### CNR IRBIM



# Deliverable 5.6

Validation and integration report on ships of opportunity

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NAUTILOS - New Approach to Underwater Technologies for Innovative, Low-cost Ocean observation is an H2020 project funded under the Future of Seas and Oceans Flagship Initiative, coordinated by the National Research Council of Italy (CNR, Consiglio Nazionale delle Ricerche). It brings together a group of 21 entities from 11 European countries with multidisciplinary expertise ranging from ocean instrumentation development and integration, ocean sensing and sampling instrumentation, data processing, modelling and control, operational oceanography and biology and ecosystems and biogeochemistry such, water and climate change science, technological marine applications and research infrastructures.

NAUTILOS will fill-in marine observation and modelling gaps for chemical, biological and deep ocean physics variables through the development of a new generation of cost-effective sensors and samplers, the integration of the aforementioned technologies within observing platforms and their deployment in large-scale demonstrations in European seas. The fundamental aim of the project will be to complement and expand current European observation tools and services, to obtain a collection of data at a much higher spatial resolution, temporal regularity and length than currently available at the European scale, and to further enable and democratise the monitoring of the marine environment to both traditional and non-traditional data users.

NAUTILOS is one of two projects included in the EU's efforts to support the European Strategy for Plastics in a Circular Economy by supporting the demonstration of new and innovative technologies to measure the Essential Ocean Variables (EOV).

More information on the project can be found at: http://nautilos-h2020.eu/.

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### **EXECUTIVE SUMMARY**

This deliverable is the result of the work carried out in Task 5.3 (Sensor integration into Ships of Opportunity). The main objectives were to carry out the field validation and integration of the DO and Chl-a sensor and WiHub developed in ST3.1.2 on fishing vessels and AdriFOOS platform. Additionally, this reports on the integration of other sensors/samplers developed in WP3 and 4 on the FerryBox systems, in order to proceed with several of the demonstrations involving platforms and ships of opportunity foreseen in ST7.1 and ST7.2.

This document provides details on the results obtained during field validation cruises carried out in various European regions and on the integration of the NAUTILOS prototypes on the AdriFOOS platform, commercial fishing vessels and ferries.

Therefore, it is organised in five main sections:

Chapter I: Introduction, provides a brief description of the idea behind the planned activities, some references to the state of the art and a list of the activities carried out;

Chapter II: Integration of DO and Chl-a Sensor and Hub System on Fishing Vessels, contains details of five validation cruises carried out by CNR, IFREMER and SYKE; these cruises were crucial to evaluate the performance of the NAUTILOS prototypes against traditional oceanographic instruments; validation was achieved by following an agreed protocol, and the steps taken to obtain the integration of the new developed system on the CNR AdriFOOS platform, and on a fishing vessel monitored by IFREMER;

Chapter III: Integration of sensors/samplers on FerryBox ships of opportunity, describes integration of carbonate sensors, downward looking optical sensors, the phytoplankton and particle sampler, and the microplastics sensor into FerryBox systems.

Chapter IV: Ethical Considerations, reports some considerations on some ethical aspects relevant to the activities described in this deliverable;

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Table 15. The POSEIDON FerryBox instrumentation.

### LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Definition						
AdriFOOS	Adriatic Fisheries and Oceanography Observing System						
ASCII	American Standard Code for Information Interchange						
C#	C sharp						
CDOM	Chromophoric dissolved organic matter						
CFP	Common Fisheries Policy						
Chl-a	Chlorophyll a						
CSV	Comma Separated Values						
CTD	Conductivity, Temperature, Depth						
DHCP	Dynamic Host Configuration Protocol						
DO	Dissolved Oxygen						

EAF	Ecosystem Approach to Fishery
EOVs	Essential Ocean Variables
EU	European Union
FB	FerryBox
FOOS	Fisheries and Oceanography Observing System
FTP	File Transfer Protocol
GPS	Global Positioning System
IBTS	International Bottom Trawl Survey
ICD	Interface Control Document
IP	Internet Protocol address
IP	Internet Protocol
LAN	Local area network
LED	Light Emitting Diod
LTER	Italian Long-Term Ecological Research Network
MSFD	Marine Strategy Framework Directive
PC	Personal Computer
PPS	Particle and Phytoplankton Sampler
R/V	Research Vessels
REST-API	Representational State Transfer - Application Programming Interface
SOOPs	Ships of Opportunity
SQL	Structured Query Language
SSID	Network Service Set IDentifier
TCP	Transmission Control Protocol
TRL	Technology Readiness Levels
VOOs	Vessels Of Opportunity
WT	Water Tracer
XML	eXtensible Markup Language

### . Introduction

The fundamental objective of the NAUTILOS Horizon 2020 project is to fill the existing gaps in marine observation through the development of new sensors and samplers (WP3/4) to be integrated on different platforms (WP5). These new instruments shall be demonstrated in a variety of applications relevant to the European Union (EU) policies, including demonstrations of fisheries and aquaculture observing systems and, in general, on vessels and platforms of opportunity (WP7).

NAUTILOS Task 5.3 dealt with the integration of sensors, samplers and new systems, developed through NAUTILOS, into ships of opportunity (SOOPs) and platforms of opportunity. The use of SOOPs, also called Vessels Of Opportunity (VOOs), in operational oceanography constitutes a consolidated approach mainly based on the use of FerryBox systems installed on ferries or other vessels that regularly travel on commercial routes; commercial vessels equipped with scientific instruments are able to record data in areas that could not reasonably often be covered by the traditional data collection activities of research vessels without a huge effort of manpower and funding (Petersen et al., 2003; Sloyan et al., 2018; Jiang et al., 2019; Rosa et al., 2021). Some routes allow for high frequency (daily to weekly) and high spatial resolution (10's-100's of meters) observations of surface ocean physics, chemistry, and biology. FerryBoxes are also generally not limited by instrument power and space requirements, and prototype instruments can be easily tested and maintained either with personnel on board or visits while at port. FerryBox systems have matured and now measure a growing number of biogeochemical variables. New sensors and analysers have been added, including those for algal composition, pH, carbon budget (pCO2, alkalinity), and nutrients like phosphate, nitrate, and silicate (Petersen et al., 2017). The collected data are used for monitoring and research in various water systems like the Baltic Sea, North Sea, Bay of Biscay, and Mediterranean Sea. Integration with satellite remote sensing provides broader-scale data. Long-term observations along fixed transects help detect trends in coastal and oceanic waters. Such time series, available for over 25 years in the Baltic Sea, have been vital in understanding the effects of eutrophication and other factors. Continuous measurements along transects, often within days, are valuable for capturing short-term events, complementing periodic research cruises. FerryBox time series data validate and improve physical models, aiding in the development of ecosystem models. Real-time data support operational models and provide early warnings for aquaculture and fishing operations, including toxic algal blooms. New sensors for alkalinity and pH enable detailed monitoring of ocean acidification and coastal carbon dynamics, which are still poorly understood. Automated water samplers in most FB systems allow regular sample collection for subsequent lab analysis. These samples can be used for trace contaminant screening and potentially the study of microplastic abundance in oceans as suitable analytical techniques develop.

A booming global approach uses specially designed sensors deployed by commercial fishing vessels to collect large amounts of data that proved to be useful for both operational oceanography and ecosystem approach to fisheries (Van Vranken et al., 2020, 2023). NAUTILOS partners CNR, IFREMER and NKE have extensive experience in this field developed through the design and implementation of observation systems based on the use of commercial fishing vessels which have already collected huge amounts of datasets since early 2000 and through participation in several EU projects (e.g. Leblond et al., 2010; Lamouroux et al., 2016; Patti et al., 2016; Penna et al. 2023).

So far these datasets have mainly concerned depth, temperature, and salinity data received in real time, capable of improving oceanographic models (Aydoğdu et al. 2016; Mourre et al. 2019). However, the possibility of extending the spectrum to other Essential Ocean Variables (EOV) would for example increase the contribution to the objectives of the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) and the Common Fisheries Policy (CFP; EU REGULATION 1380/2013), and to the knowledge of marine ecosystems; apart from producing results comparable with traditional oceanographic instruments, new sensor systems should be robust, self-powered or easy to power, cost-effective and self-contained, easy to use and install, and not require fisherman (or other users) frequent intervention in order to support participatory approach (Martinelli et al. 2014).

As will be described below, in Sub-task 5.3.1, on the basis of the experience previously acquired on the subject by the scientific partners (e.g. Martinelli et al., 2016, 2017), field validation activities were carried out on the DO and Chl-a sensors developed in Sub-task 3.1.2 to allow comparisons with standardized methodologies; furthermore the prototypes and their receiving hub were tested during an experimental fishing cruise aboard a research vessel and integrated on the AdriFOOS platform and on a commercial fishing vessel.

In Sub-task 5.3.2 samplers and sensors developed in WP3 and WP4 were integrated with and adapted for deployment on ship of opportunities. Thus, a flow through system for a pH sensor has been designed for SYKE FerryBoxes, and the phytoplankton/suspended matter and microplastic samplers system were integrated with NIVA FerryBox systems, as well as the integration of sea surface temperature downward looking sensor for operation on an HCMR FerryBox. Whereas DO and Chl-a fluorescence sensors which are intended to be deployed on aquaculture sites did not require any adaptation and can be installed as they are.

# II. Integration of Do And Chl-A Sensor and Hub System on Fishing Vessels

In Sub-task 3.1.2, NKE developed prototypes of new Dissolved Oxygen (DO) and Fluorescence (Chl-a) Sensors expressly designed to be used in fisheries and aquaculture applications (i.e. fishing vessels as platforms of opportunity and aquaculture plants); in addition to measuring depth, temperature and the specific parameter, the sensors communicate via Wi-Fi with a receiving hub (WiHub), an automatic data recovery and transmission system, which also records GPS positioning (Malardé et al. 2022).

Three sets of sensor prototypes and two hubs were produced to undergo laboratory calibrations in ST6.1.1, field validation and integration onto platforms (where needed) in T5.3 and demonstrations on fishing vessels in ST7.1.1 and in aquaculture plants in ST7.1.2.

The six sensors were factory-calibrated for dissolved oxygen (3) and Rhodamine WT (3) in ST3.1.2 Malardé et al. 2022).

Sensors specifications as declared by the producer are:

- Pressure range 0-600 dbar with accuracy 1.5% of full scale,
- Temperature range -2 to 35 °C with accuracy ± 0.020 °C,
- DO concentration measuring Range 0 23 mg/l, with accuracy  $\pm 0.1$  mg/l, resolution 0.025 mg/l and detection Limit 0.01 mg/l,
- Oxygen Saturation percentage measuring range 0 250%, with accuracy  $\pm 1\%$ , resolution 0.25% and detection limit 0.1%,

- Chlorophyll-a concentration measuring range 0 140 ppb RWT, with sensitivity 0.03 ppb and linearity  $R^2 > 0.999$ ,
- Maximum measuring frequency 0.5 Hz.

The prototypes underwent a series of laboratory calibration tests in task 6.1, carried out at IFREMER, CNR, and SYKE facilities. Tests included intercomparisons, measurements at different salinity/temperature/dissolved oxygen combinations, and fluorescein and algal cultures comparison experiments on the Chl-a sensors (see NAUTILOS Deliverable D6.1.1). Here is a list of prototypes produced by NKE, serial numbers (s.n.) and partners to whom they were entrusted to carry out field validation activities:

- 3 DO sensors (Factory calibration Date 11-22): s.n. 2222 (CNR), s.n. 1111 (IFREMER) and s.n. 4444 (NIVA),
- 3 Chl-*a* sensors (Factory calibration Date 09-22): s.n. 8888 (CNR), s.n. 6666 (IFREMER) and s.n. 7777 (SYKE),
- 2 WiHub systems: s.n. 23 0009 (IFREMER) and s.n. 23 0002 (CNR).

Sub-task 5.3.1 had two main objectives: i) perform a field validation of the prototypes to gather more information on their behaviour and to define optimal operational conditions; ii) achieve integration of prototypes and hub on the observational platforms already in place and involving fishing vessels.

CNR carried out 2 field validation cruises in the Adriatic Sea, and integration on the AdriFOOS platform (Penna et al. 2023; Patti et al. 2016). IFREMER carried out a field validation cruise in the North Sea (instead of in the Bay of Biscay initially planned due to delay in sensors delivery), and took the opportunity to carry out an additional test in deep condition (not originally foreseen in the project description of actions for this task) during a fishing cruise in the Bay of Biscay. An additional field validation cruise (not originally foreseen in the project description of actions for this task) was carried out by SYKE in the Baltic Sea to test one of the Chl-a sensors in different conditions of water productivity.

### 1 FIELD VALIDATION

A common framework for the field validation procedures, simulating use in fisheries and aquaculture applications, was defined in October 2022 (M25) by CNR, IFREMER and SYKE in agreement with NKE. The adopted procedures were based on the experience already acquired by CNR and IFREMER while carrying out field validation on previous generations of sensors to be used on fishing gears, within previous EU FP7 projects (e.g. temperature and salinity in JERICO and EAF sensors in NEXOS; Martinelli et al., 2016, 2017). The agreed protocols include comparison of the measurements acquired by the DO and Chl-a prototypes with those acquired by a high accuracy, resolution, and sampling rate commercial Conductivity, Temperature, Depth (CTD) probe. Simultaneous profiling (Fig. 1), with particular reference to the datasets acquired during stays at a fixed depth, allows overcoming bias due to different acquisition speeds. As reference points, additional data derived from water sampling carried out in some cases during the simultaneous profiling are also included.

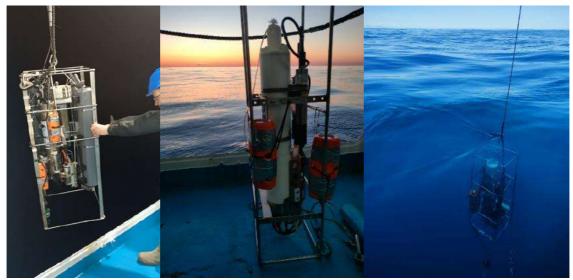


Figure 1: Examples of NAUTILOS prototypes (with orange plastic protections) mounted on a CTD frame for simultaneous deployment.

CNR and IFREMER performed casts with the DO and Chl-a sensors in various stations during research cruises in the Adriatic Sea (Mediterranean basin), the North Sea, and Bay of Biscay. SYKE performed additional field validation of the Chl-a sensor in the Baltic Sea (Fig. 2).

Here is a list of the field validation cruises carried out by partners:

- IFREMER: IBTS cruise, North Sea, January 2023 (M28),
- CNR: ANOC23 cruise, Adriatic Sea, February 2023 (M29),
- SYKE: COMBINE-kevätseuranta (spring monitoring) cruise, Baltic Sea, April 2023 (M31),
- CNR: "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise, Adriatic Sea, April 2023 (M31),
- IFREMER: PELGAS cruise, Bay of Biscay, May 2023 (M32).

In the following subsections, for each of the field validation cruises will be specified:

- Specific objectives,
- Cruise details,
- Methods,
- Results,
- Recommendations for use and future deployment/issues encountered.

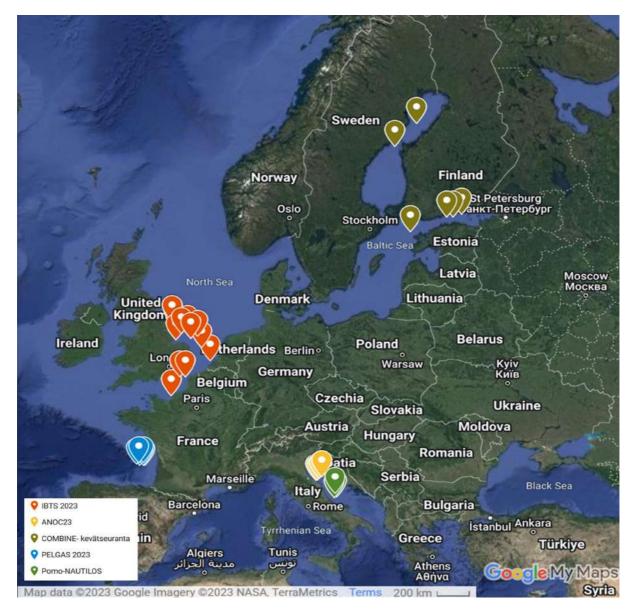


Figure 2: Map showing all stations in which simultaneous profiling of DO/Chl-a prototypes and CTD was carried out during the field validation cruises (placeholder in different colours carried out by CNR, IFREMER and SYKE in 2023.

### 1.1. IBTS cruise, North Sea, January 2023 (IFREMER)

### • Specific objectives:

To calculate offsets for temperature and pressure on both 1111 and 6666 prototypes, to calculate DO correction factors for prototype 1111 when internal salinity is set at 0, and to check Chl-*a* trends for 6666 prototype.

### Cruise details:

The International Bottom Trawl Survey Cruise (IBTS; Auber et al. 2023) was carried out by IFREMER staff in the North Sea from 25/01/2023 to 01/02/2023 on board of R/V Thalassa. The main objective of IBTS cruises is to calculate annual indices of the abundance of the main

commercial fish species exploited in the North Sea. This cruise was an opportunity to test the DO and Chl-a prototypes, comparing to the CTD on board.

Figure 3 shows the location of the stations in which field validation was carried out, while dates and coordinates for each station are reported in Table 1.



Figure 3: Map showing location of simultaneous profiling of CTD and NAUTILOS prototypes carried out in the North Sea by IFREMER staff during the IBTS cruise in January 2023.

Table 1: List of IBTS stations in which IFREMER staff carried out simultaneous sampling: date, time and coordinates are reported for each station.

Partner	Cruise	Station	Date	NMEA UTC Start Time	Latitude	Longitude
IFREMER	IBTS 2023	B2008	25/01/2023	07:46:04	49.6113	0.0896
IFREMER	IBTS 2023	B2017	26/01/2023	09:38:29	50.8717	0.9080
IFREMER	IBTS 2023	B2026	27/01/2023	09:53:21	50.8660	1.3444
IFREMER	IBTS 2023	B2033	28/01/2023	07:36:31	51.8247	3.6357
IFREMER	IBTS 2023	B2037	28/01/2023	19:48:54	52.6266	2.8434
IFREMER	IBTS 2023	B2043	29/01/2023	12:44:40	53.6649	1.5476
IFREMER	IBTS 2023	B2042	29/01/2023	07:29:49	53.2052	1.8307
IFREMER	IBTS 2023	B2052	30/01/2023	07:07:44	53.9189	0.2750
IFREMER	IBTS 2023	B2057	30/01/2023	22:06:01	54.2335	0.1803
IFREMER	IBTS 2023	B2064	31/01/2023	21:41:56	53.1407	0.5445
IFREMER	IBTS 2023	B2070	01/02/2023	10:48:30	53.4915	0.9546
IFREMER	IBTS 2023	B2073	01/02/2023	20:41:15	53.3535	2.4399

### Methods:

Simultaneous profiling of CTD and prototypes 1111 and 6666 was carried out in 12 stations (Fig. 3; Tab 1).

The instruments used for comparison were:

- Sea-Bird SBE19plus CTD (Calibration Date 14-Apr-22; reference: <a href="https://www.manualslib.com/products/Sea-Bird-Electronics-Seacat-Sbe-19-10634816.html">https://www.manualslib.com/products/Sea-Bird-Electronics-Seacat-Sbe-19-10634816.html</a>);
- Complementary sensor for oxygen measurements: SBE 43 (Calibration Date 02-Jul-22; reference: <a href="https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728">https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728</a>)
- Complementary sensor for chlorophyll measurements: Fluorometer WET Labs ECO-AFL/FL (Calibration Date 06-May-22; reference: <a href="https://www.seabird.com/eco-fluorometer/product?id=60429374754">https://www.seabird.com/eco-fluorometer/product?id=60429374754</a>).

Both prototypes were mounted on the CTD frame, with the depth detector at about 50 cm above that of the CTD (Fig. 4). The prototypes' recording was started manually before each cast. During the downcast, stops for 30 sec-1 min were performed at different depths (reported in Tab. 2). Furthermore, water samples were collected by means of a Niskin bottle attached to the CTD frame in 6 stations at 1 depth. Subsequently, water samples were analysed for oxygen and chlorophyll respectively using Winkler method (Aminot and Kérouel,

2004) and Chlorophyll-a and phaeophytin acidification method by means of a Turner Designs 10-AU fluorometer<sup>1</sup>.

Due to bad weather conditions encountered during the cruise, and wave motion with subsequent R/V fluctuations, the acquired CTD dataset showed oscillations on the pressure values. This effect was evident especially in the first part of the casts and therefore in the post-processing phase, data were filtered to eliminate anomalies using a procedure similar to that proposed by SeaBird company with the loop edit function<sup>2</sup>. NAUTILOS prototypes record 1 value every 2 seconds, therefore CTD (and complementary sensors) data were averaged to be compared with these. Oxygen concentration data collected by NAUTILOS prototypes in mg/l were converted to ml/l to be compared with CTD datasets, as well pressure (bar) was converted in depth (m).

Depth and temperature offsets for each prototype, and dissolved oxygen correction factor for sensor 1111 were calculated for each cast and depth permanence (using average measurements over each permanence depth). Depth offsets were also a posteriori corrected for the positioning of the sensors on the frame (50 cm above the CTD one; Fig. 4). The obtained results were used to build scatter plot graphs to compare the performance of the NAUTILOS prototypes versus CTD (and complementary sensors) measurements. These graphs also show possible dependence (linear regression) of the oxygen and chlorophyll measurements of the prototypes on depth or temperature recorded. Average values on all permanences were derived. Water sample results, CTD profiles, sensors' profiles and corrected DO profiles were used to build graphs for each available comparison.



Figure 4: CTD, sampling bottle, DO and Chl-a prototypes mounted on a frame used to perform water sampling and simultaneous profiling during the IBTS cruise.

<sup>&</sup>lt;sup>1</sup>https://www.turnerdesigns.com/10au-field-laboratory-fluorometer

<sup>&</sup>lt;sup>2</sup>https://www.seabird.com/cms-

portals/seabird com/cms/documents/training/Module13 AdvancedDataProcessing.pdf

### Results:

Table 2 shows average values and offsets/corrections calculated for each permanence depth according to the depth recorded by the CTD; the calculated offsets to be applied for depth to prototype 1111 were corrected to take into account the position of the sensor on the CTD frame. The calculated average ( $\pm$  standard deviation) offsets for depth and temperature and the correction for the oxygen concentration are respectively 0.237 ( $\pm$ 0.181) m, -0.017 ( $\pm$ 0.027) °C and -2.419 ( $\pm$ 0.185) ml/l (with an average water salinity recorded of 34.363.

Oxygen concentration correction is not significantly dependent on depth (p=0.884), while showed a slight positive dependence on temperature (p=0.0366). The latter was expected due to the nature of the parameter measured, and it is in agreement with results obtained during laboratory calibration in ST6.1.1 (Fig. 5 A-B). Figure 5C-D shows a substantial agreement between temperature and depth measured by sensor 1111 and CTD (orange line is a reference that represents 100% agreement). Figure 5E shows the comparison between oxygen concentration recorded by the complementary sensor on the CTD and the measurements recorded by prototype 1111 with internal salinity set at 0; there is a fair agreement between the values from both sensors. The 1111 sensor values can be *a posteriori* corrected by a linear correction equation.

Figure 6 shows the comparison between depth/oxygen concentration profiles obtained by means of CTD and prototype 1111, and for 7 stations also the values of oxygen in ml/l obtained from water samples. The average calculated oxygen concentration correction value was also used to post-process oxygen data acquired by prototype 1111, and the resulting new profiles are also shown in Figure 6.

Table 3 shows average values and offsets/corrections calculated for each permanence depth according to the depth recorded by the CTD; the calculated offset to be applied for depth to prototype 6666 was corrected to take into account the position of the sensor on the CTD frame. The calculated average ( $\pm$  standard deviation) offsets for depth and temperature are respectively 0.178 ( $\pm$ 0.339) m, -0.007 ( $\pm$  0.026) °C. For fluorometric Chl- $\alpha$  sensors the calculation of offset is not meaningful, as there is no commonly agreed method for sensor calibration and each of them measure at their own relative scale

Figures 7A-B show a substantial agreement between temperature and depth measured by sensor 1111 and CTD (orange line is a reference that represents 100% agreement). Figure 7C shows different scales for the chlorophyll measurements obtained by 6666 (in the sensor output data file it is reported in  $\mu$ g/L) and CTD complementary sensor; this is due to the fact that factory calibration of the two instruments relies on different references: sensor 6666 was calibrated with Rhodamine WT while the complementary sensor is set for chlorophyll.

Figure 8 shows the comparison between depth/chlorophyll concentration profiles obtained by means of CTD and prototype 6666, and for 4 stations also the chlorophyll values obtained from water samples. For prototype 6666, depth was corrected for the obtained average offset and Rhodamine in ppb is reported as a proxy of chlorophyll. The range used to report prototype chlorophyll values was fixed, therefore some values fall outside the graph.

Table 2: Depth and temperature offsets and dissolved oxygen correction factor calculated for sensor 1111 according to simultaneous profiling performed during the IBTS cruise for each cast and depth permanence.

Station/ Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	1111 Depth (m)	1111 Tempe- rature (°C)	1111 Oxygen (ml/l)	Corrected Depth Offset (m)	Temperature Offset (°C)	Oxygen Correcti on (ml/l)
B2008	2.398	8.510	33.359	9.190	0.593	1.600	8.530	11.755	0.298	-0.020	-2.565
B2008	3.935	8.577	33.400	8.774	0.641	3.500	8.566	11.254	-0.065	0.011	-2.480
B2008	6.698	8.607	33.400	8.782	0.605	6.000	8.595	11.125	0.198	0.012	-2.343
B2017	1.394	7.965	34.548	9.408	0.693	0.600	8.065	11.615	0.294	-0.100	-2.207
B2017	2.515	7.965	34.548	8.836	0.683	1.700	7.974	11.267	0.315	-0.009	-2.431
B2017	5.463	7.962	34.543	8.839	0.669	4.700	7.966	11.273	0.263	-0.004	-2.434
B2026	4.203	9.517	34.999	8.599	0.464	3.700	9.518	11.031	0.003	-0.001	-2.432
B2026	7.437	9.517	35.001	8.622	0.450	6.700	9.541	11.032	0.237	-0.024	-2.410
B2033	1.379	7.538	34.999	10.213	1.334	0.600	7.577	11.896	0.279	-0.039	-1.683
B2033	2.890	7.559	34.999	9.278	1.406	2.100	7.545	11.614	0.290	0.014	-2.336
B2033	5.875	7.576	35.003	9.275	1.456	5.200	7.573	11.592	0.175	0.003	-2.317
B2037	1.283	9.237	35.127	9.495	0.528	0.400	9.289	11.633	0.383	-0.052	-2.138
B2037	2.384	9.306	35.127	8.665	0.560	1.600	9.298	11.133	0.284	0.008	-2.468
B2037	5.778	9.308	35.127	8.676	0.559	5.000	9.299	11.130	0.278	0.009	-2.454
B2042	1.944	6.767	34.358	9.632	0.556	1.200	6.771	12.259	0.244	-0.004	-2.627
B2042	3.350	6.785	34.358	9.291	0.574	2.400	6.778	11.771	0.450	0.007	-2.480
B2042	5.970	6.701	34.357	9.302	0.583	5.300	6.693	11.802	0.170	0.008	-2.500
B2043	3.052	7.124	34.358	9.229	0.643	2.300	7.145	11.763	0.252	-0.021	-2.534
B2043	6.257	7.124	34.565	9.236	0.656	5.800	7.138	11.768	-0.043	-0.014	-2.532
B2052	3.272	7.321	34.481	9.112	0.381	2.400	7.340	11.584	0.372	-0.019	-2.472
B2052	7.104	7.321	34.482	9.090	0.403	5.800	7.321	11.628	0.804	0.000	-2.538
B2057	0.969	7.285	34.443	9.599	0.405	0.700	7.320	11.752	-0.231	-0.035	-2.154
B2057	3.245	7.280	34.442	9.131	0.396	2.400	7.302	11.659	0.345	-0.022	-2.528
B2057	5.931	7.289	34.443	9.148	0.394	5.300	7.295	11.651	0.131	-0.006	-2.503
B2064	2.926	5.524	33.125	9.663	0.481	2.200	5.550	12.183	0.226	-0.026	-2.520
B2064	6.128	5.521	33.123	9.680	0.474	5.400	5.540	12.194	0.228	-0.019	-2.514
B2070	4.125	6.180	33.725	9.503	0.428	3.400	6.231	12.011	0.225	-0.051	-2.508
B2073	3.404	6.757	33.725 2 1	9.405	0.693	2.600	6.830	11.937	0.304	-0.073	-2.532
B2073	5.665	6.758	33.725	9.403	0.667	5.000	6.777	11.928	0.165	-0.019	-2.525

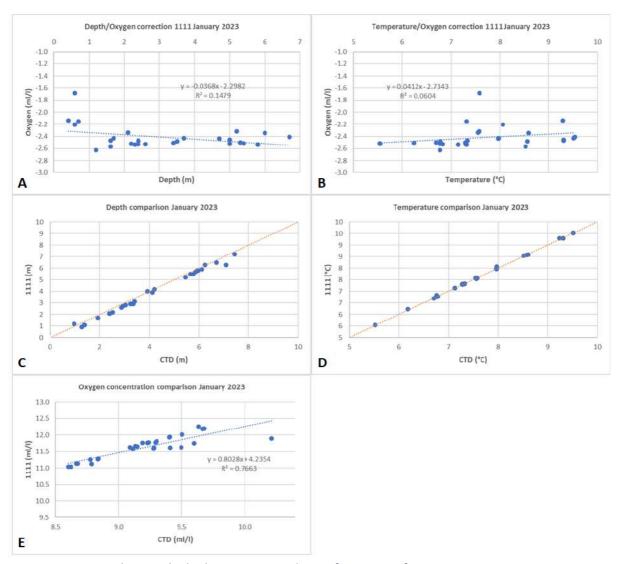


Figure 5: Scatter plot graphs built to compare the performance of NAUTILOS prototype 1111 versus CTD (and complementary sensors) measurements and to detect possible dependence of the correction factor for oxygen concentration on depth or temperature recorded.

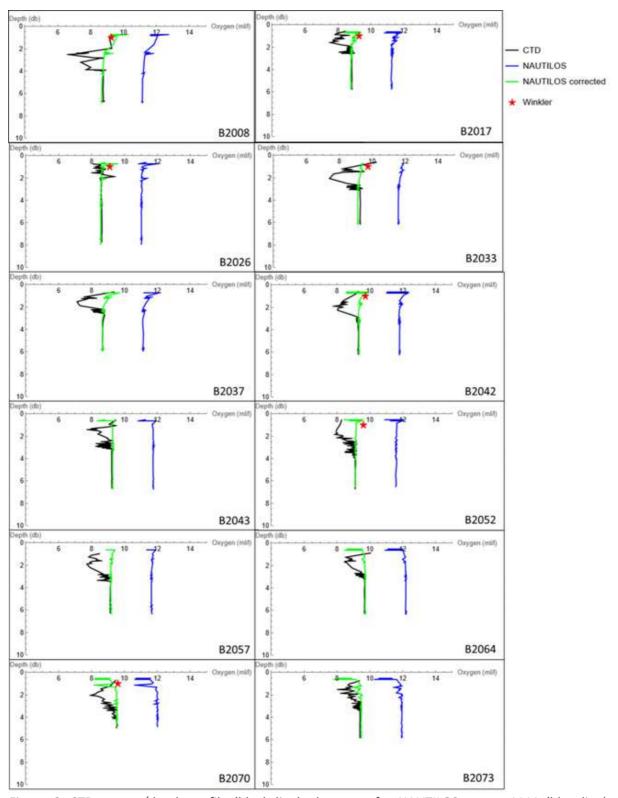


Figure 6: CTD oxygen/depth profile (black line), the same for NAUTILOS sensor 1111 (blue line), NAUTILOS sensor 1111 oxygen/depth profile corrected using the calculated correction factor (green line). The red star in some plots indicates the oxygen concentration values obtained for the sampled water by means of Winkler method. Graphs start from 0.5 m depth.

Table 3: Average values recorded and depth and temperature offsets calculated for sensor 6666 according to simultaneous profiling performed during the IBTS cruise for each cast and depth permanence.

Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	6666 Depth (m)	6666 Tempe- rature (°C)	6666 Chloro- phyll (ug/l)	Correct ed Depth Offset (m)	Tempe- rature Offset (°C)
B2008	2.398	8.510	33.359	9.190	0.593	1.900	8.544	23.151	-0.002	-0.034
B2008	3.935	8.577	33.400	8.774	0.641	3.700	8.580	21.506	-0.265	-0.003
B2008	6.698	8.607	33.400	8.782	0.605	6.300	8.601	23.115	-0.102	0.006
B2017	1.394	7.965	34.548	9.408	0.693	0.100	7.975	0.000	0.794	-0.010
B2017	2.515	7.965	34.548	8.836	0.683	1.800	7.959	0.000	0.215	0.006
B2017	5.463	7.962	34.543	8.839	0.669	4.900	7.956	22.686	0.063	0.006
B2026	4.203	9.517	34.999	8.599	0.464	3.600	9.507	21.685	0.103	0.010
B2026	7.437	9.517	35.001	8.622	0.450	6.800	9.541	14.678	0.137	-0.024
B2033	1.379	7.538	34.999	10.213	1.334	0.800	7.655	0.000	0.079	-0.117
B2033	2.89	7.559	34.999	9.278	1.406	2.200	7.563	158.230	0.190	-0.004
B2033	5.875	7.576	35.003	9.275	1.456	5.400	7.567	75.609	-0.025	0.009
B2042	1.944	6.767	34.358	9.632	0.556	0.900	6.775	27.333	0.544	-0.008
B2042	3.35	6.785	34.358	9.291	0.574	2.700	6.774	18.039	0.150	0.011
B2042	5.97	6.701	34.357	9.302	0.583	5.500	6.690	27.298	-0.030	0.010
B2043	3.052	7.125	34.358	9.229	0.643	2.000	7.136	0.000	0.552	-0.011
B2043	6.257	7.124	34.565	9.236	0.656	5.700	7.122	22.543	0.057	0.002
B2052	3.272	7.321	34.481	9.112	0.381	2.700	7.316	12.748	0.072	0.005
B2052	7.104	7.321	34.482	9.090	0.403	6.300	7.313	11.282	0.304	0.008
B2057	0.969	7.285	34.443	9.599	0.405	1.300	7.301	13.606	-0.831	-0.016
B2057	3.245	7.280	34.442	9.131	0.396	2.400	7.287	12.498	0.345	-0.007
B2057	5.931	7.289	34.443	9.148	0.394	5.200	7.284	12.748	0.231	0.005
B2070	4.125	6.180	33.725	9.503	0.428	3.200	6.188	0.000	0.425	-0.008
B2073	3.404	6.757	33.7252 1	9.405	0.693	2.300	6.765	23.508	0.604	-0.008
B2073	5.665	6.758	33.725	9.403	0.667	4.500	6.751	22.972	0.665	0.007

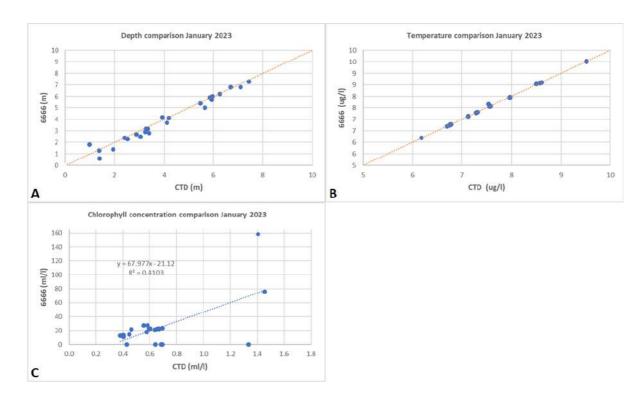


Figure 7: Scatter plot graphs built to compare the performance of NAUTILOS prototype 6666 versus CTD (and complementary sensors) measurements.

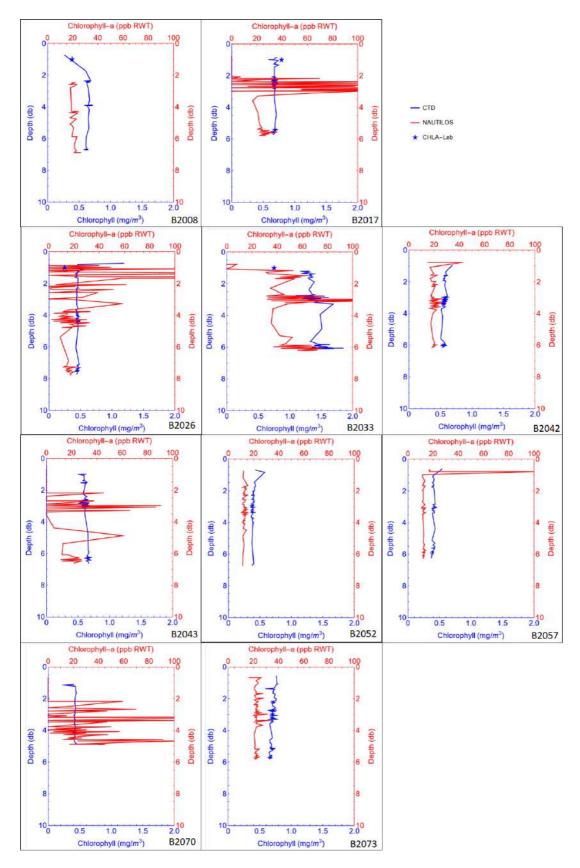


Figure 8: CTD chlorophyll/depth profile (blue line), the same for NAUTILOS sensor 6666 (red line). The blue star in some plots indicates chlorophyll concentration values obtained for the sampled water and refers to the same scale as CTD values. Graphs start from 0.5 m depth.

### Recommendations for use and future deployment/issues encountered:

The field validation was carried out in the North Sea, at different depths between 10 and 80 m, temperatures between 5 and 8 °C, salinity between 33 and 34, oxygen and chlorophyll concentrations measured by CTD complementary sensors respectively around 9 ml/l and 1  $\mu$ g/l.

The correction factor for the DO measurements obtained in laboratory (in ST 6.1.1 with the sea salinity equal to 33.2 and the internal salinity set to 0) equal to -1.2 ml/l is lower than the average correction factor obtained for the DO measurements of the sensor 1111 during this cruise.

The chlorophyll measurements of sensor 6666 show high fluctuations, which may be due to the low concentration range encountered. In fact, during this cruise the Chlorophyll- $\alpha$  concentration was very low due to seasonal conditions (1  $\mu$ g/l), therefore complementary tests at higher concentrations were necessary to be able to get a good in field qualification of the sensor on the whole range.

### 1.2. ANOC23 cruise, Adriatic Sea, February 2023 (CNR)

### Specific objectives:

To calculate offsets for temperature and pressure on both 2222 and 8888 prototypes, to calculate DO correction factors for prototype 2222 when internal salinity is set at 0, and to check Chl-*a* trends for 8888 prototype.

### Cruise details:

Prototypes 2222 and 8888 were received by CNR on 02/02/2023. The essential functions (communication, setup, parameter settings) of the prototype sensors were immediately tested in the laboratory (Fig. 9). The day after (03/02/2023), sensors were mounted on a CTD frame in order to prepare them for field testing to be carried out in the Adriatic Sea, on board of R/V Dallaporta, during ANOC23 cruise. Since 1988, CNR IRBIM collects data in 7 stations located in front of Senigallia (Ancona, Italy; Fig.10); the cruise was also linked to the LTER-Italy project (Italian Long-Term Ecological Research Network<sup>3</sup>).



Figure 9: First use test carried out at CNR on the NAUTILOS prototypes.

<sup>&</sup>lt;sup>3</sup> http://www.lteritalia.it/?q=en

The original plan was to carry out simultaneous profiling and water sampling in the 7 stations, with increasing maximum depth, using a SeaBird Electronic SBE 911plus CTD with rosette to sample water and carry out instruments intercalibration; unfortunately during the third cast (SG3; Tab. 4) some unexpected issues were encountered with the CTD. Also taking into account bad weather conditions ahead, the CNR staff decided to come back to Ancona harbour, change the CTD with the available Sea-Bird SBE19plus v2 and go back to sea to carry out more stations in the short available window of good weather.

Figure 10 shows the location of the stations in which field validation was carried out, while dates and coordinates for each station are reported in Table 1.



Figure 10: Map showing location of simultaneous profiling of CTD and NAUTILOS prototypes carried out in the Adriatic Sea by CNR staff during the ANOC23 cruise in February 2023.

Table 4: List of ANOC23 stations in which CNR staff carried out simultaneous sampling: date, time and coordinates are reported for each station.

Partner	Cruise	Station	Date	NMEA UTC Start Time	Latitude	Longitude
CNR	ANOC23	SG01	03/02/2023	12:05:38	43.764	13.217
CNR	ANOC23	SG02	03/02/2023	12:47:17	43.802	13.301
CNR	ANOC23	SG03	03/02/2023	13:29:23	43.852	13.351
CNR	ANOC23	SG04	03/02/2023	18:55:57	43.909	13.448
CNR	ANOC23	SG05	03/02/2023	19:53:53	43.962	13.545
CNR	ANOC23	SG06	03/02/2023	20:51:45	44.02	13.617
CNR	ANOC23	SG07	03/02/2023	21:44:33	44.05	13.699

#### Methods:

Simultaneous profiling of CTD and prototypes 2222 and 8888 was carried out in 6 stations (Fig. 10; Tab. 4).

The instruments used for comparison were:

- Stations 1-2: SeaBird Electronic SBE 911plus CTD (Calibration Date 20-Dec-22; reference: https://www.seabird.com/sbe-911plus-ctd/product?id=60761421595),
- Stations 1-2 complementary sensor for oxygen measurements: SBE 43 (Calibration Date 05-mar-2021; reference: <a href="https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728">https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728</a>
- Stations 1-2 complementary sensor for chlorophyll measurements: WET Labs ECO-AFL/FL (Calibration Date 17/10/2012; reference: <a href="https://www.seabird.com/eco-fluorometer/product?id=60429374754">https://www.seabird.com/eco-fluorometer/product?id=60429374754</a>),
- Stations 4-7: Sea-Bird SBE19plus v2 CTD (Calibration Date 29-Jan-13; reference <a href="https://www.seabird.com/sbe-19plus-v2-seacat-profiler-ctd/product?id=60761421596">https://www.seabird.com/sbe-19plus-v2-seacat-profiler-ctd/product?id=60761421596</a>),
- Stations 4-7 complementary sensor for oxygen measurements: SBE 43 (Calibration Date 05-mar-2021; reference: <a href="https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728">https://www.seabird.com/sbe-43-dissolved-oxygen-sensor/product?id=60762467728</a>
- Stations 4-7 complementary sensor for chlorophyll measurements: Fluorometer Turner Cyclops (Calibration Date 06/05/2020<sup>4</sup>; reference: https://www.turnerdesigns.com/c-fluor-submersible-probes).

Unfortunately, the failure of the CTD in SG3 also prevented the use of the rosette at later stations to collect water samples that would also have to be used to recalibrate sensors such as the WET Labs ECO-AFL/FL. It should be noted that even though the calibration date of the Sea-Bird SBE19plus v2  $\rm CTD^5$  is not very recent, the producer declared stability for conductivity is 0.0003 s/m per month, for temperature 0.0002 °C per month, for pressure strain-gauge  $\pm$  0.1% of full scale range, and quartz  $\pm$  0.02% of full scale range per year.

<sup>&</sup>lt;sup>4</sup> on 06/09/2023 a new calibration took place which confirmed the characteristics of the sensor

<sup>&</sup>lt;sup>5</sup> https://www.seabird.com/asset-get.download.jsa?id=54627861928).

Both prototypes were mounted on the SBE 911plus CTD frame with depth detector about 5 cm above that of the CTD, while on the Sea-Bird SBE19plus v2 CTD frame about 30 cm above that of the CTD (Fig. 11). The prototypes' recording was started manually before each cast.

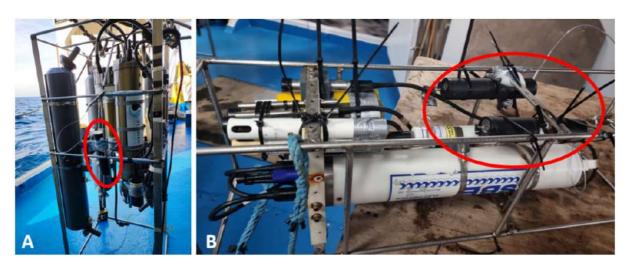


Figure 11: Prototypes 2222 and 6666 mounted on the CTD frames: A) prototypes' depth detector about 5 cm above that of SBE 911plus CTD, B) prototypes' depth detector about 30 cm above that of Sea-Bird SBE19plus v2 CTD.

During the upcast, stops for 3-4 minutes were performed at different depths (reported in Tab. 5). Water samples were collected by means of the rosette in stations SG1 and SG2 at the maximum depth and also at shallower depths. Dissolved oxygen was directly analysed on board according to Winkler (1888): samples were immediately fixed and stored in the dark and analysed within 24 h using the potentiometric method (Furuya & Harada 1995). Chlorophyll-a was measured by filtering 1-2 litres of sampled seawater through 47 mm GF/F filters and immediately frozen at -22 °C. Samples were extracted in 90% acetone and analysed at the CNR-IRBIM laboratory by spectrophotometry with a Shimadsu UV-2600 Spectrophotometer (Lorenzen and Jeffrey, 1980).

NAUTILOS prototypes record 1 value every 2 seconds, therefore CTD (and complementary sensors) data were mediated to be compared with these. Oxygen concentration data collected by NAUTILOS prototypes in mg/l were converted to ml/l to be compared with CTD datasets, as well pressure (bar) was converted to depth (m).

Depth and temperature offsets for each prototype and dissolved oxygen correction factor for sensor 2222 were calculated for each cast and depth permanence (using average measurements over each permanence depth). Depth offsets were also *a posteriori* corrected for the positioning of the sensors on the two CTD frames. Average values on all permanences were derived but kept separate for the two sensors assemblages (i.e. SG1-2, SG4-7). Water sample results, CTD profiles, sensors' profiles and corrected DO profiles were used to build graphs for each available comparison. Scatter plots will not be presented below to avoid possible overly detailed erroneous conclusions derived from the use of two different sensor assemblies during the cruise and the fact that in that period water was pretty much unstratified (Tab. 5).

### Results:

Table 5 shows average values and offsets/corrections calculated for each permanence depth according to the depth recorded by the CTD; the calculated offsets to be applied for depth to prototype 2222 were corrected to take into account the position of the sensor on the CTD frame. The calculated average ( $\pm$  standard deviation) offsets for depth and temperature when comparing with SBE 911plus (SG1-2) are respectively -0.382 ( $\pm$ 0.074) m, -0.005 ( $\pm$ 0.002) °C; the calculated offsets for Depth, Temperature when comparing with SBE19plus v2 (SG4-7) are respectively -0.127 ( $\pm$ 0.139) m, ( $\pm$ 0.002) °C. The average correction for the oxygen concentration calculated for the comparison with SBE 43 is -1.691 ( $\pm$ 0.116) ml/l with an average water salinity recorded of 38.771.

Figure 12 shows the comparison between depth/oxygen concentration profiles obtained by means of CTD and prototype 2222, and for 2 stations also the values of oxygen in ml/l obtained from water samples. The average calculated oxygen concentration correction value was also used to post-process oxygen data acquired by prototype 2222 and the resulting new profiles are also shown in Figure 12.

Table 6 shows average values and offsets/corrections calculated for each permanence depth carried out with the prototype 8888 according to the depth recorded by the CTD; the calculated offset to be applied to depth to prototype 8888 was corrected to take into account the position of the sensor on the CTD frame. The calculated average ( $\pm$  standard deviation) offsets for depth and temperature when comparing with SBE 911plus (and complementary sensor; SG1-2) are respectively -0.349 ( $\pm$ 0.043) m and -0.004 ( $\pm$ 0.006) °C, while the calculated offsets for depth and temperature when comparing with SBE19plus v2 (and complementary sensor; SG4-7) are respectively -0.094 ( $\pm$ 0.161) m and -0.006 ( $\pm$ 0.003) °C.

Figure 13 shows the comparison between depth/chlorophyll concentration profiles obtained by means of CTD and prototype 8888, and for 2 stations also the chlorophyll values obtained from water samples. For prototype 8888 depth was corrected for the obtained average offset and Rhodamine in ppb is reported as a proxy of chlorophyll; the range used to report chlorophyll values was fixed, therefore some values fall outside the graph. The values recorded by the CTD and for the sampled water are much lower than those measured with prototype 8888, which is calibrated with Rhodamine.

Table 5: Depth and temperature offsets and dissolved oxygen correction factor calculated for sensor 2222 according to simultaneous profiling performed during the ANOC23 cruise for each cast and depth permanence.

Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	2222 Depth (m)	2222 Tempe- rature (°C)	2222 Oxygen (ml/l)	Correcte d Depth Offset (m)	Temperature Offset (°C)	Oxygen Correction (ml/l)
SG1	10.860	10.993	38.379	5.773	1.339	11.200	10.996	7.123	-0.390	-0.003	-1.350
SG2	17.346	12.588	38.939	5.653	1.522	17.600	12.595	7.229	-0.304	-0.007	-1.576
SG2	9.698	13.037	38.994	5.602	1.390	10.100	13.041	7.197	-0.452	-0.004	-1.595
SG4	52.239	13.643	38.923	5.380	2.316	52.100	13.640	7.102	-0.161	0.003	-1.722
SG4	40.041	13.832	38.960	5.333	1.940	39.900	13.831	7.072	-0.159	0.001	-1.739
SG4	19.990	13.855	38.959	5.366	2.263	19.800	13.852	7.112	-0.110	0.003	-1.746
SG5	64.975	13.795	39.005	5.307	1.908	64.900	13.795	7.039	-0.225	0.000	-1.732
SG5	40.222	13.807	39.003	5.288	1.953	40.100	13.812	7.063	-0.178	-0.005	-1.775
SG5	19.945	13.822	39.006	5.243	1.967	19.800	13.823	7.098	-0.155	-0.001	-1.855
SG6	64.397	13.831	38.993	5.357	2.004	63.800	13.830	7.012	0.297	0.001	-1.655
SG6	40.215	13.838	38.992	5.339	2.112	40.100	13.842	7.044	-0.185	-0.004	-1.705
SG6	19.889	13.837	38.994	5.318	2.234	19.700	13.837	7.051	-0.111	0.000	-1.734
SG7	66.257	13.838	38.988	5.300	1.901	66.200	13.838	7.015	-0.243	0.000	-1.715
SG7	40.051	13.840	38.988	5.309	1.727	39.900	13.840	7.015	-0.149	0.000	-1.706
SG7	19.852	13.837	38.987	5.262	2.141	19.700	13.837	7.017	-0.390	0.000	-1.755

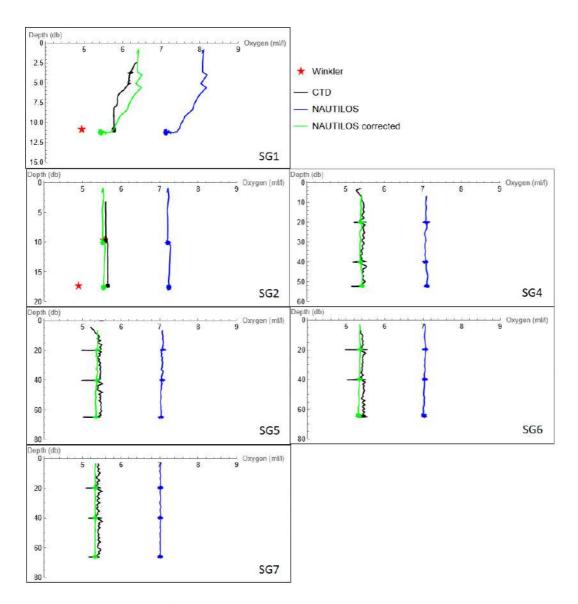


Figure 12: CTD oxygen/depth profile (black line), the same for NAUTILOS sensor 2222 (blue line), NAUTILOS sensor 2222 oxygen/depth profile corrected using the calculated correction factor (green line). The red star in some plots indicates oxygen concentration values obtained for the sampled water by means of Winkler method. Graphs start from 0.5 m depth.

Table 6: Average values recorded and depth and temperature offsets calculated for sensor 8888 according to simultaneous profiling performed during the ANOC23 cruise for each cast and depth permanence.

Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	8888 Depth (m)	8888 Tempe- rature (°C)	8888 Chloro- phyll (ug/l)	Corrected Depth Offset (m)	Tempe- rature Offset (°C)
SG1	10.860	10.993	38.379	5.773	1.339	11.200	10.990	24.412	-0.390	0.003
SG2	17.346	12.588	38.939	5.653	1.522	17.600	12.594	27.597	-0.304	-0.006
SG2	9.698	13.037	38.994	5.602	1.390	10.000	13.047	26.791	-0.352	-0.010
SG4	52.239	13.643	38.923	5.380	2.316	52.100	13.645	24.220	-0.161	-0.002
SG4	40.041	13.832	38.960	5.333	1.940	39.800	13.835	24.681	-0.059	-0.003
SG4	19.990	13.855	38.959	5.366	2.263	19.800	13.867	20.690	-0.110	-0.012
SG5	64.975	13.795	39.005	5.307	1.908	64.900	13.803	17.007	-0.225	-0.008
SG5	40.222	13.807	39.003	5.288	1.953	40.100	13.819	21.841	-0.178	-0.012
SG5	19.945	13.822	39.006	5.243	1.967	19.800	13.827	19.386	-0.155	-0.005
SG6	64.397	13.831	38.993	5.357	2.004	63.700	13.834	20.920	0.397	-0.003
SG6	40.215	13.838	38.992	5.339	2.112	40.000	13.841	18.426	-0.085	-0.003
SG6	19.889	13.837	38.994	5.318	2.234	19.700	13.844	21.803	-0.111	-0.007
SG7	66.257	13.838	38.988	5.300	1.901	66.100	13.843	16.508	-0.143	-0.005
SG7	40.051	13.840	38.988	5.309	1.727	39.900	13.844	20.613	-0.149	-0.004
SG7	19.852	13.837	38.987	5.262	2.141	19.700	13.840	17.544	-0.148	-0.003

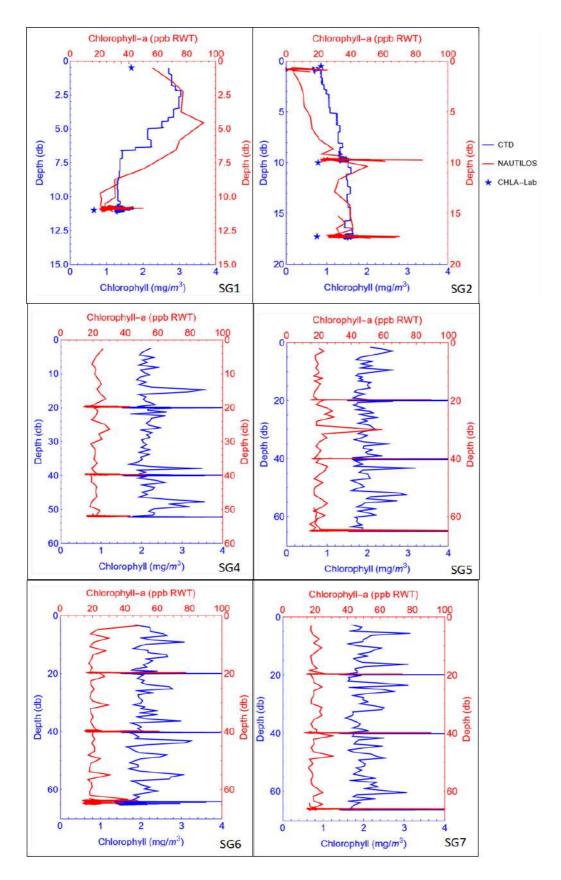


Figure 13: CTD chlorophyll/depth profile (blue line), the same for NAUTILOS sensor 8888 (red line). The blue star indicates chlorophyll concentration values obtained for the sampled water and refers to the same scale as CTD values. Graphs start from 0.5 m depth.

## Recommendations for use and future deployment/issues encountered:

This field validation experiment was carried out in the Adriatic Sea at depths lower than 70 m, temperature ranging between 11 and 14 °C, salinity between 38.4 and 39 and oxygen concentration and chlorophyll measured by CTD complementary sensors respectively around 7 ml/l and between 1.3-2.4  $\mu$ g/l.

The correction factor obtained for the DO measurements of sensor 2222 when internal salinity is set at 0 and seawater salinity is over 38 is very close to that obtained in laboratory in ST6.1.1 (the average correction with water salinity equal to 38 and internal setting equal to 0 was in that case equal to -1.78 ml/l).

Graphs in Figure 12 show that when using the DO prototype with internal salinity setting at 0, a post-processing procedure taking into account the real seawater is useful to correct the acquired data.

Figure 13 shows that the range of chlorophyll measurements of sensor 8888 is much higher than those resulting both from the CTD measurements and water sampling, because it has been calibrated using rhodamine. Post-processing of data is required to get results in comparable scales.

## 1.3. COMBINE-kevätseuranta (spring monitoring) cruise, Baltic Sea, April 2023 (SYKE)

# Specific objectives:

Additional field validation tests for the Chl-a fluorometer prototype were carried out by Syke on a monitoring cruise on R/V Aranda in the Baltic Sea. The aim of the tests was to check the fluorometer result linearity relative to the WET Labs ECO FLNTURT in local algae community conditions during the Baltic Sea spring bloom.

#### Cruise details:

COMBINE-kevätseuranta (spring monitoring) cruise in the Baltic Sea between 18.04.2023 - 28.04.2023. The cruise is a part of the HELCOM COMBINE Baltic Sea monitoring programme, with a specific focus on hydrography and the spring bloom characteristics. The monitoring stations where NAUTILOS activities were carried out are shown in Table 7 and Figure 14. The Baltic Sea is a coastal sea dominated by brackish water, the salinity in the different basins ranging from 3 to 11 in the study area. The sea is eutrophic, and the water contains a high amount of CDOM (chromophoric dissolved organic matter).

#### Methods:

Field validation was carried out on NAUTILOS prototype Chl-a 7777. Instruments used for comparison:

- SBE 911 CTD (Sea-Bird Scientific)
- External Sensors: ECO FLNTURT Chlorophyll-a & turbidity fluorometer (WET Labs).

The NAUTILOS Chl- $\alpha$  fluorometer was mounted on the CTD frame looking downwards on the opposite side from the ECO FLNTURT to minimise interference from the other sensor's LED. The sensors were on the same level, also with the CTD pressure sensor and the plumbing inlet. The exact measure was not taken, so the pressure comparison information is just shown to confirm comparability of Chl- $\alpha$  and temperature data.

On upcast the CTD was stopped at standard monitoring depths of 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100 m for 1 min 30 sec for water sampling, the deepest level depending

on the bottom depth of the monitoring station. The data from these stationary measurements were used for the analysis.

The sensor's clocks were synchronised prior to each cast. Since the NAUTILOS Chl- $\alpha$  sensor measuring frequency is max 0.5 Hz, the CTD data for matching timestamps were picked out for comparison. The sensor data are reported here with the factory calibration and are not scaled to real Chl- $\alpha$  concentration from water samples.

Table 7: List of HELCOM stations in which Syke staff carried out sampling: date, time, depth, and coordinates are reported for each station.

Partner	Cruise	Station	Date	NMEA UTC Start Time	Latitude	Longitude	Profile maxim um depth
SYKE	COMBINE- kevätseuranta	LL3A	19/04/2023	06:23	60.067167	26.346633	60m
SYKE	COMBINE- kevätseuranta	LL5	19/04/2023	12:17	59.916883	25.59705	60m
SYKE	COMBINE- kevätseuranta	LL6A	19/04/2023	15:09	59.916833	25.030217	60m
SYKE	COMBINE- kevätseuranta	LL15	20/04/2023	10:17	59.183367	21.746733	100m
SYKE	COMBINE- kevätseuranta	F18	23/04/2023	06:29	63.314333	20.272633	90m
SYKE	COMBINE- kevätseuranta	воз	23/04/2023	18:33	64.302283	22.3329	100m

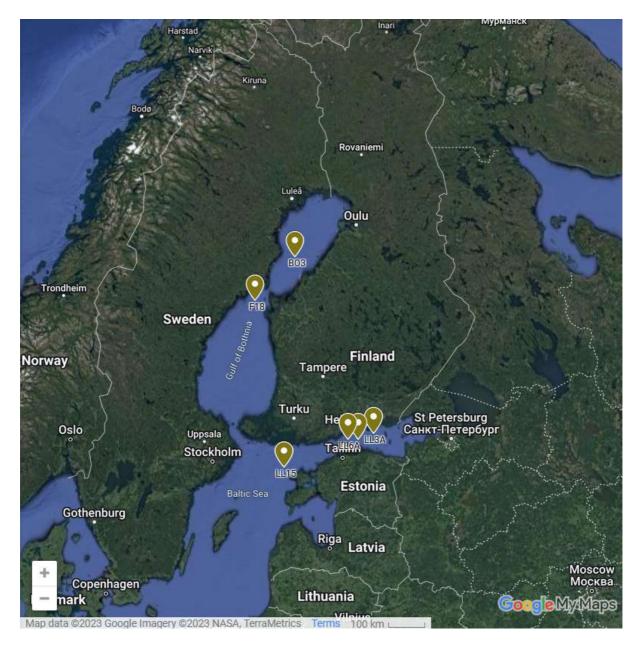


Figure 14: Map showing location of simultaneous profiling of CTD and NAUTILOS prototypes carried out in the North Sea by SYKE staff during the COMBINE- kevätseuranta cruise in April 2023.

#### Results:

The relationship of response from the two fluorometers was found to be very linear, with a correlation coefficient for the whole dataset being 0.9817. All profiles could be treated as one dataset, since the sensor's relationship was consistent throughout the entire cruise, independent of sampling area (Fig. 15).

As already indicated by the lab tests in WP6 D6.1.1, the fluorometer prototype data has a higher variability than the commercial reference sensors, as can also be seen in Figures 16 and 17.

The temperature sensors of the SBE 911 CTD and the Chl- $\alpha$  prototype were well in agreement, with an offset of 0.03 °C (Fig. 18), as were the pressure sensors (Fig. 19).

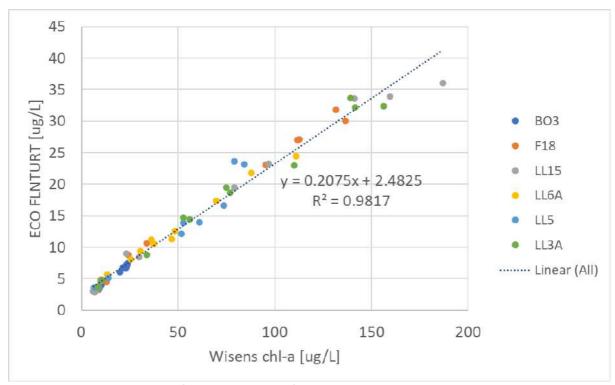


Figure 15: The relationship of the mean values from each sensor at each stationary depth. The linear fit shown is for the entire dataset. To explain the different scales of fluorescence readings, ECO FLNTURT is factory calibrated (with algae culture), while Chl-a 7777 prototype is calibrated with Rhodamine.

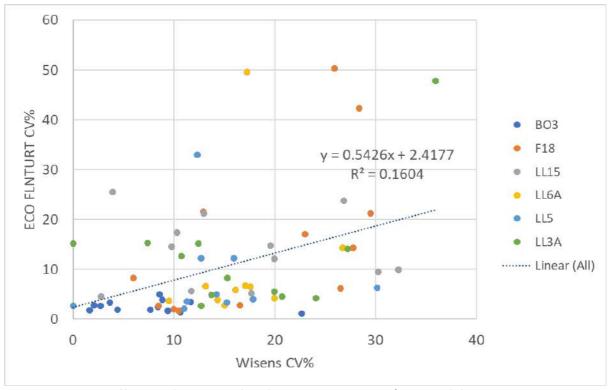


Figure 16: The coefficient of variation (100\*standard deviation / average) for both sensors at each sampling depth, sorted by station.

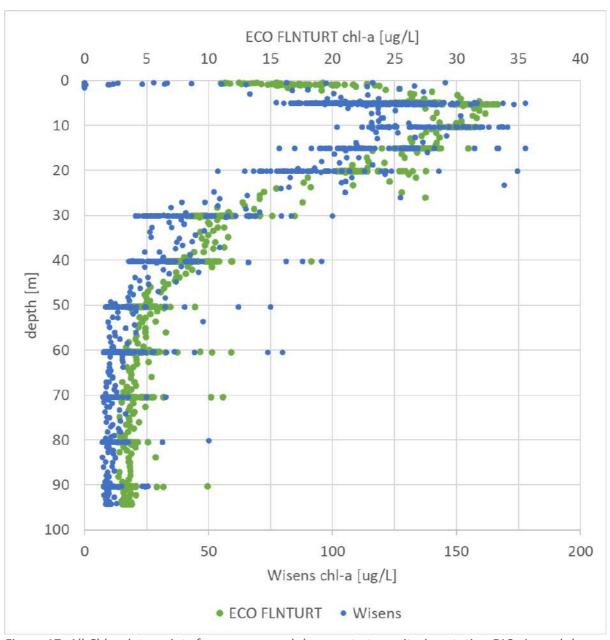


Figure 17: All Chl-a data points from an up- and downcast at monitoring station F18. A much larger variation in the Chl-a 7777 prototype results can be observed at sampling stop depths. To explain the different scales of fluorescence readings, ECO FLNTURT is factory calibrated (with algae culture), while Chl-a 7777 prototype is calibrated with Rhodamine.

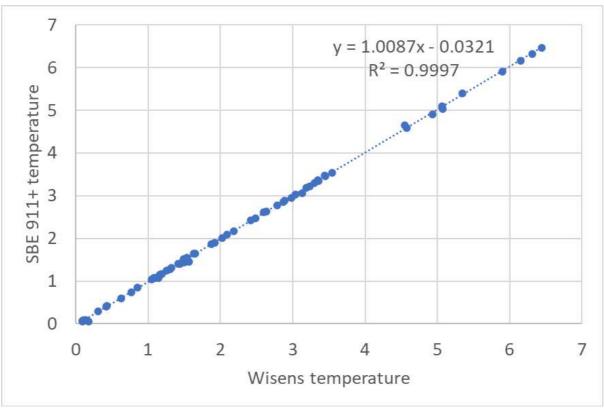


Figure 18: The temperature relation of the Chl-a 7777 prototype and SBE911+ temperature sensors.

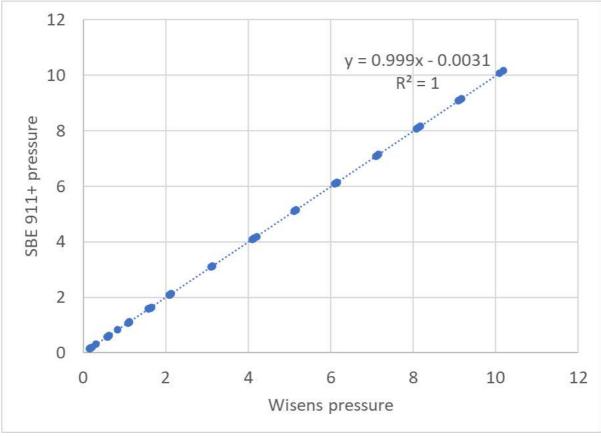


Figure 19: The pressure relation between the Chl-a 7777 prototype and SBE 911+ pressure sensors.

## Recommendations for use and future deployment/issues encountered:

As discussed in D6.1.1, the LED spectra of the NKE Chl-a prototype and that of the WET Labs ECO FLNTURT peak at the same wavelength (463 nm) and have almost the same width, so the fluorescence response of the sensors was expected to have a linear relationship. This was proven in laboratory conditions with different algae monocultures (See D6.1). Outside the laboratory conditions, the same was also proven in the field validation, independent of water basins of different hydrographic properties as well as algal community and bloom phase.

# 1.4. "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise, Adriatic Sea, April 2023 (CNR)

### • Specific objectives:

To calculate offsets for temperature and pressure on both 2222 and 8888 prototypes, to calculate DO offset when the internal salinity setting of sensor 2222 is corresponding to that of the seawater and check Chlorophyll-*a* trends for the 6666 prototype.

#### Cruise details:

"Monitoraggio Pomo 2023 & NAUTILOS trials" cruise was carried out by the CNR staff in the central Adriatic Sea (Mediterranean basin) from 21/04/2023 to 02/05/2023. Since 2012, CNR IRBIM collects data in the area in the framework of a multiannual monitoring program (Penna et al. 2022 a,b).

Figure 20 shows the location of the stations in which field validation was carried out, while dates and coordinates for each station are reported in Table 8.

Table 8: List of "Monitoraggio Pomo 2023 & NAUTILOS trials" stations in which CNR staff carried out simultaneous profiling: date, time and coordinates are reported for each station.

Partner	Cruise	Station	Date	NMEA UTC Start Time	Latitude	Longitude
CNR	Pomo- NAUTILOS	P21	27/04/2023	04:02:43	42.843	14.965
CNR	Pomo- NAUTILOS	P31	27/04/2023	07:12:12	42.584	15.068
CNR	Pomo- NAUTILOS	P30	27/04/2023	09:36:30	42.496	14.870

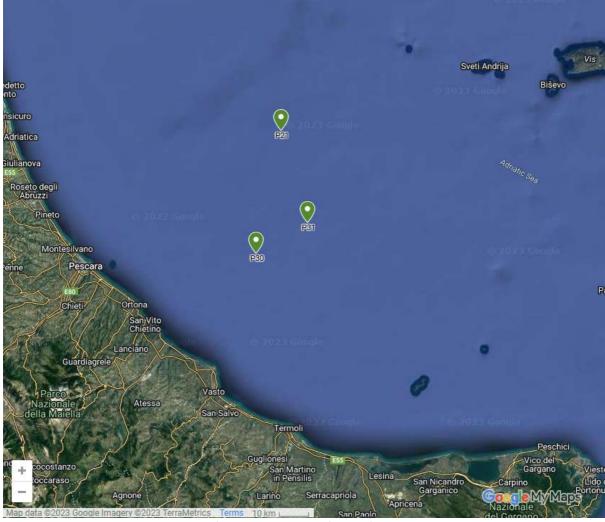


Figure 20: Map showing location of simultaneous profiling of CTD and NAUTILOS prototypes carried out in the Adriatic Sea by CNR staff during the "Monitoraggio Pomo 2023 & NAUTILOS trials cruise in April 2023.

#### Methods:

Simultaneous profiling of CTD and prototypes 2222 and 8888 was carried out in 3 stations (Fig. 20; Tab. 8). The sensors were mounted on the steel support of the CTD through the use of plastic ties and high resistance adhesive tape in order to ensure that the correct position was maintained. Sea-Bird SBE19plus v2 was used to carry out this additional simultaneous profiling test.

The instruments used for comparison were:

- Sea-Bird SBE19plus v2 CTD (see details in paragraph 1.2),
- Complementary sensor for oxygen measurements: SBE 43 (see details in paragraph 1.2)
- Complementary sensor for chlorophyll measurements: Fluorometer Turner Cyclops (see details in paragraph 1.2).

Both prototypes were mounted on the CTD frame with depth detector respectively about 5 cm above CTD depth recorder in case of prototypes 2222 and 10 cm above in case of prototypes 8888 (Fig. 21). Before the first simultaneous profiling, a few CTD casts were carried

out in order to determine which salinity setting to use in the DO prototype: the selected value was 38.785. The prototypes were manually set to *continuous* recording mode (see Fig. 42A) and start and stop recording have been activated by the operator through the available web application before and after each cast. During the upcast stops for 3-4 minutes were performed at different depths (about 150-100-50-25 m; Tab. 9).

NAUTILOS prototypes record 1 value every 2 seconds, therefore CTD (and complementary sensors) data were averaged to be compared with these. Oxygen concentration data collected by NAUTILOS prototypes in mg/l were converted to ml/l to be compared with CTD datasets, as well pressure (bar) was converted to depth (m).

Depth, temperature and dissolved oxygen offsets were calculated for each cast and depth permanence (using average measurements over each permanence depth). Depth offsets were also *a posteriori* corrected for the positioning of the sensors on the frame (Fig. 21). The obtained results were used to build scatter plot graphs to compare the performance of the NAUTILOS prototypes versus CTD (and complementary sensors) measurements. These graphs also show possible dependence (linear regression) of the oxygen and chlorophyll measurements of the prototypes on depth or temperature recorded. Average values on all permanences were derived. CTD profiles and sensor profiles were used to build graphs for each available comparison.



Figure 21: DO and Chl-a prototypes (with orange plastic protections provided by NKE, see paragraph 2.1) mounted on a frame used to perform simultaneous profiling during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise.

#### Results:

Table 9 shows average values and offsets calculated for each permanence depth according to the depth recorded by the CTD; the calculated offsets to be applied for depth to prototype 2222 were corrected to take into account the position of the sensor on the CTD frame. The calculated average (± standard deviation) offsets for depth, temperature and oxygen concentration are respectively -0.113 ( $\pm 0.066$ ) m, 0 ( $\pm 0.077$ ) °C and -0.234 ( $\pm 0.419$ ) ml/l. If compared to the correction factors calculated for internal salinity set at 0, the oxygen concentration offset obtained for prototype 6666 when the internal salinity setting is similar to real seawater salinity is very small. In this case the offset resulted to be significantly (p<0.001) dependent from both depth (negative effect) and temperature (positive effect) (Fig. 22A-B); but this is probably mainly due to the fact that temperature recorded during this cruise were decreasing approximately linearly with depth, as seawater was stratified (Tab. 9). Figures 22C-D show a substantial agreement between temperature and depth measured by sensor 2222 and CTD (orange line is a reference that represents 100% agreement). Figure 22E shows the comparison between oxygen concentration recorded by the complementary sensor on the CTD and the measures recorded by prototype 2222. Figure 23 shows the comparison between depth/oxygen concentration profiles obtained by means of CTD and prototype 2222, in which profiles are substantially in agreement, especially in stations P30-31 below 50 metres depth. The slightly greater differences detected for station P21 along the profiles could be due to sudden variations of the oxygen concentrations along the water column, not compensated for deployment speed.

Table 9: Depth, temperature and dissolved oxygen offsets calculated for sensor 2222 according to simultaneous profiling performed during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise for each cast and depth permanence.

Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	2222 Depth (m)	2222 Tempe- rature (°C)	2222 Oxygen (ml/l)	Corrected Depth Offset (m)	Temperature Offset (°C)	Oxygen Offset (ml/l)
P21	213.36	12.396	38.897	5.341	0.125	213.6	12.398	5.086	-0.290	-0.002	0.255
P21	150.22	12.852	38.864	5.332	0.098	150.3	12.867	4.774	-0.130	-0.015	0.558
P21	100.85	14.226	38.889	5.275	0.206	100.9	14.232	5.151	-0.100	-0.006	0.124
P21	50.304	14.709	38.873	5.304	1.345	50.3	14.722	5.615	-0.046	-0.013	-0.31
P21	25.002	15.181	38.835	5.229	1.289	25	15.178	5.606	-0.048	0.003	-0.376
P31	178.15	12.641	38.883	5.588	0.817	178.3	12.647	5.445	-0.200	-0.006	0.142
P31	150.45	13.399	38.835	5.391	0.595	150.5	13.428	5.444	-0.100	-0.029	-0.053
P31	100.75	14.387	38.918	5.281	0.837	100.8	14.385	5.428	-0.100	0.002	-0.147
P31	49.743	14.56	38.897	4.94	1.258	49.8	14.608	5.637	-0.107	-0.048	-0.697
P31	24.865	14.991	38.878	4.788	0.739	24.9	15.072	5.634	-0.085	-0.081	-0.846
P30	151.74	13.384	38.891	5.232	0.823	151.8	13.127	5.41	-0.110	0.257	-0.179
P30	101.7	14.365	38.885	5.1	0.365	101.7	14.396	5.402	-0.050	-0.031	-0.302
P30	50.503	14.508	38.862	4.855	1.285	50.6	14.522	5.515	-0.147	-0.014	-0.66
P30	25.081	14.591	38.855	4.831	0.752	25.1	14.605	5.612	-0.069	-0.014	-0.781

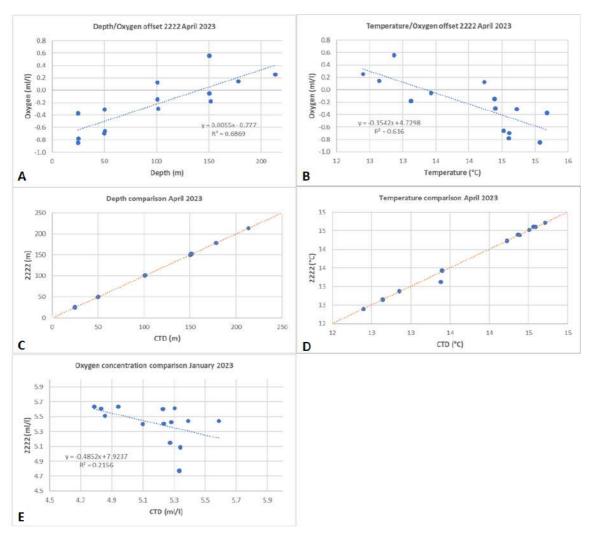


Figure 22: Scatter plot graphs built to compare the performance of NAUTILOS prototype 2222 versus CTD (and complementary sensors) measurements and to detect possible dependence of the oxygen concentration offset on depth or temperature recorded.

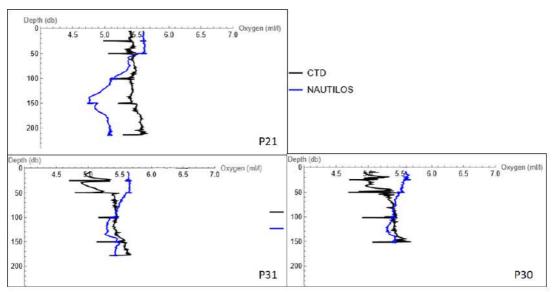


Figure 23: CTD oxygen/depth profile (black line), the same for NAUTILOS sensor 2222 (blue line). Graphs starting from 0.5 m depth.

Table 10 shows average values and offsets calculated for each permanence depth according to the depth recorded by the CTD; the calculated offsets to be applied for depth to prototype 8888 were corrected to take into account the position of the sensor on the CTD frame. The calculated average ( $\pm$  standard deviation) offsets for depth and temperature are respectively -0.133 ( $\pm$ 0.059) m and -0.014 ( $\pm$  0.079) °C. Figures 24A-B show a substantial agreement between temperature and depth measured by sensor 8888 and CTD (orange line is a reference that represents 100% agreement). Figure 24C shows linearity between chlorophyll measurements recorded by CTD and prototype 8888 even if the scale of the measurements is different, but this is again probably due to different factory calibration procedures (see above paragraphs).

Figure 25 shows the comparison between depth/chlorophyll concentration profiles obtained by means of CTD and prototype 8888. For prototype 8888 depth was corrected for the obtained average offset and Rhodamine in ppb is reported as a proxy of chlorophyll; the range used to report chlorophyll values was fixed, therefore some values fall outside the graph.

Table 10: Average values recorded and depth and temperature offsets calculated for sensor 8888 according to simultaneous profiling performed during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise for each cast and depth permanence.

Cast	CTD Depth (m)	CTD Tempe- rature (°C)	CTD Salinity	CTD Oxygen (ml/l)	CTD Chloro- phyll (ug/l)	8888 Depth (m)	8888 Tempe- rature (°C)	8888 Chloro- phyll (ug/l)	Corrected Depth Offset (m)	Temperature Offset (°C)
P21	213.36	12.396	38.897	5.341	0.125	213.4	12.414	3.269	-0.140	-0.018
P21	150.22	12.852	38.864	5.332	0.098	150.2	12.866	2.425	-0.080	-0.014
P21	100.85	14.226	38.889	5.275	0.206	100.9	14.238	3.73	-0.150	-0.012
P21	50.304	14.709	38.873	5.304	1.345	50.3	14.734	18.618	-0.096	-0.025
P21	25.002	15.181	38.835	5.229	1.289	25	15.192	13.131	-0.098	-0.011
P31	178.15	12.641	38.883	5.588	0.817	178.3	12.66	8.143	-0.250	-0.019
P31	150.45	13.399	38.835	5.391	0.595	150.5	13.436	6.608	-0.150	-0.037
P31	100.75	14.387	38.918	5.281	0.837	100.8	14.396	10.33	-0.150	-0.009
P31	49.743	14.56	38.897	4.94	1.258	49.7	14.669	15.05	-0.057	-0.109
P31	24.865	14.991	38.878	4.788	0.739	24.8	15.085	8.066	-0.035	-0.094
P30	151.74	13.384	38.891	5.232	0.823	151.8	13.147	11.75	-0.160	0.237
P30	101.7	14.365	38.885	5.1	0.365	101.7	14.407	4.881	-0.100	-0.042
P30	50.503	14.508	38.862	4.855	1.285	50.5	14.529	12.824	-0.097	-0.021
P30	25.081	14.591	38.855	4.831	0.752	25	14.619	8.181	-0.019	-0.028

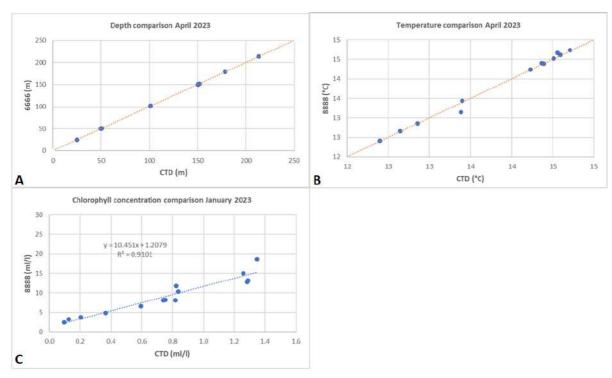


Figure 24: Scatter plot graphs built to compare the performance of NAUTILOS prototype 8888 versus CTD (and complementary sensors) measurements.

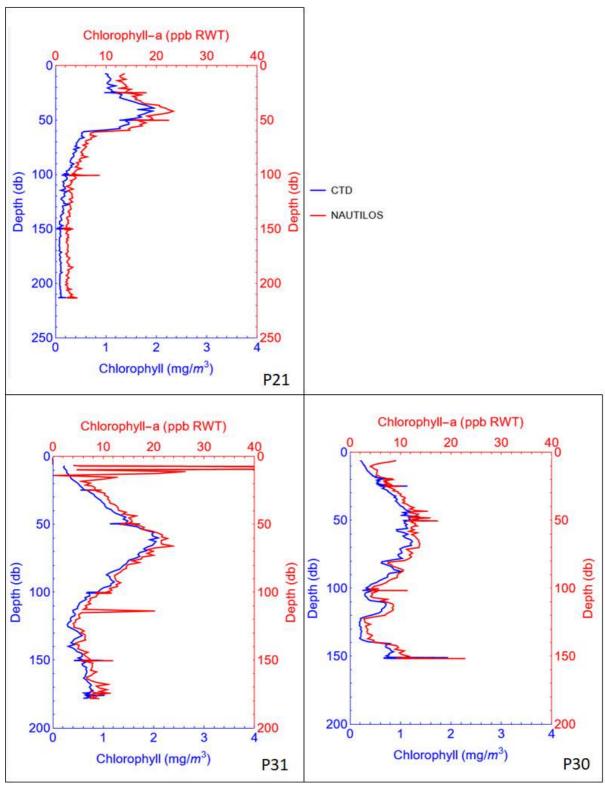


Figure 25: CTD chlorophyll/depth profile (blue line), the same for NAUTILOS sensor 8888 (red line). Graphs start from 0.5 m depth.

## • Recommendations for use and future deployment/issues encountered:

This field validation experiment was carried out in the Adriatic Sea at depths lower than 220 m, temperature ranging between 12.4 and 15.2 °C, salinity between 38.8 and 38.9, oxygen concentration and chlorophyll measured by CTD complementary sensors respectively between 4.8 and 5.6 ml/l and between 0.3-1.3  $\mu$ g/l.

If compared to the correction factors calculated when internal salinity was set at 0 (see paragraphs 1.1-1.2), the oxygen concentration offset obtained for prototype 2222 when the internal salinity setting is similar to real seawater salinity is quite small, and the depth/oxygen profiles acquired by CTD and 2222 prototype are mainly in agreement. Therefore, if the seawater salinity in which the sensors will be used is known and as well as temperature do not undergo huge variations during a deployment in a certain area, there is no need for post-processing on the acquired datasets. This applies especially if the permanence at a certain depth is enough to let the sensor compensate for differences along the water column in agreement with its response time. In future DO sensors firmware versions would be good to include the specifications on the used salinity setting in the metadata included in the bottom part of the data file produced by the sensors.

The range of chlorophyll measurements of sensor 8888 is much higher than that resulting from CTD measurements as sensor 8888 is calibrated with Rhodamine.

### 1.5. PELGAS cruise, Bay of Biscay, May 2023 (IFREMER)

### Specific objectives:

IFREMER took the opportunity to make an additional field validation of prototypes DO 1111 and Chl-a 6666 during the PELGAS deep trawling cruise in May 2023. The plan was to carry out simultaneous profiling of the sensors in deep condition and compare data recorded by the DO and Chl-a prototypes in continuous mode with pressure, temperature, and fluorescence recorded by a traditional CTD equipped with a fluorometric complementary sensor. No permanences at given depth, water sampling and no oxygen concentration comparisons were planned for this cruise.

#### • Cruise details:

The PELGAS cruise (leg 1) was carried out by IFREMER staff in the Bay of Biscay from 29/04/2023 to 13/05/2023 on board R/V Thalassa (Doray and Duhamel, 2023). The objective of the PELGAS cruise is to monitor distribution and abundance of pelagic species fished in the Bay of Biscay, using two direct evaluation methods: acoustics and spawning estimates. The cruise was conducted in coordination with France, Spain, and Portugal, in the framework of the European council regulations.

Simultaneous profiling was carried out in 25 stations whose dates and coordinates are reported in Table 11; among these, 6 stations had a maximum depth of about 500 m (Fig. 26).

Table 11: List of PELGAS stations in which IFREMER staff carried out simultaneous profiling: date, time and coordinates are reported for each station.

Partner	Cruise	Station	Date	NMEA UTC	Latitude	Longitude
				Start Time		
IFREMER	PELGAS 2023	B2190	05/05/2023	19:08	44.8662	-2.2510
IFREMER	PELGAS 2023	B2191	05/05/2023	21:32	44.8685	-2.0031
IFREMER	PELGAS 2023	B2192	05/05/2023	23:16	44.8745	-1.7921
IFREMER	PELGAS 2023	B2193	06/05/2023	1:31	44.8693	-1.5122
IFREMER	PELGAS 2023	B2194	06/05/2023	3:13	44.8673	-1.2827
IFREMER	PELGAS 2023	B2197	06/05/2023	19:04	44.8678	-2.8351
IFREMER	PELGAS 2023	B2198	06/05/2023	21:57	44.8633	-2.4959
IFREMER	PELGAS 2023	B2199	07/05/2023	0:14	45.0348	-2.4436
IFREMER	PELGAS 2023	B2203	07/05/2023	19:14	45.1818	-2.0865
IFREMER	PELGAS 2023	B2204	07/05/2023	21:45	45.1657	-1.6877
IFREMER	PELGAS 2023	B2205	07/05/2023	23:53	45.1673	-1.2783
IFREMER	PELGAS 2023	B2206	08/05/2023	2:34	45.3379	-1.6719
IFREMER	PELGAS 2023	B2207	08/05/2023	3:51	45.4178	-1.4497
IFREMER	PELGAS 2023	B2208	08/05/2023	4:58	45.4850	-1.2697
IFREMER	PELGAS 2023	B2212	08/05/2023	18:53	45.4503	-1.9215
IFREMER	PELGAS 2023	B2213	08/05/2023	21:07	45.5590	-1.6489
IFREMER	PELGAS 2023	B2214	08/05/2023	23:07	45.6553	-1.4183
IFREMER	PELGAS 2023	B2218	09/05/2023	20:36	44.9673	-3.0763
IFREMER	PELGAS 2023	B2219	09/05/2023	22:11	45.0469	-2.8830
IFREMER	PELGAS 2023	B2220	10/05/2023	0:06	45.1286	-2.6962
IFREMER	PELGAS 2023	B2221	10/05/2023	2:07	45.2380	-2.4222
IFREMER	PELGAS 2023	B2223	10/05/2023	17:50	45.7169	-2.3481
IFREMER	PELGAS 2023	B2224	10/05/2023	22:28	45.8340	-2.0687
IFREMER	PELGAS 2023	B2225	11/05/2023	1:02	45.9271	-1.8469
IFREMER	PELGAS 2023	B2226	11/05/2023	2:53	46.0164	-1.6390

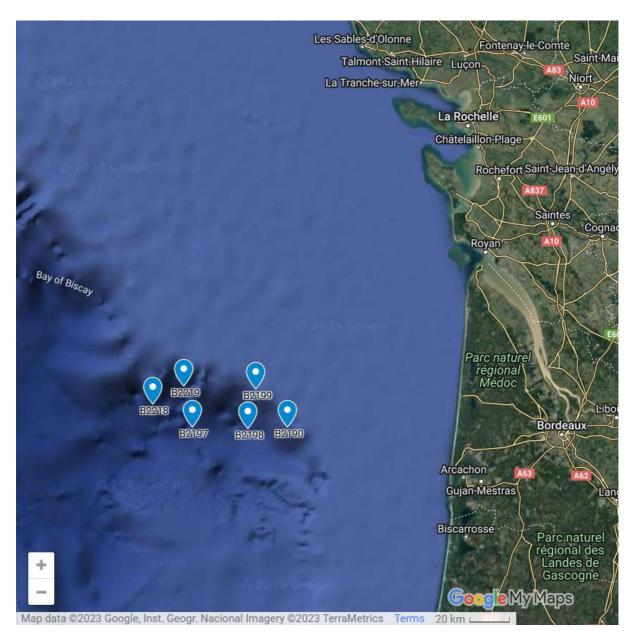


Figure 26: Map showing location of simultaneous profiling of CTD and NAUTILOS prototypes carried out in the Bay of Biscay by IFREMER staff during the PELGAS cruise in May 2023.

#### Methods:

Field validation was planned to be carried out on NAUTILOS prototypes DO 1111 and Chl-a 6666.

The instruments to be used for comparison were:

- Sea-Bird SBE19plus V2 CTD (calibration date: winter 2022; reference: see paragraphs above)
- External Sensors: SBE 43, Fluorometer WET Labs ECO-AFL/FL.

The prototypes were planned to be mounted on the CTD frame (Fig. 27) and programmed to record data in continuous mode at the lowest recording rate and acquisition mode *on* 

condition: start when pressure exceeds 0.3 bar and stop when pressure becomes lower than 0.2 bar.

Unfortunately, Chl-a prototype 6666 did not respond when trying to connect to it via Wi-Fi in order to configure it before carrying out simultaneous profiling. IFREMER and NKE staff got in contact immediately to investigate possible causes. The conclusion was that the sensor was out of order due to water infiltration, and it was necessary to send it back to the manufacturer for further inspection and repair before use in later project phases.

As a result, only the prototype DO 1111 could be tested versus CTD, but since the CTD had no oxygen sensor, only the pressure and temperature data could be compared.

Values were recorded every 2 seconds, from the surface to the bottom (max. 500 m) and during the upcast, immediately after reaching the bottom. Each profile includes a descent to 5 m and a return to the surface before the full profile in order to adjust the CTD sensors.





Figure 27: NAUTILOS prototypes (with orange plastic protections) mounted on CTD cage to be simultaneously deployed during the PELGAS cruise by IFREMER staff.

#### Results:

Simultaneous profiling of CTD and prototype DO 1111 was carried out in 25 stations. According to the settings, values were supposed to be recorded by the DO prototype every 2 seconds, but it turned out that data were recorded with a period between 4 and 6 seconds (cf. Tab. 12).

Six stations were set up at depths of up to 500 metres (B2190, B2197, B2198, B2199, B2218, B2219). As the results were stable over the duration of the mission, only detailed data from station B2190 downcast are presented below. The average temperature offset is 0,0389 °C and the average pressure offset is 0,0734 bar (Table 13).

Figure 28 shows CTD and DO prototype 1111 temperature readings according to the B2190 depth, while Figures 29 and 30 show intercomparisons between CTD and DO prototype for temperature and pressure.

Table 14 summarises the relation between CTD and DO prototype 1111 at different depth ranges for two stations.

Table 12: Example datafile as recorded by prototype DO 1111 including real recording rate (column 3).

			CH1:Temperatur	CH2:Oxyge	CH3:Dept CH4	:Oxygen_Concentratio CH5:Oxy	gen_Saturatio
Source.Name	Standard) 📶 (Standarc 🔼 e	A. Andread .	The state of the s		h(m) 👱 n(m	g/L) <u> </u>	*
1111_data_230505_210830.csv		0,68	15,533	22,264	6,7	10,4	104,471
1111_data_230505_210830.csv	05/05/2023 21:08:36	0,66	15,641	22,225	6,5	10,4	104,715
1111_data_230505_210830.csv	05/05/2023 21:08:38	0,38	16,037	22,071	3,7	10,412	105,726
1111_data_230505_210830.csv	05/05/2023 21:08:44	0,2	16,171	22,065	1,9	10,375	105,649
1111_data_230505_210830.csv	05/05/2023 21:08:48	0,11	16,182	22,084	1,1	10,355	105,465
1111_data_230505_210830.csv	05/05/2023 21:08:54	0,13	16,186	22,115	1,3	10,326	105,179
1111_data_230505_210830.csv	05/05/2023 21:08:58	0,14	16,18	22,114	1,4	10,328	105,193
1111_data_230505_210830.csv	05/05/2023 21:09:04	0,13	16,18	22,119	1,3	10,324	105,148
1111_data_230505_210830.csv	05/05/2023 21:09:08	0,52	16,118	22,179	5,2	10,29	104,665
1111_data_230505_210830.csv	05/05/2023 21:09:14	0,92	15,535	22,322	9,1	10,347	103,948
1111_data_230505_210830.csv	05/05/2023 21:09:18	1,3	15,053	22,403	12,9	10,43	103,697
1111_data_230505_210830.csv	05/05/2023 21:09:24	1,74	14,891	22,423	17,3	10,465	103,679
1111_data_230505_210830.csv	05/05/2023 21:09:28	2,1	14,701	22,447	20,8	10,505	103,652
1111_data_230505_210830.csv	05/05/2023 21:09:34	2,48	14,61	22,45	24,6	10,533	103,716
1111_data_230505_210830.csv	05/05/2023 21:09:38	2,92	14,504	22,47	28,9	10,549	103,642
1111_data_230505_210830.csv	05/05/2023 21:09:44	3,31	14,245	22,497	32,8	10,611	103,659
1111_data_230505_210830.csv	05/05/2023 21:09:48	3,72	14,029	22,538	36,8	10,646	103,512
1111_data_230505_210830.csv	05/05/2023 21:09:54	4,14	13,798	22,626	41	10,644	102,965
1111_data_230505_210830.csv	05/05/2023 21:09:58	4,55	13,569	22,661	45	10,69	102,886
1111_data_230505_210830.csv	05/05/2023 21:10:04	4,94	13,51	22,732	48,9	10,645	102,321
1111_data_230505_210830.csv	05/05/2023 21:10:08	5,36	13,367	22,866	53	10,573	101,301
1111_data_230505_210830.csv	05/05/2023 21:10:14	5,74	13,303	23,008	56,8	10,467	100,147
1111_data_230505_210830.csv	05/05/2023 21:10:18	6,15	13,181	23,156	60,9	10,377	99,014
1111_data_230505_210830.csv	05/05/2023 21:10:24	6,54	13,139	23,278	64,8	10,284	98,036
1111_data_230505_210830.csv	05/05/2023 21:10:28	6,97	13,098	23,382	69	10,207	97,216
1111_data_230505_210830.csv	05/05/2023 21:10:34	7,37	13,061	23,487	73	10,129	96,393
1111_data_230505_210830.csv	05/05/2023 21:10:38	7,79	12,997	23,578	77,1	10,073	95,715
1111_data_230505_210830.csv	05/05/2023 21:10:44	8,17	12,952	23,65	80,9	10,026	95,177
1111_data_230505_210830.csv	05/05/2023 21:10:48	8,63	12,907	23,707	85,4	9,993	94,764
1111_data_230505_210830.csv	05/05/2023 21:10:54	9	12,914	23,702	89,1	9,995	94,798
1111_data_230505_210830.csv	05/05/2023 21:10:58	9,45	12,864	23,763	93,5	9,959	94,358

Table 13: Temperature and pressure offsets obtained for sensor 1111 during downcast at station B2190.

Station	Depth (m)	CTD Temperature (°C)	CTD Pressure (bar)	1111 Temperature (°C)	1111 Pressure (bar)	Temperature offset (°C)	Pressure offset (bar)
B2190	5	16,079	0,504	16,118	0,520	-0,016	-0,039
B2190	9	15,158	0,907	15,535	0,920	-0,013	-0,377
B2190	13	14,913	1,311	15,053	1,300	0,011	-0,140
B2190	17	14,811	1,714	14,891	1,740	-0,026	-0,080
B2190	21	14,580	2,117	14,701	2,100	0,017	-0,121
B2190	25	14,500	2,521	14,610	2,480	0,041	-0,110

B2190	29	14,285	2,924	14,504	2,920	0,004	-0,219
B2190	33	14,109	3,327	14,245	3,310	0,017	-0,136
B2190	37	13,882	3,731	14,029	3,720	0,011	-0,147
B2190	41	13,635	4,134	13,798	4,140	-0,006	-0,163
B2190	45	13,449	4,537	13,569	4,550	-0,013	-0,120
B2190	49	13,359	4,941	13,510	4,940	0,001	-0,151
B2190	53	13,242	5,344	13,367	5,360	-0,016	-0,125
B2190	57	13,149	5,747	13,303	5,740	0,007	-0,154
B2190	61	13,057	6,151	13,181	6,150	0,001	-0,124
B2190	65	13,017	6,554	13,139	6,540	0,014	-0,122
B2190	69	12,995	6,958	13,098	6,970	-0,012	-0,104
B2190	73	12,934	7,361	13,061	7,370	-0,009	-0,127
B2190	77	12,888	7,764	12,997	7,790	-0,026	-0,109
B2190	81	12,839	8,168	12,952	8,170	-0,002	-0,113
B2190	85	12,825	8,571	12,907	8,630	-0,059	-0,082
B2190	89	12,806	8,975	12,914	9,000	-0,025	-0,108
B2190	94	12,785	9,479	12,864	9,450	0,029	-0,079
B2190	97	12,776	9,782	12,890	9,820	-0,038	-0,114
B2190	101	12,768	10,185	12,845	10,240	-0,055	-0,077
B2190	106	12,728	10,689	12,827	10,670	0,019	-0,099
B2190	109	12,715	10,992	12,790	11,050	-0,058	-0,075
B2190	114	12,676	11,496	12,763	11,490	0,006	-0,087
B2190	118	12,662	11,900	12,746	11,900	0,000	-0,084
B2190	122	12,653	12,303	12,743	12,300	0,003	-0,090
B2190	126	12,642	12,707	12,711	12,710	-0,003	-0,069
B2190	130	12,627	13,110	12,719	13,130	-0,020	-0,092
B2190	134	12,612	13,514	12,682	13,520	-0,006	-0,070
B2190	137	12,604	13,817	12,672	13,830	-0,013	-0,068
B2190	141	12,595	14,220	12,665	14,210	0,010	-0,070
B2190	144	12,588	14,523	12,659	14,580	-0,057	-0,072
B2190	149	12,569	15,027	12,636	15,010	0,017	-0,067
B2190	152	12,560	15,330	12,638	15,370	-0,040	-0,078
B2190	157	12,544	15,834	12,621	15,820	0,014	-0,078
B2190	160	12,533	16,137	12,621	16,210	-0,073	-0,088
B2190	165	12,511	16,642	12,587	16,630	0,012	-0,076
B2190	169	12,495	17,045	12,578	17,030	0,015	-0,083
B2190	173	12,477	17,449	12,567	17,440	0,009	-0,090
B2190	177	12,462	17,852	12,534	17,880	-0,028	-0,072
B2190	181	12,460	18,256	12,529	18,250	0,006	-0,069
B2190	185	12,451	18,660	12,519	18,670	-0,010	-0,068
B2190	189	12,445	19,063	12,511	19,090	-0,027	-0,066

B2190	193	12,425	19,467	12,497	19,490	-0,023	-0,072
B2190	197	12,415	19,871	12,475	19,900	-0,029	-0,060
B2190	201	12,402	20,274	12,465	20,270	0,004	-0,063
B2190	205	12,365	20,678	12,431	20,730	-0,052	-0,066
B2190	209	12,357	21,082	12,414	21,110	-0,029	-0,057
B2190	213	12,346	21,485	12,403	21,550	-0,065	-0,057
B2190	217	12,321	21,889	12,374	21,940	-0,051	-0,053
B2190	222	12,305	22,394	12,360	22,390	0,003	-0,055
B2190	225	12,273	22,696	12,332	22,780	-0,084	-0,059
B2190	230	12,222	23,201	12,284	23,200	0,001	-0,062
B2190	234	12,207	23,605	12,263	23,620	-0,015	-0,056
B2190	238	12,184	24,008	12,242	24,020	-0,012	-0,059
B2190	242	12,171	24,412	12,234	24,440	-0,028	-0,063
B2190	246	12,161	24,816	12,212	24,850	-0,034	-0,051
B2190	250	12,152	25,220	12,205	25,270	-0,050	-0,053
B2190	254	12,130	25,623	12,192	25,670	-0,047	-0,062
B2190	258	12,117	26,027	12,173	26,070	-0,043	-0,056
B2190	262	12,114	26,431	12,168	26,480	-0,049	-0,054
B2190	266	12,103	26,835	12,150	26,890	-0,055	-0,047
B2190	270	12,083	27,239	12,129	27,310	-0,071	-0,046
B2190	274	12,045	27,642	12,095	27,700	-0,058	-0,050
B2190	278	12,019	28,046	12,073	28,140	-0,094	-0,054
B2190	282	12,009	28,450	12,063	28,510	-0,060	-0,054
B2190	286	11,997	28,854	12,051	28,940	-0,086	-0,055
B2190	291	11,996	29,359	12,038	29,370	-0,011	-0,042
B2190	294	11,997	29,662	12,049	29,750	-0,088	-0,052
B2190	299	11,969	30,166	12,026	30,180	-0,014	-0,057
B2190	303	11,950	30,570	12,007	30,590	-0,020	-0,057
B2190	307	11,915	30,974	11,976	30,980	-0,006	-0,061
B2190	311	11,898	31,378	11,959	31,430	-0,052	-0,061
B2190	315	11,872	31,782	11,936	31,810	-0,028	-0,064
B2190	319	11,865	32,186	11,924	32,220	-0,034	-0,059
B2190	323	11,838	32,590	11,905	32,640	-0,050	-0,067
B2190	327	11,807	32,994	11,864	33,060	-0,067	-0,057
B2190	331	11,768	33,397	11,823	33,450	-0,053	-0,055
B2190	335	11,695	33,801	11,757	33,880	-0,079	-0,062
B2190	340	11,659	34,306	11,714	34,320	-0,014	-0,055
B2190	343	11,643	34,609	11,701	34,670	-0,061	-0,059
B2190	348	11,625	35,114	11,678	35,130	-0,016	-0,054
B2190	351	11,620	35,417	11,677	35,520	-0,103	-0,057
B2190	355	11,616	35,821	11,682	35,930	-0,109	-0,066

				aver	age value	-0,0389	-0,0734
B2190	494	11,026	49,864	11,059	49,910	-0,046	-0,033
B2190	493	11,038	49,763	11,105	49,890	-0,127	-0,067
B2190	492	11,052	49,662	11,119	49,710	-0,048	-0,067
B2190	489	11,083	49,358	11,128	49,440	-0,082	-0,045
B2190	485	11,095	48,954	11,145	49,090	-0,136	-0,050
B2190	482	11,104	48,651	11,150	48,690	-0,039	-0,047
B2190	477	11,132	48,146	11,171	48,270	-0,124	-0,039
B2190	474	11,139	47,843	11,184	47,880	-0,037	-0,045
B2190	469	11,148	47,337	11,193	47,450	-0,113	-0,045
B2190	465	11,156	46,933	11,206	47,040	-0,107	-0,050
B2190	461	11,163	46,529	11,211	46,640	-0,111	-0,048
B2190	457	11,170	46,125	11,224	46,230	-0,105	-0,054
B2190	453	11,182	45,721	11,235	45,780	-0,059	-0,053
B2190	449	11,197	45,317	11,245	45,410	-0,093	-0,048
B2190	445	11,231	44,912	11,278	44,970	-0,058	-0,047
B2190	441	11,248	44,508	11,294	44,550	-0,042	-0,046
B2190	437	11,280	44,104	11,319	44,150	-0,046	-0,039
B2190	433	11,303	43,700	11,351	43,730	-0,030	-0,048
B2190	428	11,320	43,195	11,372	43,310	-0,115	-0,052
B2190	425	11,323	42,892	11,369	42,920	-0,028	-0,046
B2190	420	11,335	42,387	11,379	42,490	-0,103	-0,044
B2190	416	11,350	41,983	11,394	42,090	-0,108	-0,044
B2190	412	11,350	41,578	11,393	41,690	-0,112	-0,043
B2190	408	11,353	41,174	11,405	41,260	-0,086	-0,052
B2190	404	11,361	40,770	11,420	40,850	-0,080	-0,059
B2190	400	11,396	40,366	11,457	40,420	-0,054	-0,061
B2190	396	11,413	39,962	11,460	40,050	-0,088	-0,047
B2190	392	11,436	39,558	11,498	39,610	-0,052	-0,062
B2190	388	11,445	39,154	11,500	39,220	-0,066	-0,056
B2190	384	11,476	38,750	11,519	38,820	-0,070	-0,043
B2190	380	11,499	38,346	11,579	38,390	-0,044	-0,080
B2190	376	11,546	37,942	11,600	38,000	-0,058	-0,054
B2190	372	11,584	37,538	11,628	37,570	-0,032	-0,044
B2190	368	11,597	37,134	11,652	37,170	-0,036	-0,055
B2190	364	11,602	36,730	11,655	36,770	-0,040	-0,053
B2190	360	11,611	36,326	11,653	36,360	-0,034	-0,042

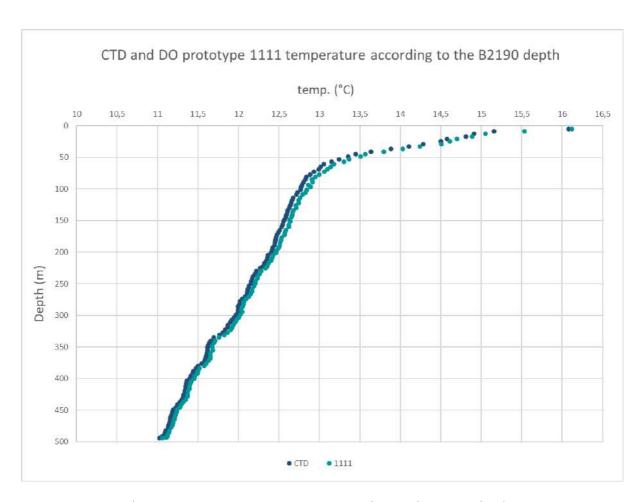


Figure 28: CTD and DO prototype 1111 temperature according to the B2190 depth.

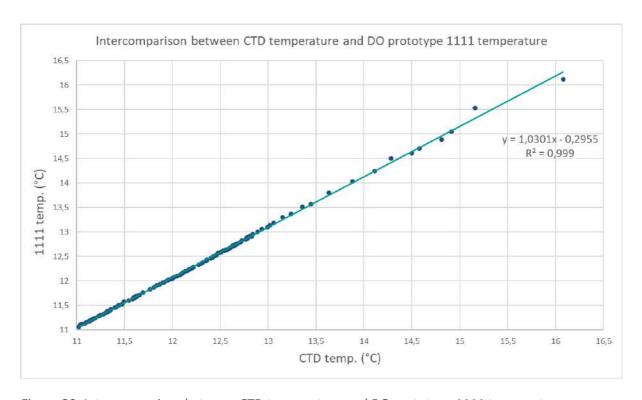


Figure 29: Intercomparison between CTD temperature and DO prototype 1111 temperature.

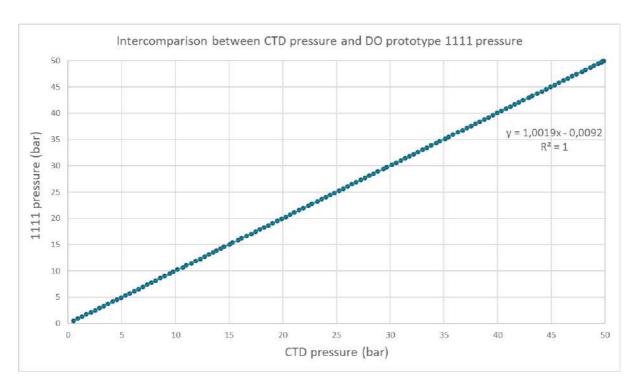


Figure 30: Intercomparison between CTD pressure and DO prototype 1111 pressure.

Table 14: Coefficient of determination ( $R^2$ ) between CTD and DO prototype 1111 temperature (T) and pressure (P) at different depth for station B2190 and station B2219.

				R <sup>2</sup>	
		[0-500m]	[0-50m]	[300-500m]	[400-500m]
B2190 (05/05/23)	T CTD vs 1111	0,9999	0,9887	0,9992	0,9952
	P CTD vs 1111	1	0,9998	1	0,9999
B2219 (05/10/23)	T CTD vs 1111	0,9998	0,9989	0,9995	0,9977
	P CTD vs 1111	1	0,9998	1	0,9999

# Recommendations for use and future deployment/issues encountered:

Even at depths of several hundred metres, the prototype demonstrated robustness and linearity when compared with traditional instruments for measuring depth and temperature. For future use, it is suggested to improve the data timestamping feature.

#### 1.6. General conclusions

Field validation tests were performed in more than 30 stations in various European seas in different ranges of depth, temperature, salinity, oxygen and/or chlorophyll concentration. The acquisition speeds of both DO and Chl-a sensors suggest their use in relatively stable conditions and for prolonged deployments at fixed depths. They are for example suitable for being used on fixed fishing gear such as creels, set nets or longlines which remain on the seabed in the same position for a long time during the fishing operation. They are also suitable for use on towed gear to record measurements as fishing operations take place at stable depths. For the same reasons, the sensors could be deployed for a certain period on fixed buoys in aquaculture facilities or lowered with stops at different depths. In general, the prototypes demonstrated good performance for both depth and temperature, comparable to those of traditional oceanographic instruments, even during the downcast or upcast profiling phases. As far as the DO is concerned, the possibility of setting a known salinity of the seawater internally to the sensors allows their use in known stable salinity conditions without the need for post-processing. Conversely, under possible known variations, it would be preferable to use sensors with internal salinity setting at 0, together with other available sensors in order to derive useful information for post-processing of the datasets.

The Chl-a prototypes showed a linear response compared to other traditional instruments. The noise was somewhat larger than reference sensors. Their usability in low Chl-a levels needs further attention. Therefore, given the conditions listed above, the acquired Chl-a trends could be considered reliable, but if accurate measurements are required, the dataset can be post-processed as it is normally done also with other commercial products. In lab tests, one of the fluorometer prototypes showed an unexpectedly high response time (see D6.1), but such effect was not explicitly noted in field tests. Reasons for this discrepancy should be investigated with additional tests.

# 2 INTEGRATION ON ADRIFOOS/FISHING VESSELS

NKE, IFREMER and CNR started the definition of the requirements for the integration of the DO and Chl-a sensors and WiHub on fishing vessels through a series of conference calls held in February 2023. For what concerns the integration on the CNR AdriFOOS platform, information already available in deliverable D2.3 (Integrated ICD- Interface Control Document for partners' vehicles, platforms, and infrastructure) was also taken into account.

DO and Chl-a prototypes were previously delivered to CNR and IFREMER in order to carry out laboratory calibration (see D6.1) and field validation tests (see paragraph 1); WiHub receivers and plastic protections for sensors were received by IFREMER in Brest and CNR in Ancona on 20/04/2023 (Fig. 31).



Figure 31: NKE equipment received by CNR: DO and Chl-a prototypes, plastic sensor protections, WiHib receiver, relative accessories for mounting and connection and quick start guide.

#### 2.1. Integration on AdriFOOS (CNR)

AdriFOOS consists of Fishery & Oceanography Observing Systems (FOOS; Patti et al. 2016) installed on commercial fishing vessels operating in the Adriatic Sea and a multifunctional dedicated on land datacenter, located at CNR IRBIM Ancona. The datacenter receives daily data sets of environmental parameters collected through the water column and near the seabed (e.g. temperature, salinity, etc.), together with GPS haul tracks, catch amounts per haul, target species sizes and meteorological information (Penna et al. 2023).

AdriFOOS includes in its fleet various kinds of fishing vessels, targeting different resources, and producing a huge amount of data useful both for oceanographic and fishery biology purposes. In this case, a bottom trawler was selected to participate in NAUTILOS demonstrations on Fisheries Observing Systems in ST7.1.1, therefore some integration tests in controlled conditions were necessary before installation on the commercial vessel.

For this reason, upon receiving the equipment and given the imminent start of the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise on 21/04/2023 (see paragraph 1.4) and the possibility of conductiong integration tests during this experimental fishing cruise, the CNR-IRBIM staff promptly arranged mechanical integration. Tests were also conducted on the instruments manufactured by NKE before installing them on board the research vessel used for the scientific survey (R/V Dallaporta).

## 2.1.1 Mechanical integration

During the cruise, a bottom trawl net equipped with otter doors was to be used for experimental fishing, similar to what will be done during the demonstrations on the commercial fishing vessel. Before the cruise, 2 steel threaded rods were welded onto the otter doors in order to facilitate the fixing of the sensors (Fig. 32,a). The sensors, equipped with plastic protections supplied by NKE, were then fixed, using two nuts, on the inner face of the otter doors and positioned so as to face the flow of water during towing (Fig. 32,b). As recommended by NKE, four anti-shock rings were used for each sensor in order to minimize the possible vibrations transmitted to the sensors by the gear during the fishing activities, while the otter door is towed on the seabed.

After initial testing (see the following paragraph), on 21/04/2023 the WiHub was mounted on board R/V Dallaporta on one of the vessel decks. This was fixed by two curved steel threaded rods to a flange supplied by NKE, powered and set in accordance with requirements (Fig. 33) and in line of sight with the Wisens sensor.

CNR-IRBIM and NKE staff were in constant contact during all the equipment installation and setting operations.

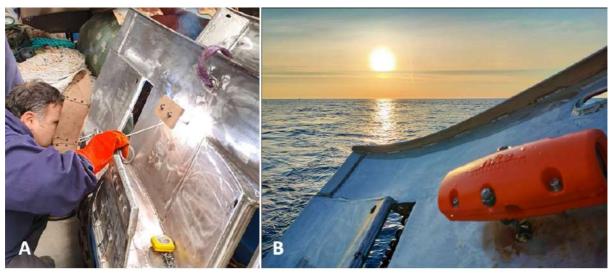


Figure 32: A) Installation operations of the steel supports on the otter doors for the positioning of the sensors; B) Prototype fixed on the otter door on board R/V Dallaporta.



Figure 33: A) WiHub installation operations on board R/V Dallaporta; B) WiHub web interface; C) Setting of the sensor through the web application.

# 2.1.2 Equipment assembly, electronic integration, configuration, and preliminary tests

According to the quick start guide provided by NKE (see Appendix 2), the following steps were followed:

- Completion of the electrical system: the terminals of the WiHub power cord were soldered to the terminals of a bipolar extension cord respecting the wiring diagram provided (red positive, blue negative/ground; see Fig. 34);
- Communication: a cat 6 Ethernet cable was soldered with the WiHub cable network pins respecting the RJ45 pinout shown in Figure 34; the correct physical functioning of the new network cable was then tested using a commercial LAN (local area network) cable tester (Fig. 35);

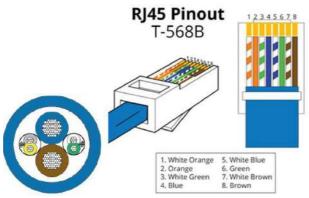


Figure 34: Blue cable of the WiHub receiver (left) and Ethernet pinout (right).



Figure 35: LAN cable tester used to test the correct physical functioning of the network cable.

- Powering: the WiHub was connected to a power supply set at 12V (it is possible to power it from 9V to 27V) and a LED positioned on it started to flash slowly with regularity, indicating a correct initialization;
- Connection: at this stage a Wi-Fi connection to the Hub was available through the WiSens-SRV service set identifier (SSID) (Fig. 36), using the protection key reported in the quick start guide; several connection issues were experienced using DELL-branded notebooks, due to some security checks specific to these laptops., while there are no connection problems with other brands (e.g. Lenovo, Asus, Android, IOS, Apple, etc.);
- Configuration: the WiHub parameters were checked and configured by accessing via browser (IP: 192.168.56.1; Fig. 37); GPS acquisition was enabled and set to the minimum available acquisition rate (5 minutes; Fig. 38).

In this first phase, some issues were encountered with the LAN connection: the LAN parameters were not modifiable, and the Ethernet button was not activated. A further check revealed that the WiHub's address could be identified, however, the data exchange failed for each attempt at a connection. Given the limited time available, it was decided to stop the LAN test while continuing to use onboard the WiHub with a Wi-Fi connection for the anticipated activities.



Figure 36: WiHub Wi-Fi SSID.



Figure 37: WiHub web application.



Figure 38: GPS settings.

In order to test the WiHub before the scientific survey, the product was then started by means of the virtual ON button (Fig. 39). When the WiHub is active, its LED flashes in a heartbeat pattern (i.e. two quick lights followed by a long light out). In this phase, the hub was connected to the CNR local network to allow remote control and check (Fig. 40). During the test there were no problems with connection, and GPS data was regularly recorded and saved into the WiHub local folder.

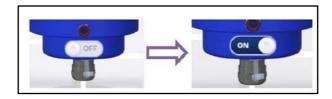


Figure 39: WiHub Virtual ON button (right).



Figure 40: WiHub testing phase.

## 2.1.3 Tests carried out on board the research vessel Dallaporta

Tests (including those described in paragraph 1.4) were carried out in the Adriatic Sea on board R/V Dallaporta until 02/05/2023, during the scientific survey "Monitoraggio Pomo 2023 & NAUTILOS trials".

After the field validation tests described in paragraph 1.4, 5 fishing hauls with sensor prototypes mounted on the trawl otter doors were carried out:

- On 28/04/2023, start 03:12 am UTC, end 04:44 am, maximum depth 256 meters;
- On 28/04/2023, start 17:10 pm UTC, end 18:40 pm, maximum depth 188 meters;
- On 29/04/2023, start 17:15 pm UTC, end 18:40 pm, maximum depth 212 meters;
- On 30/04/2023, start 03:15 am UTC, end 04:40 am, maximum depth 219 meters;
- On 30/04/2023, start 17:20 pm UTC, end 18:50 pm, maximum depth 202 meters.

The prototypes, fixed on the otter doors, were lowered and hoisted with particular attention, with the aim of monitoring activation and activity by flashing blue LEDs (Fig. 41).

Before each haul, the correct settings, and after each haul the correct data collection were checked though the NKE web application via smartphone.

The DO and Chl-a prototypes correctly recorded data from the first test casts, but started to communicate with the WiHub only after 25% of the internal memory buffer was used (this information is visible under the storage section of the web application dashboard). Beyond this point, the data was sent to the WiHub promptly at the conclusion of each haul.

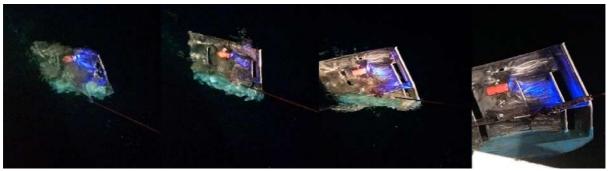


Figure 41: NKE prototype (with orange plastic protection) mounted on an otter door during a fishing operation; it is possible to see the sensor in activity through the blue LED.

While testing NAUTILOS prototypes during the fishing operations, the recording options selected through the sensor's web application were *on condition* and *fishing mode*. This type of setting allows two different data capture ratios within the same haul. In this case, the sensors were set to start recording data while in the water at pressure >0.3 bar every 2 seconds for 10 minutes, and then every 30 seconds until reaching again a pressure <0.2 bar, namely the end of the fishing operation (Fig. 42 b,c).

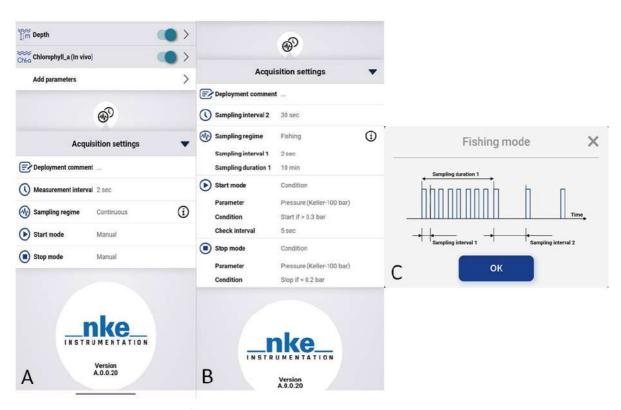


Figure 42: Sensor settings; A) Manual on Continuous sampling regime setting used during the simultaneous profiling with CTD; B) on Condition in Fishing sampling regime used during the fishing operations; C) graphic view of the different time-step of the data acquisition settings used during the fishing test.

Such an acquisition rate setting allows recording information during the downcast (i.e. the deployment of the fishing gear) and while the fishing gear remains for a while at the fishing depth, and to save battery. To correctly set the different phases of the prototype acquisition rate to be used during the fishing operations, it is necessary to also take into account the area (i.e. the depth) at which the selected fishing vessel normally operates. Figure 43a shows the case in which 10 minutes were more than enough for the fishing gear to reach the bottom and the sensor, correctly set, changed the data acquisition rate from every 2 seconds to every 30 seconds after reaching the bottom. Then the sensor switched off when the depth condition was exceeded (<2.0 bar). However, in the fishing test shown in Figure 43b, the time set for the first acquisition phase was not sufficient to reach the bottom.

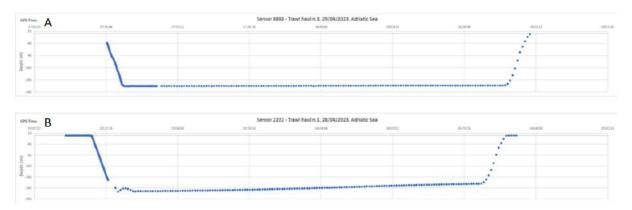


Figure 43: Depth profile recorded by the prototypes during the fishing tests. A) depth profile acquired by prototype 8888 during a fishing operation in which the fishing depth was reached before the change in data acquisition settings, B) depth profile acquired by prototype 2222 during a fishing operation in which the fishing depth was reached before the change in data acquisition settings.

## 2.1.4 Further electronic integration and configuration tests carried out after the cruise

After the scientific survey, laboratory tests resumed in May 2023. First, the LAN issue previously encountered was investigated: analyses were carried out to verify the correct functioning of the interface and the Ethernet LAN functionality. The WiHub usage instructions previously received, claimed a static IP (Internet Protocol) address (192.168.56.1), but an accurate inspection revealed that it was set to use DHCP (Dynamic Host Configuration Protocol). The setting was perhaps a holdover from tests carried out at NKE laboratories. After successfully setting the static IP and enabling the Ethernet communication (in the LAN section of the NKE web application), it was possible to connect the hub to a PC (personal computer) and download the data collected during the previous testing phases. Afterwards, a static IP was configured, and the network parameters were changed (IP address: 192.168.10.50, Subnet mask: 255.255.255.0, Gateway: 192.168.10.254) to prepare it for connection with the AdriFOOS platform components (Fig. 44).



Figure 44: LAN settings in the specific section of the NKE web application.

Data received by the WiHub can be retrieved locally in FTP (File Transfer Protocol; Fig. 45a) via Wi-Fi (IP: 192.168.56.1) or Ethernet connection (host: 192.168.10.50; port: 2221). The FTP root directory (Fig. 45b) includes 4 folders: /FTP (files received by the system if Hub is off), /MEASURE (measurement files received by sensors), /GPS (GPS recording files in progress), /ARCHIVE (contains the files transmitted in case of mobile connection is on). The GPS points recorded by the hub are appended to a single file until a preconfigured quota/file size is reached. When the quota is exceeded, a new file is generated. In case the GPS file is moved from the /GPS folder (cut-and-paste function) a new GPS file is automatically generated. The FTP connection and integrity of file transfer between the hub and multiple devices was tested positively in view of the AdriFOOS electronic logbook software update (see paragraph 2.1.5).

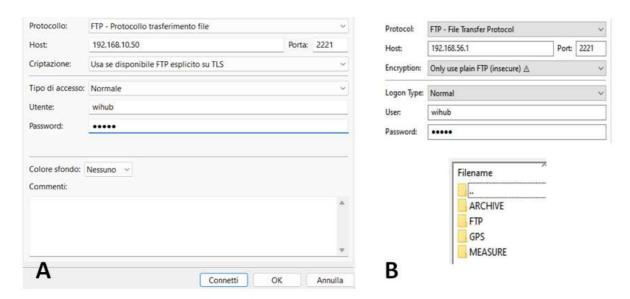


Figure 45: WiHub FTP parameter via Ethernet (A) and root folder (B).

For the purposes of integration with AdriFOOS, following the analysis of the data collected during the test cruise and the consultations between CNR and NKE staff, on 05/25/2023, the firmware of the WiHub delivered to the CNR was updated in coordination with NKE (via TeamViewer). The update ensures that GPS data is recorded on a minute basis instead of every 5 minutes. This allows for better tracking of the vessel, the geolocation of the acquired datasets, and alignment with the data already collected by AdriFOOS.

#### 2.1.5 AdriFOOS Electronic Logbook Software, Database, and Procedures Update

FOOS is a multifunction system that is able to collect different types of data from fishing operations. It can send back information to the fishermen through an electronic logbook with an *ad hoc* software embedded, which integrates information from sensors installed on the fishing gears and other data sources on board (Patti et al. 2016). Through a series of services, the AdriFOOS datacenter receives and integrates data in near real time from the fishing vessels, offers the possibility to carry out quality control on the datasets and send them directly to NAUTILOS services (Penna et al. 2023). Parallel to the scientific survey, the electronic logbook acquisition software (Fig. 46) and the AdriFOOS web viewer (Penna et al. 2023) were updated to integrate the data acquisition and processing steps necessary to include the prototypes DO and Chl-*a* and the WiHub in the architecture.

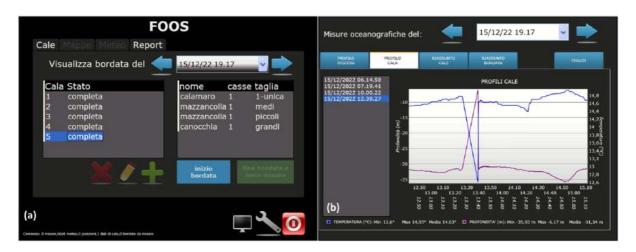


Figure 46: Electronic logbook graphical user interface used on board by fishers: screenshot examples from the catch module (a) allowing to enter the start and end of the trip (in Italian inizio and/or fine bordata) and caught species quantity (casse) and size (taglia) by haul (cala). The oceanographic real-time data visualisation module (b) showing fished depth (purple line) and relative temperature (blue line) by haul (source Penna et al. 2023).

To update the FOOS electronic logbook acquisition software, the following steps were taken:

 electronic logbook configuration menu update: information on the new sensors were added in the electronic logbook configuration menu and a check button to enable the connection to the WiHub was included (Fig. 47); new options were added to make the activation and management of the WiHub connection dynamic and more user-friendly (e.g. IP, port selector. User and password field, etc);

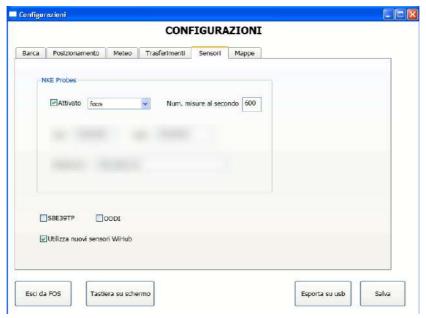


Figure 47: FOOS electronic logbook configuration menu sensors' section.

- FTP over TCP/IP (architecture client-server): the FOOS architecture normally includes an NKE product which communicates with the electronic logbook via serial link (i.e. the concentrator<sup>6</sup>), however the standard communication protocol used by the WiHub differs from that for the type of connection used (TCP/IP over Ethernet vs serial RS232); furthermore, the procedures in use envisaged a client-server architecture in which the concentrator acted as an FTP client and the logbook as an FTP server, while the WiHub itself acts as FTP server; it was therefore necessary to modify the data extraction software (programmed in C sharp; C#) to manage the FTP connection (Fig. 48);

Figure 48: Part of the code used for managing the FTP connection.

<sup>&</sup>lt;sup>6</sup> https://nke-instrumentation.com/wp-content/uploads/2018/02/CONCENTRATEUR\_UK2.pdf

new software functions for the management of the acquired data files: the architecture of the WiHub FTP includes 4 subdirectories: /ARCHIVE, /FTP, /GPS and /MEASURE (while the concentrator included in the FOOS architecture places the acquired data files directly in the root folder); the GPS folder contains the position data files (i.e. the tracking of the vessel), in MEASURE are stored the files containing the oceanographic measurements (i.e. pressure, temperature, dissolved oxygen and chlorophyll a concentration) collected by the DO and Chl-a prototypes; new software functions were developed to manage this structure (Fig. 49) and now, once the FTP connection of the WiHub is activated, an automated procedure checks for the presence of new GPS and/or measurement files, performs a primary validation for each file (checks if it is damaged), copies it to a local temporary folder in the electronic logbook (Fig. 50) and moves it to a zipped folder named "\_old" to save memory storage in the WiHub;

```
using (WebClient ftpClient = new WebClient())
{
   ftpClient.Credentials = new System.Net.NetworkCredential(user, pass);

   for (int i = 0; i <= directories.Count - 1; i++)
   {
      if (directories[i].Contains("."))
      {
            string path = "ftp://" + ipNke + ":" + port + "/" + fold + "/" + directories[i].ToString();
            string trnsfrpth = @"C:\ftp\" + directories[i].ToString();
            ftpClient.DownloadFile(new System.Uri(path), trnsfrpth);
            DeleteFile(path);
      }
}</pre>
```

Figure 49: Part of the C# code used for the management and local transfer of the files included in the MEASURE and GPS folders of the WiHub.

```
🗖 59da_gps_230719_215205.gpx
                                    9 KB File GPX
🗖 59da_gps_230720_084245.gpx
                                      1 KB File GPX
2222_data_230719_195925.csv
                                      26 KB File CSV
8888_data_230719_195855.csv
                                      23 KB File CSV
gps04b5083835200723.txt
                                      1 KB Documento di testo
gps04b5083935200723.txt
                                      1 KB Documento di testo
gps04b5084035200723.txt
                                      1 KB Documento di testo
🗐 gps04b5084135200723.txt
                                      1 KB Documento di testo
gps04b5084235200723.txt
                                      1 KB Documento di testo
🗐 gps04b5084335200723.txt
                                       1 KB Documento di testo
🖺 gps04b5084435200723.txt
                                      1 KB Documento di testo
                                      1 KB Documento di testo
gps04b5084535200723.txt
gps04b5084635200723.txt
                                      1 KB Documento di testo
🗐 gps04b5084735200723.txt
                                       1 KB Documento di testo
🗐 gps04b5084835200723.txt
                                       1 KB Documento di testo
🗐 gps04b5215416190723.txt
                                       4 KB Documento di testo
```

Figure 50: FOOS electronic logbook local folder. All data files from concentrator, WiHub and sensors are imported here.

- implementation of data parsing functions: given the difference between the data formats acquired by prototypes and WiHub and the formats previously used in AdriFOOS, it was necessary to implement a new feature; the WiHub GPS file format is XML (Fig. 51), while the concentrator one is a non-standard ASCII text format (.txt), therefore, a new GPS management software module was created; in the files produced by the DO and Chl-a prototypes, metadata are contained in a footer positioned after the recorded values (Fig. 52; instead of in a header as data normally managed within the electronic logbook collected by previous versions of NKE temperature, salinity and pressure recorders) and are collected in an XML tag enclosed between <WISENS> and <\WISENS>, therefore a new software module was developed for reading and processing according to this format;
- software management procedure update: considering the need to process the new datasets, the software management procedure (i.e. check, insert & update) of both the local electronic logbook database (SQLite, Fig. 53) and the AdriFOOS on land datacenter databases (MySQL, Fig. 54) were updated (architecture described in Penna et al. 2023); subsequently, the procedures aimed at managing REST-API calls between the logbook and the datacenter were also updated to guarantee the correct data transfer.

```
<gpx.version="1.0".creator="nke-instrumentation">
 <<trk data-id="101">
 · · <trkseq>
 ···<trkpt·lat="43.609350"·lon="13.491210">III
      <time>23-04-21.08:57:11</time>
    ·</trkpt>III
<time>23-04-21.09:20:54</time>
   ·</trkpt>
-<time>23-04-21.09:36:07</time>
   ·</trkpt>
····<trkpt·lat="43.609330"·lon="13.491150">
     .<time>23-04-21.09:41:09</time>ITT
    .</trkpt>IF
····<trkpt·lat="43.616630"·lon="13.505100">
     <<time>23-04-21.11:43:57</time>III
····</trkpt>IF
....<trkpt.lat="43.616630".lon="13.505090">
     <time>23-04-21 11:48:58</time>
   ·</trkpt>
....<trkpt.lat="43.616630".lon="13.505090">
     ·<time>23-04-21.11:53:59</time>
····</trkpt>IF
```

Figure 51: Example of GPS XML file produced by the WiHub.

```
| Dimestamp (Standard) JCB01Pressure(bar) /CB11Pressure(deg0] /CB21Orgyes(deg) JCB31Depth(n) /CB41Orgyes_Concentration(ng/b) /CB51Orgyes_Esturation(s) /CB21Orgyes_Esturation(s) /CB21Orgyes_Esturation(
```

Figure 52: Example of data file produced by the DO prototype.

datetime	sensorName	temp	depth	sal	value4	status	serial	value5	serial2
13/07/2023 10.30	wisense	34,404	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,409	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,393	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,354	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,417	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,398	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,453	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,437	-0,2		0	inviato	8888		clorophyll
13/07/2023 10.30	wisense	34,545	-0,2		0	inviato	8888		clorophyll

Figure 53: Example of data produced by prototype 8888 and stored in the local SQLite database.

datetime	idBoat	sensorName	temp	depth	sal	value4	value5	status	serial	serial2	gerico_status
2023-07-13 10:30:30	AN-04	wisense	34.404	-0.2	0	0	0	HULL	8888	dorophyll	sended
2023-07-13 10:30:32	AN-04	wisense	34,409	-0.2	0	0	0	HULL	8888	dorophyll	sended
2023-07-13 10:30:34	AN-04	wisense	34.393	-0.2	0	0	0	NULL	8888	dorophyll	sended
2023-07-13 10:30:36	AN-04	wisense	34.354	-0.2	0	0	0	HULL	8888	dorophyll	sended
2023-07-13 10:30:38	AN-04	wisense	34.417	-0.2	0	0	0	MULL	8888	dorophyll	sended
2023-07-13 10:30:40	AN-04	wisense	34.398	-0.2	0	0	0	HULL	8888	dorophyll	sended
2023-07-13 10:30:42	AN-04	wisense	34.453	-0.2	0	0	0	NULL	8888	dorophyll	sended
2023-07-13 10:30:44	AN-04	wisense	34.437	-0.2	0	0	0	HULL	8888	dorophyll	sended
2023-07-13 10:30:46	AN-04	wisense	34.545	-0.2	0	0	0	HUEL	8888	dorophyll	sended

Figure 54: Example of data produced by prototype 8888 and stored in one of the on land datacenter databases.

The AdriFOOS web viewer<sup>7</sup> was modified. The scripts for displaying the GPS track now are able to show the position data source generated by the WiHub (Fig. 55). A new function was added in the "sensori nella rete" section to show graphs generated with data from all the activated sensors dynamically, subdividing them by sensor and physical parameters. In addition to the displays of pressure, temperature and salinity parameters previously included in the AdriFOOS viewer, the possibility of displaying the concentration of dissolved oxygen and chlorophyll was added (Fig 56-57). A sample of data collected by AdriFOOS in the last period can be accessed using username: foosample and password: fsA@23.mp.

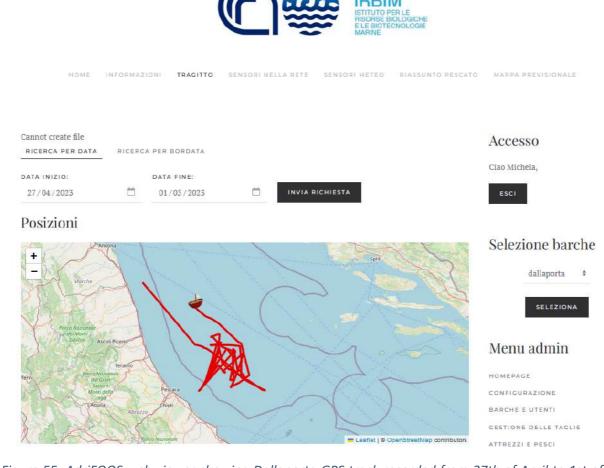


Figure 55: AdriFOOS web viewer showing Dallaporta GPS track recorded from 27th of April to 1st of May 2023 by the WiHub installed on board during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise (see paragraph 2.1.3).

<sup>&</sup>lt;sup>7</sup> http://foosweb.irbim.cnr.it/



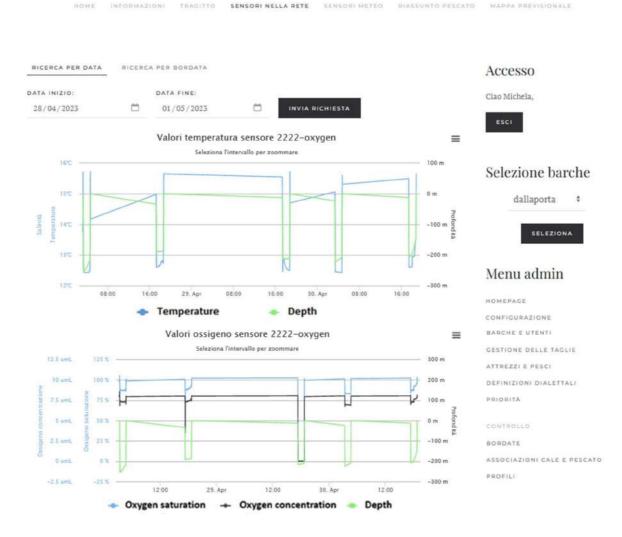


Figure 56: AdriFOOS web viewer showing depth and temperature (top panel) and dissolved oxygen concentration and saturation profiles (bottom panel, depth is shown again) acquired by prototype 2222 over 5 fishing hauls carried out from 28 to 30 April 2023 during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise (see paragraph 2.1.3).

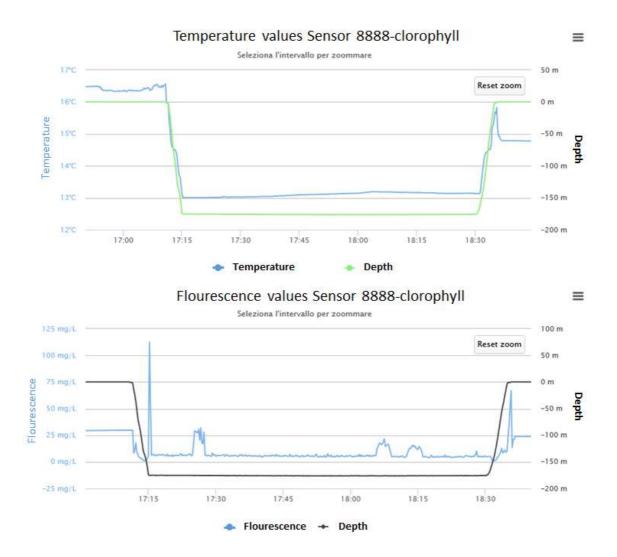


Figure 57:The AdriFOOS web viewer allows users to select and zoom in on a specific section of the acquired profiles. The upper panel shows a zoom on the depth and temperature data acquired by the 8888 prototype during one of the test hauls carried out as part of the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise (see paragraph 2.1.3), while the lower panel shows depth and chlorophyll-a (referred to as fluorescence) for the same haul.

#### 2.1.6 Installation on a commercial bottom trawler

The installation of the DO and Chl-a prototypes and of the WiHub on a commercial fishing vessel of the AdriFOOS fleet based in the port of Ancona is scheduled for 29/06/2023 (with a second option on 06/07/2023, depending on the availability of vessel's captain) and will be carried out taking into account all the integration phases described above.

#### 2.2 Integration on Fishing vessel (IFREMER)

During the PELGAS cruise (see paragraph 1.5) it was originally planned to trial the DO prototype 1111 and the Chl-a prototype 6666 during a fishing haul set in *fishing* acquisition mode (double recording rate). After the issues encountered with prototype 6666, due to fear of losing prototype DO 1111, it was decided not to carry out tests on the fishing gear during this cruise. NKE is currently conducting hyperbaric chamber tests on the Chl-a 6666 prototype and already has plans to replace it.

In the meantime, the DO prototype 1111 and the receiving Hub developed by NKE in ST3.1.2 were installed on a commercial fishing vessel used by IFREMER as VOO. This is a fishing vessel using pots, whose captain is a long time partner of IFREMER, namely involved in the ex-RECOPESCA program (Lamouroux et al. 2016; Leblond et al. 2010 - ended in January 2023). The DO sensor equipped with plastic protection was put in a meshed bag fixed inside a trap. It was configured by means of the web application developer by NKE using the settings shown in Figure 58.

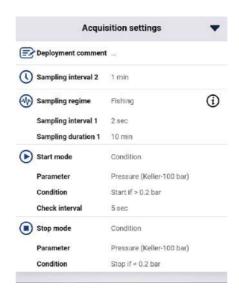


Figure 58: Prototype 1111 setting on Condition in Fishing sampling regime.

The WiHub was powered by an inverter 24V-12V (Hub electrical pinout: RED - positive, BLUE - negative/ground) and fixed with a flange on a stick on the deck (Fig. 59).

Once out of the water after the fishing operations, the DO sensor sends the data acquired during the fishing phase to the hub via Wi-Fi. The hub transfers the sensor's dataset and the GPS time stamp file that it produces to the NKE FTP server (as a first step) using file transfer protocol FTP port 2221. Figure 60 shows the web interface through which the WiHub settings can be selected.



Figure 59: WiHub installed on the commercial fishing vessel.



Figure 60: Web interface of the WiHub settings.

#### 2.3. General conclusions

The orange plastic protections supplied by NKE together with the DO and Chl- $\alpha$  prototypes (Fig.31-32) seem to make the sensors sufficiently safe against shocks due to deployment, especially during fishing operations. During the demonstration phase, the robustness of the material used will be verified after prolonged application on fishing gear and exposure to ultraviolet rays, marine salinity and sediments. The brackets supplied by NKE for the WiHub (Fig. 31, 33) make its fixing very simple and fast. In fact, it can be installed on various points of the boat where pipes or fixing rods for flags and electronic instruments are certainly available.

The electrical wiring, foreseen only for the WiHub, is very simple, having only 2 power cables and 2 pairs of cables for the Ethernet connection. Inside the WiHub, the cables are securely fixed using a dedicated power connector and a standard RJ45 connector. The IP protection of the cable entry into the WiHub appears to be adequate for both dust and water and, in any case, will be checked over time during the demonstrations.

Another positive note is the possibility of autonomously replacing the internal batteries of the prototypes using commercial lithium batteries. It would be worthwhile in the next updates to include the battery charge level in the metadata part of the files generated by the sensors. This would allow maintenance personnel to schedule their replacement, significantly improving management operations and drastically reducing sensor downtime.

Compared to previously available commercial products, an important step forward was made regarding the communication interface with LAN connection; the connection with the hub takes place not only with the LAN cable, but also via the standard Wi-Fi protocol. This allows the users to connect to the device via PC or mobile phone using a web browser, making the system easy to operate even for non-expert users.

The data transfer protocol used is FTP and an FTP server is installed on the WiHub; it is possible to choose the file formats between XML or CSV, standard formats that can be easily integrated in various services.

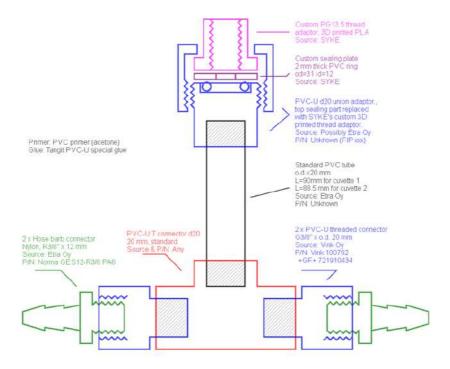
# III. INTEGRATION OF SENSORS/SAMPLERS ON FERRYBOX SHIPS OF OPPORTUNITY

#### 1. CARBONATE SENSORS

A flow through cuvette has been developed to deploy the Memosens CPS77E pH ISFET sensor, produced by Endress+Hauser (D4.1 Development of carbonate chemistry/ocean acidification sensors). It will be deployed on SYKE FerryBox Silja Serenade, sailing between Helsinki-Stockholm in WP7 demonstrations.

It has been designed with standard components, to be compatible with most FerryBox or flow through system fittings. The cuvette is built using stock PVC-U parts with three exceptions/special cases:

- The custom part for fixing the sensor to the PVC-U union has been 3D printed. The FreeCAD drawing (FCStd) and production file (stl) will be soon published.
- The flat sealing ring, that fits between the O-rings of the sensor and the PVC-U union, was cut from a thin PVC sheet.
- The PVC-U union part dimensions vary depending on the manufacturer. Using any other manufacturer's part would likely require changing the dimensions of the two parts mentioned above.



*Figure 61: Scheme of the flowthrough cuvette.* 

The cuvettes have PSB 3/8" threads and come fitted with plastic 3/8" x 12 mm barbed hose fittings that can be easily replaced. Two angled fittings are also supplied separately, as well as a silicon sealing disc that can be used to seal the cuvette while the sensor is not installed. The angle of the sensor can be freely selected. Moreover, the use of standard stock PVC parts keeps the assembly costs very low.



Figure 62: Versions of the cuvette that have been produced. Cuvette nr. 2 has been made about 2 mm shorter than nr. 1, in case it is found that the sensor needs to be slightly deeper inside.



Figure 63: The sensor, its transceiver and cuvette being tested in series with AFT-pH sensor.

The sealing of the cuvettes has been tested at 1.5 bar pressure.

The data is transmitted in real-time from the Memosens CML18 meter to the FerryBox computer in ASCII format via M12-USB A Serial connection. Data will be processed using salinity and temperature measured by the FerryBox system as well as Tris-seawater calibration buffer data.

#### 2. DOWNWARD LOOKING SENSORS

#### 2.1. Platform description

The POSEIDON FerryBox system<sup>8</sup> was initially installed on the Piraeus–Heraklion route in 2003 and operated for one year as a part of the European network for FerryBox measurements9, running the Piraeus-Heraklion route. In 2012 and most recently in 2019, it was upgraded and relocated to a new high-speed ferry on the same route, crossing the Cretan Sea, the largest and deepest basin (2500 m) in the south Aegean Sea. The HCMR FerryBox (Fig. 64) is the only one in the Eastern Mediterranean and serves as a pilot case for studying regional and subbasin scale physical phenomena, such as coastal upwelling and mesoscale cyclones north of Crete. It is also valuable for studying water circulation, especially when integrated into numerical circulation models for improved accuracy (Korres et al., 2014). The 4H-JENA engineering FB is located next to the main engine department, 2 m below the waterline, and samples water from the ship's main bow inlet without interference from ship equipment. The updated system is equipped, apart from the initial temperature, conductivity and fluorescence sensors, with dissolved oxygen and pH sensors and can be remotely controlled during the transect as long as there is mobile phone network coverage (Table 15). These sensors are operating continuously in a closed measuring circuit with seawater passing at a rate of 25 lt/min, while a debubbling unit removes air bubbles, which may enter the system during heavy seas. Bio-fouling is prevented by automated cleaning of the sensors with fresh water and acidified water rinsing after each cruise. The system is controlled through a computer, and is equipped with internal pressure and flow control sensors, which can shut it down in case of severe errors and malfunctions. The data are transmitted daily, when the ship reaches the destination harbour. The FB system average data sampling rate is set to 1 min, along Piraeus (37° 58'N 23° 38'E) to Heraklion (35° 20'N 25° 10'E) route (Fig. 64b). The hosting ship travels the 165 nautical miles distance at a speed of 20 knots collecting 430 samples on average. The trip duration is ~ 9 h and the FB is geographically initiated to log over the 200 m isobath, 6 miles (ca. 10 km) north of Heraklion and stops 4 miles (ca. 6 km) south of Piraeus at a depth of 100 m. Scheduled routes are performed once a day, during the night, with the exception of July and August, the peak tourism season months, when daily cruises are also added to the program. Although the ship's track is rather stable, currents, winds and traffic can affect it. To date, bad weather conditions and FB computer malfunction are the major reasons for missing data.

<sup>&</sup>lt;sup>8</sup> https://poseidon.hcmr.gr/

<sup>9</sup> http://www.ferrybox.org

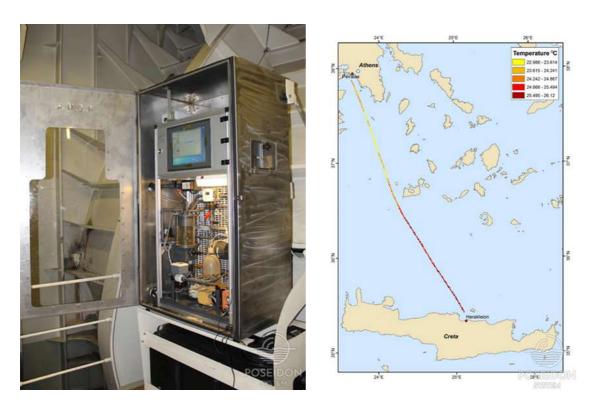


Figure 64. The POSEIDON Ferrybox system (a) and the transect along Piraeus to Heraklion (b).

Table 15. The POSEIDON FerryBox instrumentation.						
Instrument	Measured Parameter(s)	Elevation / Depth	Sampling average	Frequency of data recovery		
Scufa II Turner	Fluorescence /Turbidity	Surface	1 min	Daily		
Aanderaa optode 3835	Dissolved Oxygen	Surface	1 min	Daily		
Meinsberg electrode	рН	Surface	1 min	Daily		
SBE 45 MicroTSG Thermosalinograph	Temperature /Conductivity	Surface	1 min	Daily		
SBE 38 Digital Oceanographic Thermometer	Temperature	Surface	1 min	Daily		

#### 2.2. Sensor integration

As part of the NAUTILOS Sub-task 5.3.2, which involves integrating sensors and samplers onto FerryBox SOOPs, we will incorporate an infrared sea surface temperature sensor into the POSEIDON-HCMR FB system. Currently, the system already houses two highly accurate temperature sensors, with one positioned in the seawater inlet of the ship and the second operating within the main FB module. The Calex Pyro Mini Bus (Fig. 65) will be deployed on the open deck of the ship's right (starboard) side for WP7 demonstrations, ensuring that it directly faces the sea surface for data collection. The choice of installation point aligns with the manufacturer's recommendations, so that the sensor exclusively captures infrared radiation from the intended target without any interference from ship-related sources like smoke, fumes, or dust, which could potentially affect temperature measurements. Additionally, to minimise electromagnetic interference or 'noise,' the sensor will be positioned away from motors, generators, and similar equipment.

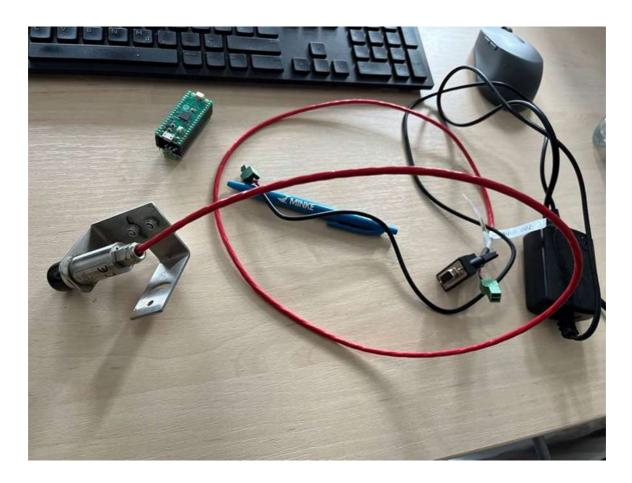


Figure 65. The Calex Pyro Mini Bus and the installation brackets attached on the sensor.

Regarding mechanical integration, the sensor will be securely mounted using two stainless steel brackets and fastened with safety nuts and bolts supplied by the manufacturer. These brackets serve the purpose of adjusting the pitch and roll of the sensor (as depicted in Fig. 66).

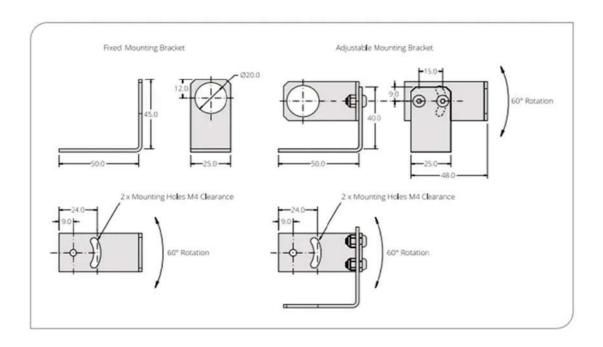


Figure 66. The adjustment brackets schematics provided by the manufacturer (source: calex.co.uk).

For power supply, the sensor will draw power from the FB's 12 V DC power supply unit, located within the main module of the FB system (Fig. 67). Already existing cable channels connecting the main machinery room where the FB is located and the side decks of the ship will be used. In terms of data storage and telemetry, real-time data will be transmitted and stored on the FB computer through the RS485 Modbus serial protocol via a DB9 connector. Furthermore, an automated timestamp will accompany the data to facilitate correlation with the time-stamped coordinates provided by the GPS module during the ship's transect.



Figure 67. The POSEIDON Ferrybox system power and telemetry module.

#### 3.1. Introduction

The NAUTILOS phytoplankton and particle suspended matter sampler (PPS) will be deployed on a FerryBox ship of opportunity as part of WP7 demonstrations. For the integration activity, the PPS has been integrated with a stationary FerryBox system at NIVA's Solbergstrand field station in Drøbak, Norway as well as on NIVA's M/S Color Fantasy (Oslo, Norway-Kiel, Germany) FerryBox. On the stationary FerryBox, the PPS was mechanically and electrically integrated and sampled surface and 1 m depth seawater. On M/S Color Fantasy, the PPS was physically and electrically integrated and also operated in a cooling chamber that will be used for sample preservation. The sampler will later be integrated with an analogous FerryBox system on M/S Richard With (Bergen-Kirkenes, Norway) for WP7 demonstrations.

#### 3.2. Water source

Seawater is sourced from the FerryBox distribution manifold with an input pressure of approx. 0.5 bar. The internal volume of the flow path section between the FerryBox distribution manifold supply valve and PPS intake is about 175 ml. This volume is flushed out directly before sampling by keeping the multi-port valve at the neutral port between samples (port 0, which allows water to flow through the system to drain without passing through a sampling filter), then pumping the required volume to bring in fresh seawater before moving to the next sampling port (Fig. 68).

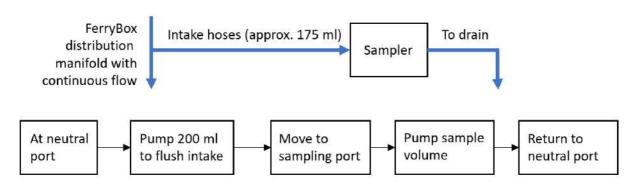


Figure 68: Water intake schematic and flushing procedure.

#### 3.3. Nitrogen/air filter flushing

To enable post-sample drying of the filters in the filter holders prior to retrieval, pressurised nitrogen is fed to the system as follows: the PPS pump is relocated in the flow path from its original position between the multi-port-valve and water/fixative selection valve to downstream of the selection valve. Then  $N_2$  or filtered air is connected to the fixative input port of the selection valve, and the remaining sample is expelled via the water exhaust hose which is connected to the fixative exhaust port of the selection valve (Fig. 69).

# Original config. Fixative Valve System Intake Fixative System Exhaust Fixativ

# Alternative config. for air/N<sub>2</sub> filter drying

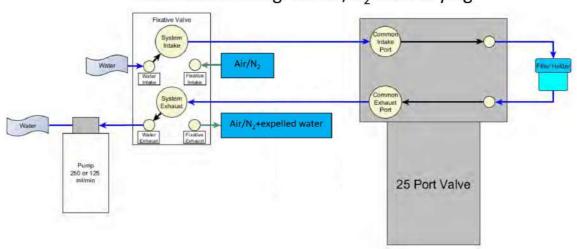


Figure 69: Modified configuration for nitrogen filter flushing (modified from McLane 2022).

#### 3.4. Cooler integration

Preliminary cooler integration for sample preservation was done with an ER Kyl cooler permanently installed on board M/S Color Fantasy that was previously used for the ChemMariner passive sampler (Fig. 70). For more compact, lightweight and portable cooling, the sampler is integrated into a Dometic CFX3 100 cooler that can be controlled and monitored over Wi-Fi or Bluetooth and can cool its 88 litre compartment down to -22 °C in ambient temperatures up to 43 °C. The PPS' 24 filter holders are dismounted from their original baseplate and installed on a baseplate dimensioned to fit in the cooler (Fig. 71). The control pressure housing, consisting of an anodized aluminium alloy cylinder and end cap rated to a depth of 5500 metres, is made shorter and lighter. The housing contains an electronics stack and a battery holder designed for 16 D-cell batteries (which is not required for FerryBox deployment; external power integration described in next section). The battery holder is dismounted from the electronics stack and the aluminium 387 mm long aluminium cylinder is replaced by a 95 mm long plastic cylinder.

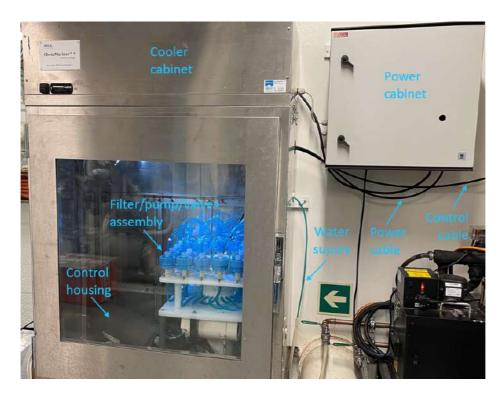


Figure 70: Sampler installed in ER Kyl cooler.

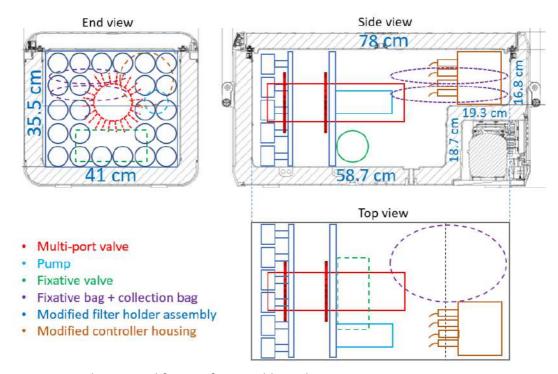


Figure 71: System layout modification for portable cooler integration.

#### 3.5. External power integration

The off-the-shelf PPS is designed for battery-powered deployments and does not have an external power input connector. External power delivery is enabled by replacing the control housing's communication bulkhead connector with a new MacArtney/SubConn 5-PIN MCBH5F wet-mate connector that enables both power and communication transfer. A diode board is added to the system's internal electronics to enable automatic selection of whichever voltage input is higher of external power or battery (battery use will be rare but possible). The original external communication cable is replaced with a Teledyne Impulse power+comms Y-cable with an MCIL-5-MP-IM male pin connector in the control housing end, branched to identical MCIL-5-FS-IM female socket connectors for power supply and RS-232 communication in the opposite end (Fig. 72).

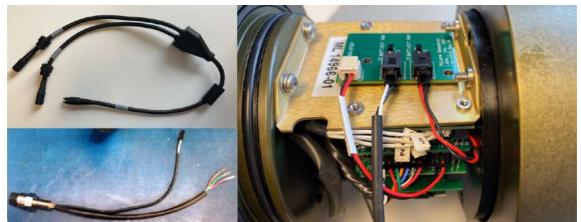


Figure 72: Battery/external power diode board (right), external power+comms Y-cable (top left), and MCBH5F bulkhead connector with internal power/comms pigtail and MTE-2F connector (bottom left).

#### 3.6. Power supply

The female socket power connector of the Y-cable described in section 3.5 is mated with a MCIL-5-MP-IM male pin connector moulded to a 2 conductor cable ending as a pigtail. The pigtail end is passed through a cable gland and into the FerryBox control cabinet and terminated at the 24VDC distribution terminals supplied by an XP DNR60 AC/DC power supply. The PPS system accepts 18-36VDC input and has a maximum current consumption of approximately 415 mA.

#### 3.7. Communication path

The female socket communications connector of the Y-cable described in section 3.5 is mated with a MCIL-5-MP-IM male pin connector moulded to a 5 conductor cable ending as a pigtail. The pigtail end is passed through two cable glands and into the FerryBox wet cabinet, and from there, to the system's serial-to-Ethernet interface junction box. Internally in the box, the three pigtail RS-232 conductors are terminated at the screw terminals of an available port on NIVA's in-house developed E-box v1.0 connection board. The board connects to a Moxa NPort 5650-8-DT Serial Device Server, which integrates the PPS system together with other sensors into the FerryBox Ethernet network (Fig. 73).



Figure 73: FerryBox serial-to-Ethernet interface junction box with Moxa NPort and serial device connection board.

#### 3.8. Control/monitoring interface

Control/monitoring scripts are implemented in Matlab and will later be integrated into the FerryBox LabView control application. Scripts are written to partly automate the priming process, to automate the intake flush  $\rightarrow$  sampling  $\rightarrow$  drying process described below, and to partly automate the filter retrieval process. Sampler control is linked with cooler temperature control and monitoring.

#### 3.9. Priming process

All sampling ports to be used during a deployment are primed manually, to wet filters, fill fluid lines with neutral (distilled/deionized) water and remove all trapped air, as follows: First, the system's water exhaust hose is placed in the neutral water reservoir. For each port to be primed, its filter holder is disconnected from the baseplate by opening its quick-release, auto-sealing connector. The filter holder is opened, and a 150 ml syringe filled with neutral water is connected to the bottom half with a quick-connector. A filter is placed on the filter holder and saturated by injecting water upwards with the syringe while the top half of the holder is slowly reattached. The sampler multi-port valve is moved to the port being primed. The remaining water in the syringe is now injected in reverse through the fluid path upstream of the filter holder while gently tapping the holder against the baseplate, to fill the upstream path with water and remove all trapped air.

While/after doing these steps, one end of a hose is connected to the port's baseplate quick connector, and its other end to the drain. The sampler pump (which is at the downstream end of the system) is commanded to pump 100-150 ml of neutral water in reverse, to push all air out of the fluid path downstream of the filter holder. The filter holder is then connected to the baseplate and the process is repeated for the next ports.

#### 3.10. Sampling process

Sampling is triggered by the FerryBox control computer based on vessel position, other sensor inputs or manually by remote control from shore. Parameters issued to the sampler comprise sample port, volume, flow rate, minimum flow rate (pump to stop if flow rate drops to this limit due to filter getting clogged) and time limit (pump to stop if time limit reached). The system rests at the neutral port between samples, to flush the intake path just prior to moving to a port for sampling. After sampling, the repurposed fixative selection valve switches to  $air/N_2$  to dry the filter for one minute, then switches back to water.

#### 3.11. Filter retrieval

Filters are retrieved one by one manually when the vessel is in port, and preserved in a buffer and by freezing. Specific method depends on the type of sample taken. Further details related to filter retrieval and sample analysis are provided in detail in D6.1.

#### 4. MICROPLASTIC SENSOR

#### 4.1. Integration of the microplastic sampler

Sub-task 5.3.2 describes the integration of the developed microplastic samplers and sensors on FerryBox ships of opportunity. The combined sampler and detection system was developed by NIVA and CSEM in task 4.4. The sampling part was tested at an early stage at the M/S Colorline Fantasy showing the robustness to sample large volumes of subsurface water through the existing FerryBox system. One of the major challenges in developing the sampler and the laser detector system is the large sampled volumes (> 8000 L) needed to obtain significant numbers on the amounts of microplastics in relation to the small volumes and flows through the laser detector unit. Integrating the system on FerryBox systems showed this weakness of the system, and it was not possible to achieve satisfactory recovery rates of microplastics added to the system. For this reason, only the sampler system was integrated and installed on the M/S Trollfjord for a sampling campaign around the Norwegian coast and back and forth to Svalbard. Samples have to be taken back to NIVA's specialised microplastic lab and can not be processed on-line through the FerryBox systems on board ships of opportunity. This is a deviation to the original description of the task. Alternative use of the sampler and detection system will be further exploited in Task 11.2 Instrumentation Roadmap. Scalability, replicability and transferability study, where the focus will be on the use of the system for drinking water sampling. This application will require less sampling volumes and contain less interfering biological material. This application is in line with the demand of monitoring drinking water according to EU's water directive.

#### 4.2. Mechanical integration microplastic sampler (Hurtigruten M/S Trollfjord)

The system was installed on the Hurtigruten M/S Trollfjord in the beginning of June 2023. The microplastic sampler was added as a module to the FerryBox in the pumping room on deck 2, this is illustrated in Figure 74. The FerryBox pumps water from about 4 metres under the surface through several sensors. A connection was added before the FerryBox, so the samples were undisturbed by any contamination from the other sensors, which might contain plastic or polymer materials.



Figure 74: The installation of the microplastic sampling module in the pump room of the Hurtigruten M/S Trollfjord.

One of the advantages of the low-cost filter systems is that it is possible to exchange whole filter systems, which reduces contamination and allows several sample transects to be monitored. This is illustrated in Figure 75 where the filter unit and changing and closing of the filter unit is shown. All materials are in stainless steel and do not contain any plastic or polymer materials. In total, 10 transects can be sampled and remotely operated on-line from the FerryBox. The ship's crew can easily change the filter units without contamination of the samples.



Figure 75: The filter unit and replacement of the whole filter unit to avoid contamination.

#### 4.3. Integration of the microplastic sampling module with the FerryBox

An illustration of the NAUTILOS microplastic consisting of a separate pump, an inlet valve, a flow metre, a draining valve, the filter unit and an air valve is given in Figure 76. This module is completely integrated in the FerryBox and can be operated on-line through the connection with the FerryBox or operated on board. The FerryBox gathers the total volume sampled, the sampling time in addition to the GPS coordinates at the start of sampling and at the end of the sampling. The start and stop of the sampling can also be triggered by the GPS coordinates. All values are visible on the displays in Figure 76. In addition to the output of the microplastic module, metadata from the other sensors is collected. This includes inlet temperature (Seabird), oxygen concentration, oxygen saturation, temperature (Optode Anderaa), temperature, conductivity, salinity (CT seabird), Chl-a fluorescence, CDOM fluorescence, turbidity, temperature (Optical Sensor Turner, C3).



Figure 76: The parts of the microplastic module and integration of the with the FerryBox, including the metadata from the other sensors.

#### 4.4. Planned usage of the low cost NAUTILOS microplastic sampler

On behalf of the Norwegian Environment Agency (MilDir), NIVA will perform research on the amounts of microplastics along the Norwegian coast and Svalbard. NIVA will use the NAUTILOS microplastic sampler on board the Hurtigruten M/S Trollfjord. On the M/S Trollfjord we plan to take samples on the Hurtigruten Svalbard express and the NorthCap express during the period September-November 2023. The microplastic samples taken on the M/S Trollfjord will be part of the MikroNor monitoring program of microplastic pollution in the Norwegian aquatic and marine environment. The program is one of the largest surveys of microplastic in the environment in Europe.

The first sampling plan for the period 10-22 September 2023 consists of a total of 10 samples which will be taken with the focus on part of the coast and the transect to and from Svalbard as outlined in Figure 77. Samples will be taken when the ship is in transit and moving to avoid contamination in the harbours. The FerryBox stops sampling automatically when in harbour or when the speed of the ship is too low (<5 kts). After the sampling, the filter holders will be collected by NIVA when the Trollfjord arrives back in Bergen.

Location	MP Unit
Bergen	Test NIVA
Åndalsnes	Sample 1
Træna	Sample 2
Stokmarknes	Sample 3
Tromsø	Sample 4
Honningsvåg	Sample 5
Til sjøs rundt Bjørnøya	Sample 6
Longyearbyen	Sample 7
Ny-Ålesund	Sample 8
Til sjøs (Bjørnøya)	Sample 9
Tromsø og Senja	Sample 10
Svolvær	
Brønnøysund	
Ålesund og Urke	
Bergen	Collection

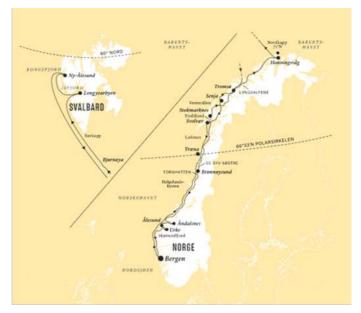


Figure 77: The sampling first campaign planned for the Norwegian Environment Agency using the Nautilos microplastic sampler along the Norwegian coast and Svalbard, planned from the 9th to the 20th of September 2023.

#### 5. DO AND CHL-A

Even though the project description of actions states, in ST5.3.2, that NKE DO/Chl-a sensors would be integrated with NIVA and HCMR FerryBoxes for the aquaculture demonstration, there was a discrepancy with the text of ST7.1.2. The latter, "Novel approach to Aquaculture Observing Systems", clearly states that those DO/Chl-a sensor prototypes from T3.1 (and the carbonate system sensors from T4.1) will be put in operation at mariculture and fish farming sites to provide complementary data of high temporal resolution to meet the purposes of the planned demonstration. Therefore, NIVA and NKE have defined new deployment strategies in agreement with use planned in T7.1.2. Eventually, it was agreed among all partners that the DO/Chl-a prototypes will be deployed in a fish farm located in mid-Norway at the Norwegian Aquaculture Academy fish aquaculture cages containing approximately 1.5 million salmons. A FerryBox equipped with complementary observational systems will pass about 2 km east of the aquaculture site every 5 days on average.

#### IV. ETHICAL CONSIDERATIONS

#### 1. DATA PROTECTION

Within ST5.3, raw data and analysis results were exchanged among partners to carry out joint analyses and/or evaluation related to prototype field validation and instruments integration phases. Ethical questions related to data protection when exchanging some information may arise, however, raw dataset and information were only used when voluntarily shared and only to meet the project goals. All the processed information that will be publicly shared outside the project for the purposes of project dissemination, will follow the procedures already established in D13.2 and all relationships with people or private companies external to the project followed and will follow in further developments what was recommended in D13.7.

#### 2. ENVIRONMENTAL PROTECTION

As regards the impact on the seabed of the otter doors used for the experimental fishing carried out during the "Monitoraggio Pomo 2023 & NAUTILOS trials" cruise, and the collection of the biological sample deriving from the catches, they are part of the normal monitoring activity carried out by the CNR IRBIM in the study area in agreement and with the authorizations of the managing bodies.

As regards the lithium batteries, inserted in the prototypes, precautions have been taken to avoid the loss of the instruments at sea and the sensors have a waterproof pressure housing which should prevent leakage towards the outside.

#### 3. HEALTH AND SAFETY

As regards the field validation cruises, all the safety requirements of the various leading institutions for the on-board personnel have been respected. The same goes for the integration of the DO and Chl-a and WiHub prototypes, during which the personnel involved used the provided personal protective equipment. Connections via cellular network and Wi-Fi should follow the expected standards.

When seeking to install a FerryBox system or sensors in a commercial SOOP, it is vital to adeptly navigate the operator organisational structure and establish positive connections with key individuals. Ideally, the involvement of senior management, particularly those who own and operate the ships, can facilitate approval from lower-level personnel. However, it's important to acknowledge that ship ownership and operation might be separate entities, which could complicate the chain of command. Consequently, direct communication with the ship's captain and first engineer becomes crucial, as they hold the responsibility for ship operations. As the highest-ranking officials responsible for ship operations, they have a deep understanding of the vessel's safety protocols and operational requirements. Their primary concern is the safety of the ship, its crew, and the cargo it carries. Any modifications or additions to the ship's equipment must be carefully evaluated to ensure they do not compromise the vessel's stability or integrity, and requests for support related to the installation must go through them for consideration. Involving and collaborating with the ship's crew, based on the size and management structure, may also provide valuable assistance during the process.

#### 4. PROTECTION OF MARINE LIFE

No particular harms to marine life were identified (except for fishery catches). Some prototypes emit a flashing blue light for a very short time, as already discussed in D3.1.

#### 5. DUAL USE POTENTIAL

No dual use potential has been identified for the DO and Chl-a sensors, the WiHub could potentially be used for GPS tracking of vessels.

### V. SUMMARY

In ST5.3.1, five field validation cruises were carried out by three NAUTILOS partners (CNR, IFREMER and SYKE). They took place in the North Sea, Adriatic Sea, Baltic Sea and Bay of Biscay to test the performances of the DO and Chl-a prototypes developed by NKE in ST3.1.2. The data collected, and the following joint analyses allowed to highlight strengths, weaknesses, possible improvements, attention to be paid in data processing and the suggested deployment strategies.

One of the cruises carried out by the CNR in the Adriatic Sea was also used to test for the first time the use of these new prototypes on fishing gear, together with their receiving WiHub, and the integration of this newly developed system on the AdriFOOS platform. All the steps necessary for the integration on the latter, and also on a fishing vessel, that will be used by IFREMER for the demonstrations foreseen in ST7.1.1, are described in this document. Indeed, this system allows the acquisition of datasets of depth, temperature, dissolved oxygen and chlorophyll automatically in near real time during fishing operations and can also be used in other applications such as aquaculture plants.

The integration activities completed in ST5.3.2 have successfully prepared the pH sensor, the phytoplankton/suspended matter sampler, the microplastics sampler/sensor, and sea surface temperature downward looking sensor for the demonstrations on Ship of opportunity in task 7.2 and aquaculture environment in subtask 7.1. Several lab-based and field trials were performed to check various aspects of physical and electrical/communications integration for the various instrument-FerryBox systems. Additional integration was not required for DO and Chl-a sensors as these will be deployed in situ at aquaculture sites in WP7. The results regarding integration of the DO and Chl-a prototypes on research and commercial fishing vessels and aquaculture operations suggest that a Technology Readiness Levels (TRL) of 6 was achieved for these sensors at this stage of the NAUTILOS project. The pH sensor, phytoplankton/suspended matter sampler, and microplastics sampler/sensor also achieved TRL 5/6. And the sea surface temperature sensor achieved a TRL 5 as the hardware/software integration was also tested in a large outdoor seawater tank which is reported in Deliverable 6.1.

# Appendix 1: References and Related Documents

ID	Reference or Related Document	Source or Link/Location
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3	Jiang, ZP., Yuan, J., Hartman, S.E., Fan, W., Enhancing the observing capacity for the surface ocean by the use of Volunteer 510 Observing Ship, Acta Oceanol. Sin., 38, 114–120, 2019.	https://doi.org/10.1007/s13131-019-1463-3
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5	Van Vranken, C.H., Vastenhoud, B.M., Manning, J.P., Plet-Hansen, K.S., Jakoboski, J., Gorringe, P., Martinelli, M., Fishing 585 Gear as a Data Collection Platform: Opportunities to Fill Spatial and Temporal Gaps in Operational Sub-Surface Observation, Front. Mar. Sci., 7: Article 485512, 2020.	https://doi.org/10.3389/fmars.2020.485512
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8	Lamouroux, J., Charria, G., De Mey, P., Raynaud, S., Heyraud, C., Craneguy, P., et al. Objective assessment of the contribution of the RECOPESCA network to the monitoring of 3D coastal ocean variables in the Bay of Biscay and the English Channel. Ocean Dyn. 66, 567–588, 2016.	https://doi.org/10.1007/s10236-016-0938-y
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11	Aydoğdu A., Pinardi N., Pistoia J., Martinelli M., Belardinelli A.,	https://doi.org/10.1016/j.jmarsys.2016.03.0 02
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21	Aminot A., Kérouel R. Hydrologie des écosystèmes marins. Paramètres et analyses. Ed. Ifremer. 2004.	https://books.google.it/books?id=dd7qZIWQ Gb8C&printsec=frontcover&hl=it
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28	Deliverable D2.3 (Integrated ICD-Interface Control Document for partners' vehicles, platforms and infrastructure)	h2020.eu/index.php/f/1529
29	D13.2 POPD - Requirement No. 2	NAUTILOS Gdrive
30	D13.7 GEN-Requirement No. 10	NAUTILOS ownCloud
31	D3.1 - Report and fabrication of a dissolved oxygen sensors based on fluorescence quenching	
32	McLane Research Labs, Inc. Particle & Phytoplankton Sampler User Manual, Rev.22.L.07.	https://mclanelabs.com/wp- content/uploads/2023/08/McLane-PPS- Manual.Rev .22.L.07.pdf

## APPENDIX 2: NKE DATA SHEETS

#### 2.1 WiHub SRV – Quickstart Guide (NKE)

# **Quick start WiHub**

#### First step

Connect the product electrically: Red, positive terminal (9V->27V accepted). Blue, GND.

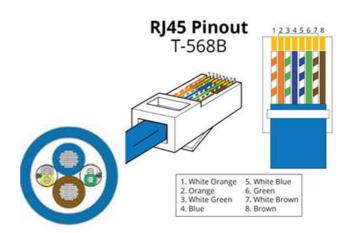
Connect the Ethernet connector (nke):

Wire 1 RJ45 -> Connected to yellow wire

Wire 2 RJ45 -> Connected to grey wire

Wire 3 RJ45 -> Connected to green wire

Wire 6 RJ45 -> Connected to white wire



#### **Second step**

Once the power is on, when the LED flashes slowly at regular intervals: the product is correctly started.

Connect to the product's Wi-Fi. Protection key: 12345678



#### Third step

On the main page, Activate/Set as required:

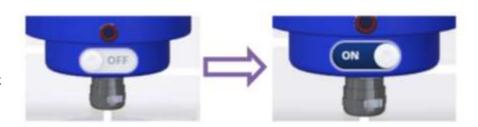
- GPS acquisition,
- 3G/4G file transmission,
- Ethernet link.





#### Fourth step

Start the product.
The product LED flashes in a heartbeat (two quick lights followed by a long light out) if it has been started successfully.





#### Info

The data received by the WiHub can be retrieved

locally in FTP via:

Host: 192.168.56.1 in Wi-Fi or Ethernet IP.

Port: 2221 User: wihub Password: wihub

#### 4 folders available:

#### /FTP

-> files received by the system if Hub is off.

#### /MEASURE

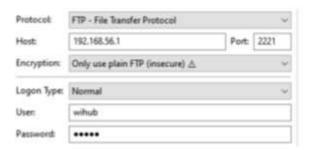
-> Measurement files received while waiting for 3G/4G transmission if Hub is on.

#### /GPS

-> GPS recording files in progress.

#### /ARCHIVE

-> Contains the files transmitted in 3G/4G





#### Connection and identification code



These informations allow you to connect to the embedded WiMo configuration interface.

Wi-Fi hotspot: SSID-SN from information plate

Connexion url: 192.168.56.1

or QRCode from information plate



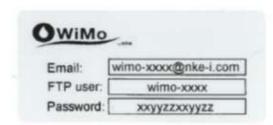
These informations allow you to retrieve data from the 3G/4G modem with nke default configuration.

Email: Email from information plate

FTP user: SSID-SN from information plate

Password: Password from information plate

#### Information plate



You can use email, SFTP or FTPS to retrieve or access to your data from nke server.

Domain name	Protocol	Port
smtp.lonos.fr	Email	465
sftp.nke-i.com	SFTP	22
ftp.nke-l.com	FTPS explicit	21



#### 2.2 WiSens – Quick Start Guide (NKE)

# **Quick start WiSens**

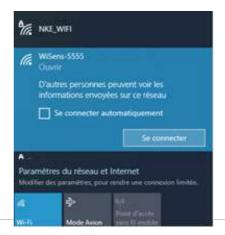
#### First step

Activation via a magnet placed on the Wi-Fi symbol



#### **Second step**

Connection to the WiFi of the WiSens Chl-a or DO sensor from a PC or a tablet



#### Third step

Enter the following in the address bar

http://192.168.56.1

Web Browser version





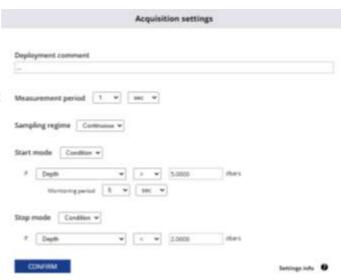
#### Fourth step

On the main page (System status):
Set the desired measurement rate,
Set the product to start mode and stop mode
on condition. These two values must be set
carefully to avoid unwanted start/stop when
the product is close to the surface.

Set the scan period of the measurement start condition.

Do not forget to press "Confirm".

⚠ The scan period and measurement period have an influence on the product's autonomy.



#### Five step

On the "System settings: Set the product to time, Set the product to automatic transmission mode

# Clock synchronization

2022-07-20

Synchronize with local clock (2022-07-20 15:36:54)	65	
Automatic trans	fer	

Local "WiSens-SRV"

#### Six step

On the main "System status" page: Turn on the product by clicking on the activation button.

Disconnect the product from Wi-Fi. Remove the magnet.



