowing intercomparisons of, and conclusions from different sensors.

A characterization and evaluation of the different sensors and systems has been performed and thereby, a foundation towards harmonization was established through intense exchanges during the two respective workshops.

The next steps will be to work on defining more homogeneous best practices on the different aspects described above. The final objective is now to reach some consensus on methods and best practices in the utilization and deployment of the sensors. The results will be reported in Deliverable 2.5: *Report on Best Practice in the in the utilization of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling*.