The Research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 262584, JERICO

TNA PROJECT REPORT
3rd Call of Proposals

A) General Information

Proposal reference number	CALL_3_4
Project Acronym (ID)	TOFU
Title of the project	new Tools for Oxygen, Fluorescence and tUrbidity sensors testing and intercomparison
Host Research Infrastructure	Hellenic Centre for Marine Research – Calibration Laboratory
Starting date - End date	19.07.2014 - 02.08.2014
Name of Principal Investigator **Home Laboratory** **E-mail address** **Telephone**	Roberto Bozzano Consiglio Nazionale delle Ricerche roberto.bozzano@cnr.it +39.010.6475656
Additional users	Roberto Bozzano, Sara Pensieri Consiglio Nazionale delle Ricerche Via de Marini 6 16149 Genoa (ITALY)

B) Project objectives (max. 250 words)

The project aimed at testing innovative software and hardware tools to inter-compare sensors for oxygen, fluorescence and turbidity measurements.

For the test of oxygen sensors CNR developed a hardware/software tool able to simultaneously acquire up to 8 oxygen probes in the same calibration tank without the need of several multiparametric probes hosting the sensors, but simply one CTD instrument to acquire temperature and salinity data to be used as reference. The tool allowed for the acquisition of the raw voltage output of all sensors and for performing an inter-comparison in physical units devoted to the verification or the update of the calibration coefficients (or only the drift) provided by the manufacturer and to an evaluation of the performance of different type of oxygen sensors.

The second objective of the proposed project aimed at testing a package for the inter-calibration of multiple fluorescence and turbidity sensors in the same chamber acquiring real time data simultaneously by all sensors and with the same known chlorophyll/turbidity concentration.

Both new tools allowed for the calibration of sensors based on an inter-comparison with reference probes and Winkler tritation for dissolved oxygen, with chlorella and fluorescence dye solutions diluted with distilled water for fluorescence acquisitions and with Formazine diluted with deionised water for turbidity data.

All sensors used in the experiment provided by CNR are operationally and routinely deployed on the W1-M3A offshore observatory and this contributes to the long term monitoring of biological parameters in the Mediterranean Sea.

C) Main achievements and difficulties encountered

The experiment allowed to test two new different software and hardware tools for laboratory calibration/inter-comparison of oxygen and fluorescence/turbidity sensors.

The oxygen probes test was carried out in the HCMR calibration tank equipped with an immersion circulator and two aerators. The developed tools allowed the simultaneous comparison of four SBE43 oxygen sensors using only one CT(D) measurement as reference instead of using one CT(D) sensor for each oxygen probe without being able to check dissolved oxygen values in real time. Using such configuration, several calibration points, also with different sensors set-up, were performed, thus optimizing the time needed for reaching a steady temperature inside the tank.

The new chamber used to test fluorescence/turbidity sensors was an efficient tool allowing the simultaneous acquisition of multiple devices. Real-time raw voltage and counts measurements of the sensors under test were acquired using the ad-hoc developed software tool. Hence, several different concentrations were used in a shorter time with respect to use of a chamber hosting only one sensor each time.

The new developed tools and the facilities provided by HCMR proved to be really efficient and particularly useful for calibrating sensors before the deployment and for testing sensors after the recovery (i.e., verifying and correcting any offset or drift in the data).

No particularly difficulties have been encountered and the working programme was done as planned except for the sea trial that was not performed due to bad weather conditions at sea.

D) Dissemination of the results

The proposed project performed an operational, although in laboratory conditions, test of innovative software and hardware tools aiming at performing inter-comparison/calibration exercises of sensors for oxygen, fluorescence and turbidity measurements.

Preliminary results show some discrepancies between the calibration sheets provided by the manufacturer and the operational validation of the sensors that can be partially ascribed to different operational conditions and configurations of the sensors, but also evidence the need of a laboratory/field calibrations (especially for chlorophyll-a measurements) before deploying the instruments.

The contribution “New tools for dissolved oxygen, fluorescence and turbidity sensors testing and intercomparison” summarizing the preliminary outcomes of the project has been already accepted to the 7th EuroGOOS conference “Operational Oceanography for Sustainable Blue Growth” (Lisbon, October 28-30 2014).

A plan for producing a technical paper describing the facilities used at the hosting, the developed hardware and software tools as well as the main findings was already prepared.

E) Use of the Infrastructure/Installation

	In situ	By remote
Nr. of Users involved	2	
Access units (days/months/etc)	Days	
In situ stay day / Remote Access duration	10	

F) User project scientific field

Main field	Earth Sciences & Environment
Scientific description	Marine Science / Oceanography

H) Technical and Scientific preliminary Outcomes

The project dealt with testing of two different innovative software and hardware tools developed by CNR aiming at performing inter-comparison exercises of sensors for oxygen, fluorescence and turbidity measurements based on analytical laboratory techniques to assess known concentrations of the parameters to be measured by the tested sensors.

The assessment of these new tools have been performed through laboratory tests for intercomparing/calibrating sensors measuring dissolved oxygen, fluorescence, and turbidity.

Dissolved oxygen experiment

For dissolved oxygen sensors the according calibration routine relies on comparing lab analysis via Winkler tritation which need some experience in proper water sampling and specific knowledge about the related chemistry. A wide range of different concentration levels and different temperature values are usually reached by varying temperature and introducing oxygen into the water using bubblers.

The main objective for the inter-comparison of oxygen sensors adopting different methodologies and measuring techniques consists in testing a hardware/software tool able to simultaneously acquire up to 8 oxygen probes in the same calibration tank without the need of several multiparametric probes hosting the sensors, but simply one CT(D) instrument (e.g., SBE37sm from Seabird) to acquire temperature and salinity data to be used as reference. The tool allows for the acquisition of the raw voltage output of all sensors and the possibility to perform an inter-comparison in physical units devoted to the verification or the update of the calibration coefficients (or only the drift) provided by the manufacturer and to an evaluation of the performance of different type of oxygen sensors.

The first day of the experiment was dedicated to the instrumental set-up for dissolved oxygen experiment and preliminary tests were carried out in order to verify the assessment of sensors, the ancillary instrumentation and the software program developed to collect and visualize data in real time. The second, third and fourth days were completely devoted to the dissolved oxygen measurements.

The facility used in the experiment was composed by a tank (800x500x500 mm) with glass walls filled by filtered sea water. Homogenous mixing in the tank was obtained using an Haake N2 immersion circulator whereas two aerators were switched on to increase oxygen concentration (by introducing oxygen into the water) or turned off to facilitate low dissolved oxygen concentrations.

One CT(D) sensor (SBE37sm s/n 5372) was connected to two pairs of dissolved oxygen sensors (Sea-Bird Electronics Inc., SBE43) and a submersible pump (SBE5T) using pipes and 1/2" Y-fittings. Three sensors (s/n 0541, 2050, 2281) were provided by CNR and one by HCMR (s/n 1163). None of the used sensor was deployed after their original calibration. SBE43 s/n 2281 and s/n 2050 were flushed by a SBE5T pump whereas SBE43 s/n 541 and s/n 1163 were flushed by a SBE5P pump.

Since SBE43 is an in-line sensor it has to be flushed with the same stream of a CT-cell providing temperature and salinity measurements. Using this configuration it is expected that all four oxygen probes measure the same flux of the TC duct. No toxic tributyl-tin leaching tips, usually employed at sea for avoiding biofouling, were used at the intake and discharge of the duct.

Two Aanderaa Instruments AS Optode models 3975 (s/n 794, 1647) were used as references in real time. One Optode was positioned as close as possible to the water intake of the

dissolved oxygen duct.

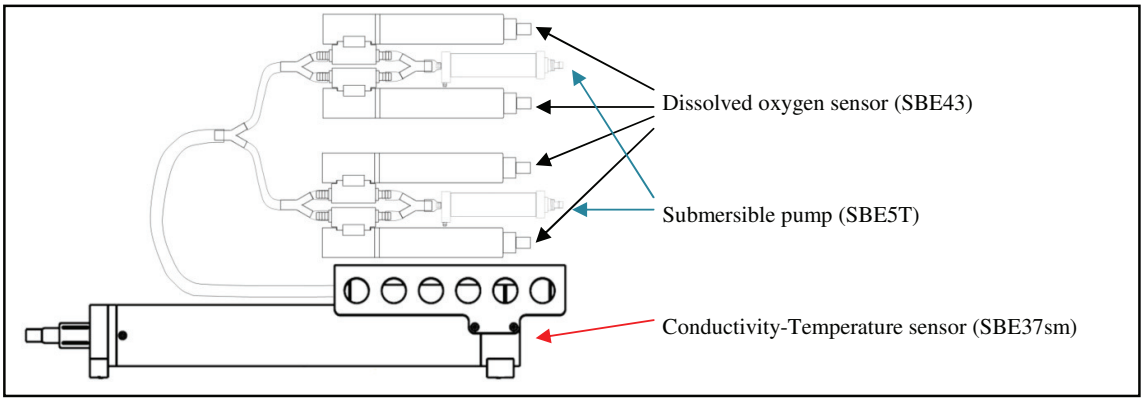

Figure 1. Sketch of the first and second used configuration with the two pairs of SBE43s and submersible pumps connected to the SBE37 outtake.

For evident practical reasons, sea water into the tank was refrigerated during the night and progressively warmed during the day to reach the desired set point in temperature. Several temperature gradients were obtained by varying oxygen concentration but only to create 100% stable mixture as monitored in real time through the Optode devices.

Reference values using Winkler tritation were collected from water samples at sufficiently stable oxygen concentration and continuously measured (in µM) with two Aanderaa Instruments AS Optode model 3975.

Simultaneous analogue voltage provided by SBE43 sensors were collected by a datalogger, processed and displayed in real-time through the software programme developed by CNR. The datalogger consists of a National Instruments board (NI-8205) having multiple channel acquisition capability with adjustable voltage ranges and an accuracy of 0.15 mV for the range 0-5 V. The detected voltage was converted into the oxygen concentration by using a modified version of the algorithm by Owens and Millard (1985).

During the whole experimental phase, temperature ranged from 15 °C to 32 °C and the salinity from 38.8 PSU to 39.2 PSU.

The SBE43 device is constituted of a polarographic membrane oxygen sensor which is an upgraded Clark cell redesigned and optimized to reduce drift and hysteresis. This sensor has an analogue output voltage signal proportional to the temperature-compensated current flow occurring when oxygen is reacted inside the membrane.

During the experiment, three different set-ups were used to evaluate the behaviours of the sensor and to minimize the gap between real-time dissolved oxygen concentration of SBE43s and reference values.

In the first configuration, two pairs of SBE43s in an almost vertical position were connected through a pipe to the SBE37 outtake; in the second configuration the same package was horizontally positioned close to the bottom of the tank, whereas in the third configuration the pipe connecting the two oxygen sensor pairs to the SBE37 was removed.

Figure 2 summarises the results showing that the usage of the pipe connecting the oxygen sensor intake to the CT(D) cell is not necessary when an homogenous mixing is reached inside the tank provided that oxygen cell is properly flushed. Indeed, the second pair (SBE43 s/n 541, 1163) measured wrong oxygen concentration in the second configuration when we measured a very low flow inside their cells.

Optode acquisitions are generally in a very good agreement with oxygen concentration determined by Winkler method whereas SBE43 data show an increasing offset as oxygen concentration increases.

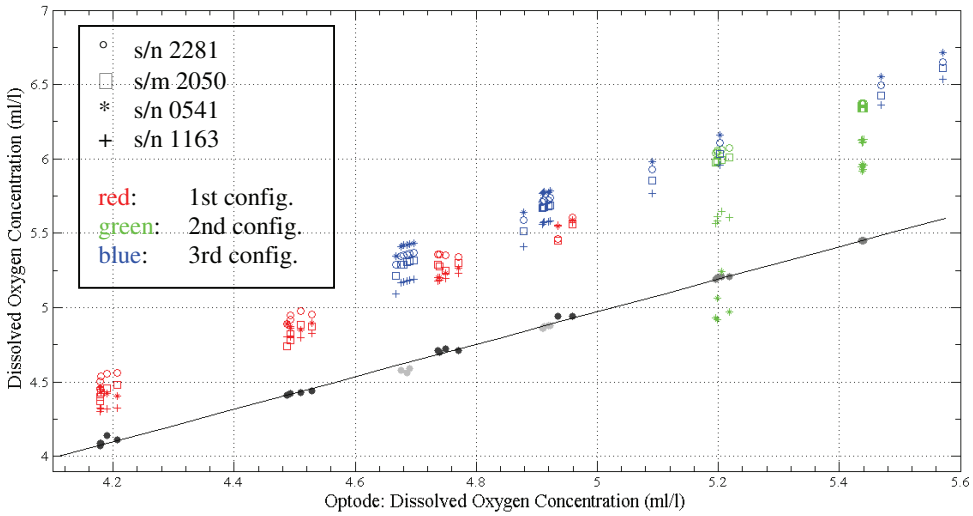

Figure 2. Dissolved oxygen concentration measured by four SBE43 sensors (circle s/n 2281, square s/n 2050, star s/n 0541, plus s/n 1163) vs. Optode 3975 (s/n 1647) concentration in the three configurations (red, green, blue). Grey dots correspond to the concentrations obtained through Winkler tritation.

Fluorescence experiment

For the fluorescence tests, a new chamber developed by CNR was used (Figure 3): it is composed by a box with an inlet and outlet from which a pipe pass through a magnetically coupled centrifugal pump (Totton model NEMP50/7) having a flow rate of 50 liters/minute. Some cable feed-through have been used in the door of the box for the cables of the 5 sensors that can be hosted inside. The hardware setup is completed by a National Instruments board (NI-8205) for collecting the raw voltage signal from the sensors and by a serial device driver for logging serial data from the same sensors. The software programme was developed for acquiring data from Wet LABS fluo/turbidimeters. This configuration allows to log in real time both the converted decimal output and the raw analogue voltage signal.

The Wet LABS Environmental Characterization Optics (ECO) combination fluorometer and turbidity sensor allows the user to measure chlorophyll fluorescence at 470 nm and turbidity at 700 nm within the same volume. The sensors used in the TOFU experiment were all same model (ECO-FLNTUS) having analogue and serial output capabilities with 4000-count range and an integrated anti-fouling bio-wiper that was removed before the test.

The fluorometer allows the user to monitor chlorophyll concentration by directly measuring the amount of chlorophyll-*a* fluorescence emission from a given sample volume of water. Chlorophyll, when excited by the presence of an external light source, absorbs light in certain regions of the visible spectrum and re-emits a small portion of this light as fluorescence at longer wavelengths. Two bright blue LEDs (centered at 455 nm and modulated at 1 kHz) provide the excitation source. A blue interference filter is used to reject the small amount of red light emitted by the LEDs. The blue light from the sources enters the water volume at an angle of approximately 55–60 degrees with respect to the end face of the unit. Fluoresced light is received by a detector positioned where the acceptance angle forms a 140-degree intersection with the source beam. A red interference filter is used to discriminate against the

scattered blue excitation light. The red fluorescence emitted is synchronously detected by a silicon photodiode.

Figure 3. The experimental set-up prepared to perform the fluorescence test. On the foreground is the chamber with the sensors inside. In the background, on the table, the power suppliers, the analogue acquisition board and the serial device driver are installed.

Operationally, when deployed at both E1M3A and W1M3A sites, output voltage from ECO-FLNTUS sensors is provided as an input voltage to the SBE CTD and included in the raw data string retrieved by the multiparametric probe. The analogue voltage is converted to a fluorometry value (as µg/l chlorophyll-a) using a factory scale factor determined from a Dark Count and a Chlorophyll Equivalent Concentration (CEC).

The relationship between fluorescence and chlorophyll-*a* is highly variable, and is not easy to determine in the laboratory. Species distribution, ambient light level, and health of the stock are just some of the factors that affect the relationship in addition to condition of the plankton population, highly variable with season and geographic location. Hence, accurate measurements of chlorophyll-*a* concentration with a fluorometer can be obtained using regular and periodical calibrations on seawater samples with concentrations of plankton populations equal, or at least similar, to what is expected in situ.

The first test was carried out using reference concentrations of chlorella culture. Chlorella is a genius of single-cell green algae belonging to the phylum Chlorophyta containing the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. A solution with a high concentration of Chlorella was prepared by the Institute of Aquaculture of HCMR.

The chamber was initially filled with 28 liters of ultrapure distilled water and a blank reading was acquired; afterwards, different volumes of Chlorella solutions were progressively poured into the chamber in order to get several different increasing chlorophyll concentration standards. Table 1 reports the steps of the described process. Three samples of water were collected for laboratory analysis through chemical analysis and high-performance liquid chromatography (HPLC) in order to identify and quantify each component of the mixture.

Step	Volume (ml)	Added Volume (ml)	Subtracted Volume for Laboratory Analysis (ml)	Average Replicate Values (st. dev.) from HPLC Analysis (µg/l)	Average Replicate Values (st. dev.) from Chemical Analysis (µg/l)
1	28000.0	0.0	0.0		
2	28001.0	1.0	0.0		
3	28002.0	1.0	3000.0	0.6735 (0.0312)	0.6980 (0.0558)
4	25003.0	1.0	0.0		
5	25004.0	1.0	0.0		
6	25005.0	1.0	2000.0	1.6660 (0.142)	1.5200 (0.0252)
7	23005.8	0.8	0.0		
8	23006.6	0.8	0.0		
9	23007.6	1.0	0.0		
10	23009.6	2.0	0.0		
11	23011.6	2.0	2000.0	4.4303 (0.1958)	3.9720 (0.5875)
12	23014.6	3.0	0.0		
13	23019.0	4.4	0.0		
14	23025.0	6.0	0.0		
15	23035.0	10.0	0.0		
16	23055.0	20.0	0.0		
17	23095.0	40.0	0.0		

Table 1. Steps applied Volumes of 500 NTU Formazine primary standard added to the chamber and the corresponding expected turbidity concentration.

Figure 4 shows the scatter plots and the linear fit between the reference concentration of chlorella determined through the HPLC analysis and the analogue voltage output of the three sensors. Two interpolated curves (for sensors s/n 2776 and s/n 615) are almost superimposed whereas the other (referring to sensor s/n 615) has a larger slope since the first two have a narrow range of 0-25 µg/l whereas the latter has a full scale range of 50 µg/l.

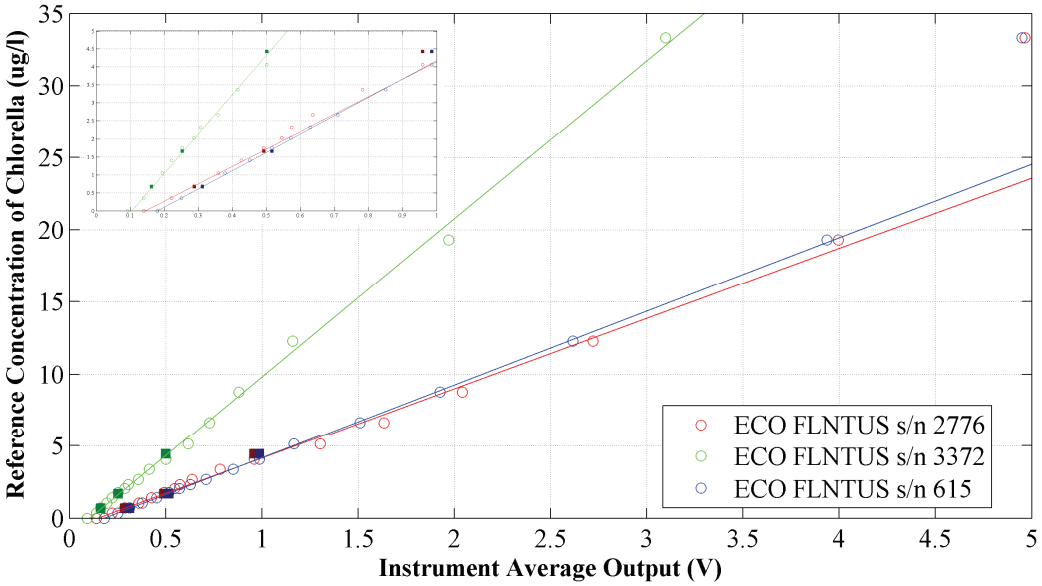

Figure 4. Reference concentration of chlorella as raw analogue voltage output of the three fluorometers varies. In the upper left corner, a zoom focuses on voltage output lower than 1 Vdc corresponding to concentration of chlorella lower than 5 µg/l. Filled squares correspond to the concentrations measured through the HPLC analysis.

Table 2 includes the nominal (extracted from the datasheet) and the new computed scale factor and dark count coefficients with the correlation coefficient between the analogue voltage data and the reference concentration of chlorella.

	Manufacturer Coefficients		New Calibration Coefficients		
ECO FLNTUS s/n	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Corr. Coef.**
2776	4.0	0.074	4.8571	0.1459	0.9982
3372	10.0	0.066	10.9583	0.1054	0.9989
615	4.0	0.104	5.0940	0.1808	0.9999

Table 2. Original coefficients from the datasheets and the new calibration values.

Having established the zero point (dark count) and the scale factor for each instrument, the conversion of analogue volts to chlorophyll concentration can be carried out using the equation:

$[Chl]_{sample} = (V_{output} - V_{dc}) * SF$

being

$[Chl]_{sample}$ the concentration of a chlorophyll sample of interest (µg/l), V_{output} the raw analogue voltage value when measuring a sample of interest , V_{dc} the blank value (the measured signal output of the instrument in clean water), and SF the multiplier in µg/l/volts.

After the first test with chlorella, the chamber and all parts (pipes, pump, etc.) were accurately cleaned with a diluted soft soap solution and dried in order to be ready on the next day to carry out a second test using a solution of disodium salt form of fluorescein (Na-fluorescein, also known as uranine).

Fluorescein is a synthetic organic compound slightly soluble in water and alcohol widely used as a fluorescent tracer in many applications. The produced fluorescence is very intense with a peak excitation at 494 nm and a peak emission at 521 nm. With respect to chlorella, the outstanding advantage is that fluorescein can be easily bought by any chemical supplier and the preparation of the solution is relatively easy and the initial standard is stable for an enough long time to perform the experiment.

Starting from a solution having a high concentration (20 mg/l), different volumes were progressively poured into the chamber in order to get different increasing fluorescence concentrations (Figure 5).

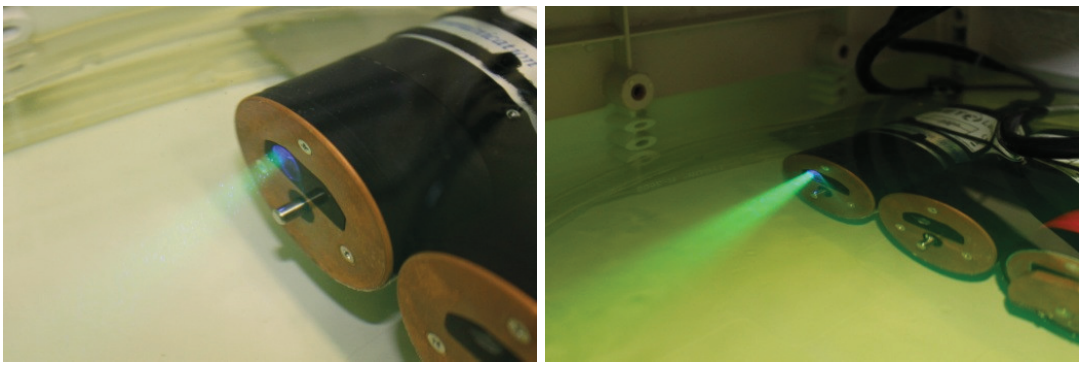

Figure 5. The beam of the fluorometer with a fluorescein concentration of (left) 75 µg/l and (right) 300 µg/l.

Figure 6 shows the scatter plots between the reference concentration of fluorescein as the raw

output voltage from the fluorometers varies. Voltage values in the horizontal axis are 1 minute averages acquired sequentially one sensor after the other when solution inside the chamber is supposed to be homogenous. Trend is linear throughout the scale although at very low concentrations the variability of the output voltage signals is higher.

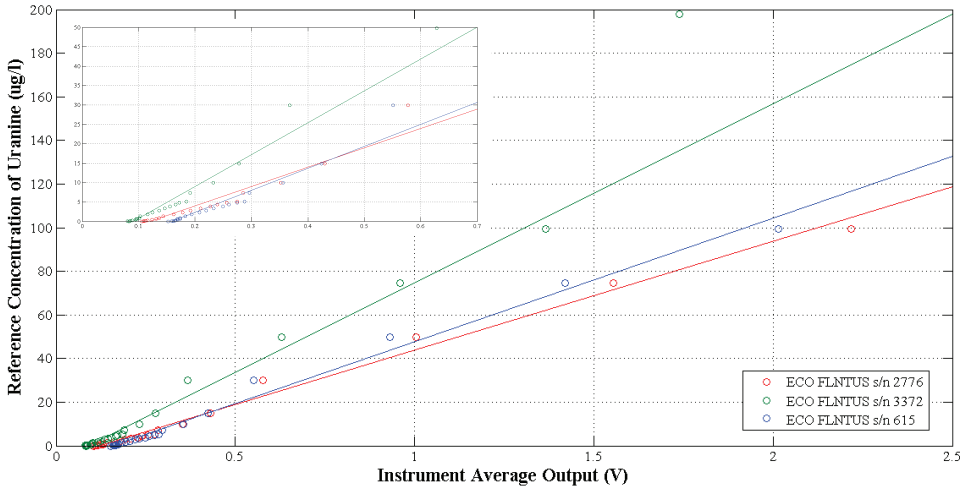

Figure 6. Reference concentration of uranine as raw analogue voltage output of the three fluorometers varies. In the upper left corner, a zoom focuses on voltage output lower than 0.7 Vdc corresponding to concentration of uranine lower than 50 µg/l.

Table 3 includes the nominal (extracted from the datasheet) and the new computed scale factor and dark count coefficients with the correlation coefficient between the analogue voltage data and the reference concentration of uranine. With respect to those reported in the datasheet or computed using chlorella concentrations, scale factor is about one order magnitude higher.

	Manufacturer Coefficients		New Calibration Coefficients		
ECO FLNTUS s/n	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Corr. Coef.**
2776	4.0	0.074	49.8431	0.1202	0.9953
3372	10.0	0.066	82.0756	0.0905	0.9952
615	4.0	0.104	56.6366	0.1591	0.9950

Table 3. Original coefficients from the datasheets and the new calibration values obtained working with different concentration of fluorescein.

The test carried out using fluorescein provided results having the same very good linearity (concentration vs. instrument voltage) of the chlorella test. However, similar raw output voltage values were obtained with a lower concentration of chlorella (in terms of µg/l) with respect to the fluorescein concentration. Fluorescein is a derivate chemical product and its fluorescence characteristic differ from real culture.

Both calibration exercises showed a high variability of the acquired raw voltage signal for all instruments (Figure 7). The recorded noise level (considered as 1 minute raw output voltage standard deviation) was lower during the fluorescein test and this can be explained considering the fluorescein solution more stable and well mixed with respect to different

chlorella solutions. However, in both cases, instrument noise is higher (one order magnitude) with respect to the one declared in the datasheet (0.8 mV), but this can be due to the testing conditions that were specifically adopted to match the operational condition of use of the sensors when deployed at sea.

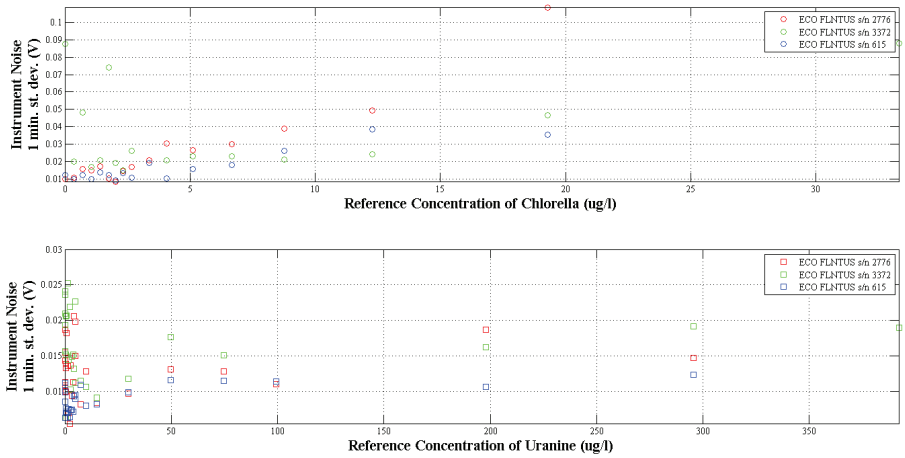

Figure 7. Instrument noise considered as 1 minute standard deviation of the raw output voltage for (top) chlorella and (bottom) uranine tests as concentration of the two different solutions varies.

Fluorescence and pH

Another test was performed to qualitatively evaluate the dependence of fluorescence measurements using an initial stable and known concentration of disodium salt form of fluorescein (Na-fluorescein, also known as uranine) with respect to pH variations. For this test another experimental setup was prepared without using any chamber (Figure 9): the three fluorometers were packed together and suspended into a 10 liter beaker. A submersible pump was put at the side of the package to ensure an homogenous mixing of the water volume: it was switched on to mix the water volume and off during the data acquisition. Starting volume of deionized water was added by few tenth of milliliter of Hydrochloric acid (HCl) solution 0.5 M to make it more acid or by few tenth of Sodium hydroxide (NaOH) solution 0.5 M to make it more alkaline. pH in the beaker was monitored using a Latch HQ40d portable meter.

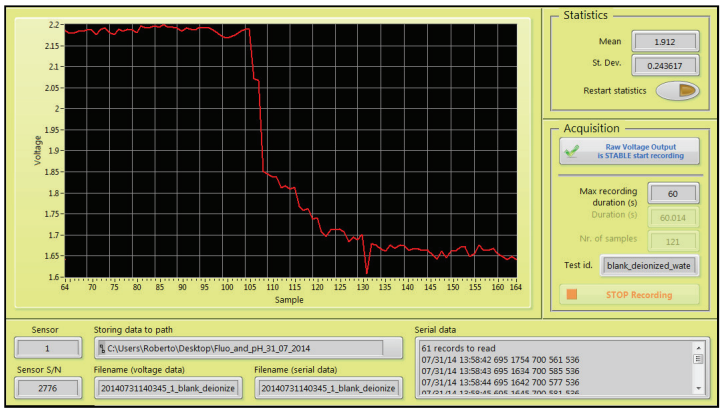

Figure 8. User interface of the programme acquiring and storing real time data from a fluorometer showing the variation of the voltage signal in response to the dilution of 1 ml HCl 0.5 M into the beaker.

The same programme used for the fluorescence experiment was used to collect raw data in real time from the ECO-FLNTUS sensors. Figure 8 shows the rapid drop in the voltage signal of sensor s/n 2776 when 1 ml of HCl 0.5 M was added into the beaker making pH decreasing from 4.7 down to 4.4.

Figure 9 shows the obtained curve with all collected data, specifically the 1 minute raw averaged voltage data as pH varies. All curves show a non-linear behavior, especially for acid solution and in particular just below pH 7: nonetheless, also for pH around the normal seawater values (pH 8±0.5), the curves are not flat and a negative slope of -0.1725, -0.1068, -0.1762 V/pH characterizes the trend of the three sensors in the range 7.32 – 8.27 of pH.

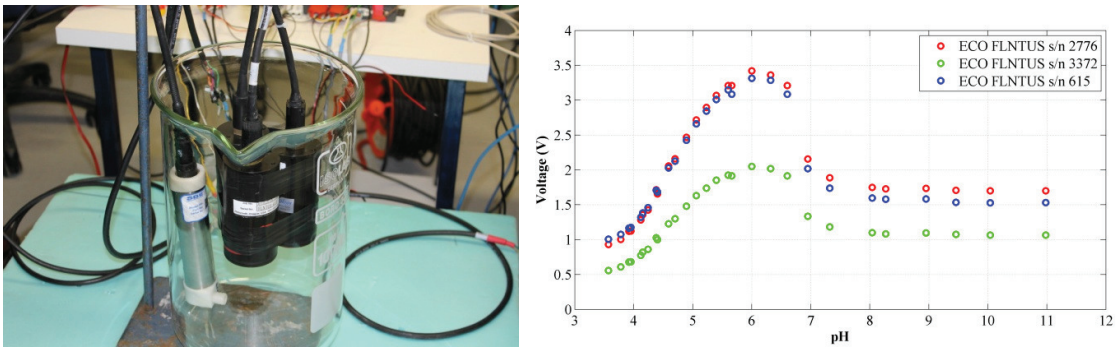

Figure 9. (left) The three ECO FLNTUS sensors forming a unique package at the center of a 10 liter beaker with a submersible pump for mixing water after every dilution of acid or alkaline solutions. (right) Measured averaged output voltage from the three sensors as the pH varies.

Turbidity experiment

Turbidity can be defined as the phenomena where a specific portion of a light beam passing through a liquid medium is deflected from undissolved particles. The deflection is generally a function of the size and shape of the particles. This is the reason why although the factory-supplied calibration coefficient can be used to obtain approximate values, field calibration is highly recommended. Moreover, the relationship between turbidity and nephelometric turbidity unit (NTU) is highly variable, and is not easy to determine in the laboratory.

In general, performing calibrations on seawater samples with distributions of particles that are similar to what is expected in situ and, successively, making periodical in-situ measurements of comparison is the best way to have guarantee of good measures.

Alternatively, the method followed in this experiment can be used consisting in checking sensor performance by exposing it Formazin with several concentration targets achieved by sequential additions of known concentrations of particle stock solution.

The same ECO FLTNUS sensors used for the chlorophyll-a experiment were employed also for the turbidity test. The sensor detects back-scatter across an inferred range of 0-10 (or 25) Nephelometric Turbidity Unit (NTU) at a wavelength of 700 nm. Calibration constants are used to convert the voltage to NTU using a scale factor calculated from a Dark Count and a known Formazine concentration standard. All these coefficients are the factory calibrated values shipped with each of the ECO-FLNTU units, but localized determination of these values is highly recommended to enable a more “realistic” determination of relative chl-a concentrations and turbidity from the sensor voltage outputs at each observatory site.

Turbidity is measured simultaneously to fluorescence by detecting the scattered light from a 700 nm LED at 140 degrees to the same detector used for fluorescence. The measured volume

of water is at few centimeters far from the sensor. The turbidity measurement is performed at the same 140 degree angle as the chlorophyll fluorescence.

Also the same chamber was used: it was filled with 20 liter of nanopure deionized water. A preliminary blank measurement to determine the so-called dark count provided extremely high voltage values probably due to multiple reflections from the walls of the chamber. Thus, a piece of black rubber was inserted into the chamber to suppress these reflections. Then, other blank measurements were performed providing more consistent values although greater with respect to the one in the instruments datasheets. The tests were conducted at a room temperature of 22 °C controlled by air-conditioning.

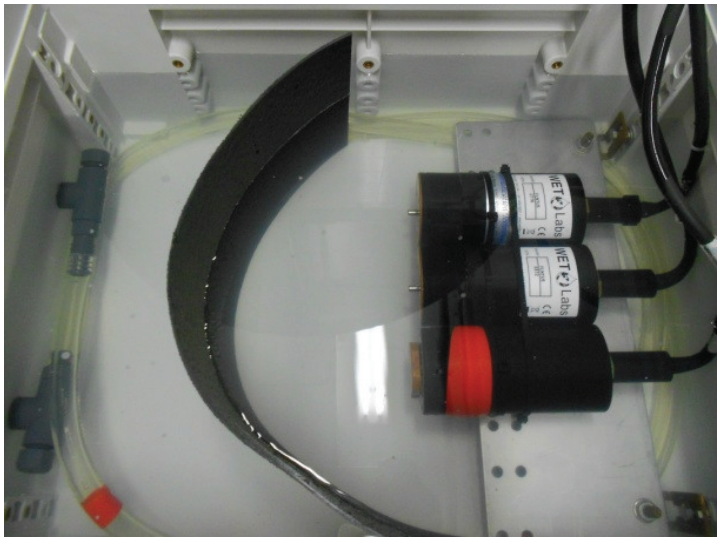

Figure 10. The three ECO FLNTUS sensors (s/n 2776, s/n 3372, s/n 615) in the chamber during the blank measurements. The bent rubber sheet used to suppress multiple reflections from the walls of the box is visible.

Table 4 includes the "blank" analogue values recorded by the three sensors (s/n 2776, s/n 3372, s/n 615) in different testing conditions. The obtained values are considerably greater than those reported as the so-called "analogue dark count" in the datasheet (0.062, 0,055, 0,058 V, respectively), but the latter were obtained by covering the detector with a black tape as suggested by WET Lab. The last configuration used (black shield / pump off / chamber close) provided the most stable results to be considered as the blank values and this configuration was chosen for performing the tests. In particular, closing the door of the chamber assured to have a totally dark measuring environment inside the chamber, although it was already assessed that down-welling irradiance hadn't a significant impact on instrument response.

TESTING CONDITIONS	ECO FLNTUS Anal. output (V) s/n 2776	ECO FLNTUS Anal. output (V) s/n 3372	ECO FLNTUS Anal. output (V) s/n 615
Pump on / Chamber open	1.710224	0.445138	0.873437
Black shield / Pump on / Chamber open	0.308619	0.125720	0.219982
Black shield / Pump off / Chamber open	0.279515	0.199138	0.246755
Black shield / Pump off / Chamber close	0.292308	0.145022	0.239440

Table 4. Analogue values for the blank correction recorded by the three ECO FLNTUS sensors (s/n 2776, s/n 3372, s/n 615) using different testing setups.

Different volumes of a 0,5 liter Formazine primary standard at 500 NTU were progressively poured into the chamber in order to get a turbidity standard (Table 5), a liquid sample with a defined and reproducible turbidity.

Step	Added Volume (ml)	Turdibity (NTU)
1	80	1.992
2	80	3.968
3	80	5.929
4	80	7.874
5	80	9.804
6	50	11.002
7	45	12.076

Table 5. Volumes of 500 NTU Formazine primary standard added to the chamber and the corresponding expected turbidity concentration.

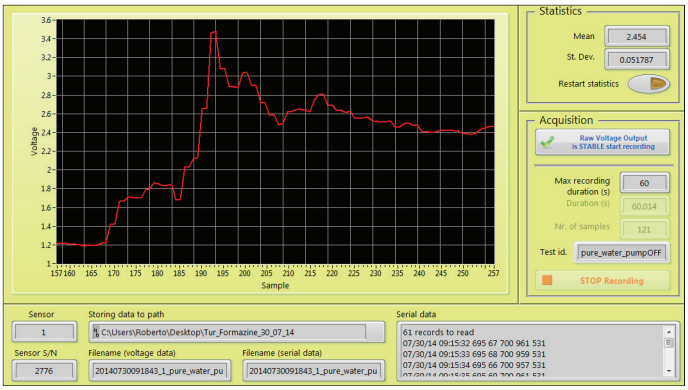

Figure 11. Snapshot of the user interface of the programme managing the acquisition of analogue and serial data from a ECO FLNTUS sensor. The plot shows the increase of the voltage signal of the s/n 2776 sensor corresponding to the addition of 80 ml of Formazine at 500 NTU into the chamber.

Each time the pumped circuit was activated for 30 seconds to mix the water inside the chamber. Then, analogue voltage outputs were acquired sequentially from each sensor for 1 minute at a sampling rate of 2 Hz. At the same time, the instrument stored raw data in the internal memory whose content is retrieved and cancelled through a serial link.

As an example, Table 6 shows the raw data collected from ECO FLNTUS s/n 2776 at two different Formazine concentrations, 5.929 and 12.076 NTU, respectively.

Ref. Turbidity	Date (mm/dd/yy)	Time (hh:mm:ss)	Chl Ref	Chl Signal	NTU Ref	NTU Signal	Thermistor
5.929	07/30/14	09:42:23	695	54	700	2454	526
	07/30/14	09:42:24	695	58	700	2451	526
	07/30/14	09:42:25	695	55	700	2455	526
	07/30/14	09:42:26	695	56	700	2461	526
	07/30/14	09:42:27	695	51	700	2455	526
12.076	07/30/14	10:43:11	695	60	700	4130	526
	07/30/14	10:43:12	695	60	700	4130	526
	07/30/14	10:43:13	695	63	700	4130	526
	07/30/14	10:43:14	695	63	700	4130	526
	07/30/14	10:43:15	695	60	700	4130	526

Table 6. Raw data acquired by ECO FLNTUS s/n 2776 at two different turbidity concentrations.

Collected data were used to verify the scale factor calculated by obtaining a consistent output from a solution with a known concentration, then subtracting the meter's dark counts (blank). The scale factor is applied to the output signal to provide the direct conversion of the output signal to turbidity concentration through the following equation:

$$[NTU]_{sample} = (V_{output} - V_{dc}) \cdot SF$$

where

$[NTU]_{sample}$ is the level of turbidity (NTU), V_{output} is output when measuring a sample of interest, V_{dc} is the blank value (the measured signal output in clean water), and SF is scale

factor multiplier in NTU/volts.

The collected average analogue data are shown in Figure 12 with respect to the known turbidity concentrations obtained by progressively adding volumes from a Formazine primary standard. Sensors s/n 2776 and s/n 615 have a full scale range of 10 NTU and this explains the last two saturated values for reference concentrations greater than 10 NTU. Black lines correspond to the theoretical linear scales of the sensors.

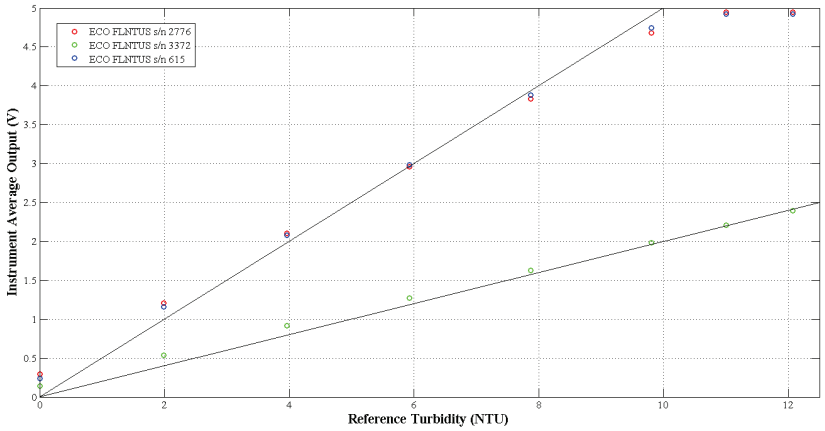

Figure 12. Average analogue data with respect to known turbidity concentrations obtained by progressively adding volumes from a Formazine primary standard to the measuring chamber filled with deionized water.

The instruments response over the range 0 - 10 NTU was modeled by linear regression and the results are included in Table 7 compared with the coefficients included in the instrument datasheets.

	Manufacturer Coefficients		New Calibration Coefficients		
ECO FLNTUS s/n	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Scale Factor (NTU/V)**	**Dark Count (V)**	**Corr. Coef.**
2776	2.0	0.062	2.2362	0.30875	9.9997e-001
3372	5.0	0.055	5.3815	0.16214	9.9991e-001
615	2.0	0.058	2.1714	0.24457	9.9998e-001

Table 7. Original coefficients from the datasheets and the new calibration values.

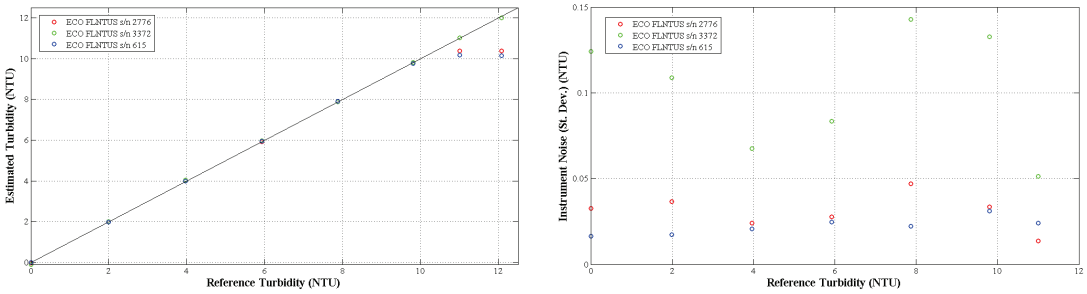

Figure 13. WET Labs turbidimeters calibrated points and instrument noise in response to increasing turbidity derived from Formazine added to nanopure deionized water: (left) 1-minute average calibrated points in response to sequential additions of known Formazine concentrations; (right) instrument noise (standard deviation) as reference turbidity concentration varies.

Figure 13 shows the calibrated signals of the three turbidimeters and the standard deviations (in NTU) of the time series used to compute the average analogue values per each calibration points. Sensors (s/n 2776, s/n 615) having a narrow scale had a saturated signals and showed a lesser instrumental noise (always < 0.05 NTU throughout the entire scale).

The use of the new calculated calibration coefficients provides a better match between reference and measured turbidity values (Figure 14) with deviations always within ±0.1 NTU for all three sensors and with no dependence on turbidity value. Indeed, the use of manufacturer coefficients introduces a linear trend with positive differences as turbidity increases.

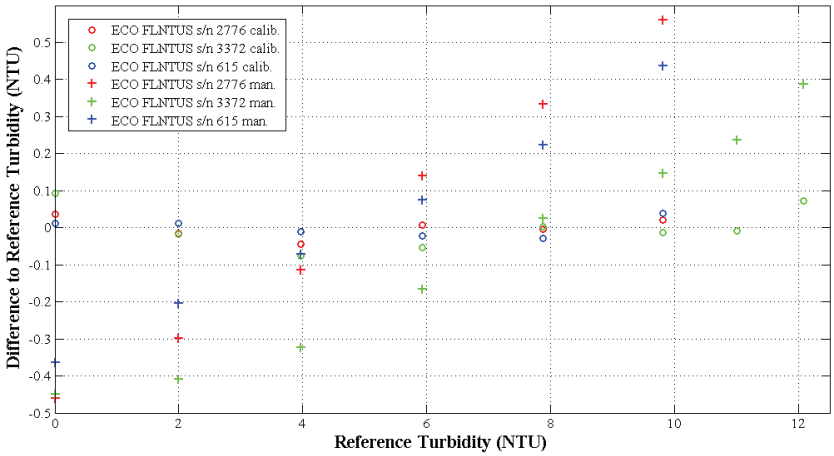

Figure 14. Difference between reference and estimated turbidity values as reference turbidity varies. Circles correspond to estimated turbidity values obtained with new calibration coefficients ("calib.") whereas crosses indicate estimated values obtained using coefficients in the sensor datasheets ("man.").

Results demonstrated the usefulness of the developed calibration tool including the software programme, able to dynamically configure each sensor and to log simultaneously analogue and serial data, and that the new designed chamber capable of providing a perfectly mixed volume of water in few seconds guarantees very stable and repeatable measurements.