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 PRELIMINARY REPORT

1st Call of Proposals
12 January – 3 April, 2012

A) General Information

Proposal reference number	CALL_1_12/1210185
Project Acronym (ID)	CIEBIO
Title of the project	Calibration and inter-calibration exercise of bio-geochemical sensors
Host Research Infrastructure	Hellenic Centre for Marine Research – Calibration Laboratory
Starting date - End date	26.11.2012 - 30.11.2012
Name of Principal Investigator	Roberto Bozzano
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B) Project objectives (max. 250 words)

The experiment addresses the main scope of performing a calibration and inter-calibration exercise of bio-geochemical sensors to be operationally and routinely deployed on off-shore marine observatories making part on a continuous basis of the marine monitoring network of the Mediterranean Sea.

In particular, the first objective consists in enhancing the accuracy of the in-situ observations on a long term basis of dissolved oxygen, chlorophyll-a and turbidity in the Ligurian basin collected by a multiparametric probes installed on the W1-M3A offshore observing system, constituted by the "ODAS Italia 1" spar buoy and by a close subsurface mooring.

The opportunity to install carefully calibrated probes will improve the knowledge about the biogeochemical processes in the upper thermocline and can support with real-time quality controlled observations the developing biogeochemical forecast models for both the phases of assimilation and calibration/validation.

The W1-M3A observatory, together with the E1-M3A buoy moored in the south Aegean Sea and the E2-M3A buoy positioned in the South Adriatic, is part of the M3A network, developed within the framework of the MFSTEP project in order to answer to the needs of the Mediterranean Forecasting System of real-time physical and biogeochemical observations of the upper thermocline.

Indeed, the possibility to use sensors calibrated with the same procedures installed on the different sites belonging to the M3A network makes feasible a comparison between the involved sites thanks to an homogenous database in order to verify at a quantitative level the observed differences and to enhance the quality of the in-situ observations.

C) Main achievements and difficulties encountered

The experiment allowed to obtain a calibration in laboratory and at sea for oxygen and fluorescence sensors. More in details in laboratory, the calibration of oxygen probes has been carried out in a tank (800x500x500 mm) furnished by an Haake N2 immersion circulator and two aerators. Two SBE43 oxygen sensors were tested together and Winkler chemical titration served as the reference standard for evaluating performance characteristics. Five calibration points (at 14°C – 17.7 °C – 20.2 °C) have been chosen and three samples for each point have been used for the Winkler analysis. During the one day cruise onboard the R/V Philia three water samples were acquired for the evaluation of both oxygen and chl-a parameters. The results show an underestimation of oxygen probes respect to Winkler samples with an average difference of about 0.43 ml/l for the laboratory test and 0.49 ml/l for the samples taken at sea. The chl-a calibration were performed by means of two reference of chlorella culture and eight concentration points of uranine solution in laboratory and with water samples at sea. The results show a good agreement between tests in laboratory and in field and allowed to calculate a new scale factor for the analyzed sensors. The need of very steady temperature for the oxygen calibration and of accurate reference concentration for chl-a tests extended the schedule of the experiment and didn't allow the calibration of turbidity sensors that has been postponed and has been performed only by HCMR team.

D) Dissemination of the results

The "Calibration and inter-calibration exercise of bio-geochemical sensors project" in the framework of Jerico TNA allows to perform a calibration of oxygen, chlorophyll-a and turbidity sensors both in laboratory and at sea. The achieved results show several discrepancies between the calibration sheet provided by the manufacturer and the in-situ validation and evidence the need of a field calibration especially for chlorophyll-a and turbidity measurement before the deploying of the instruments. The finalization of a technical report describing the methodologies and the tests performed during the experiment is foreseen by all the involved teams and an abstract containing the description of the M3A network and the results of the experiment has been submitted to OCEANS'13 MTS/IEEE conference.

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	5	

F) User project scientific field

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

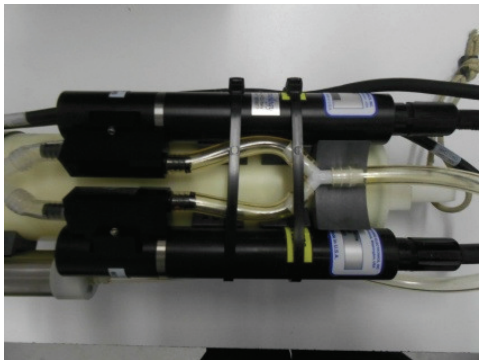
H) Technical and Scientific preliminary Outcomes

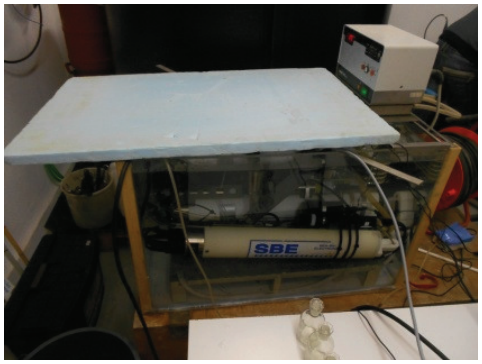
The performed experiment was based on analytical laboratory techniques to assess known concentrations of the parameters to be measured by the tested sensors. The first day was dedicated to the instrumental set-up for the oxygen calibration and preliminary tests were carried out in order to verify the assessment of sensors and ancillary instrumentation.

The second and the third days were completely devoted to the oxygen experiment: two SBE16plus pumped CTD equipped with SBE43 oxygen sensors, one Optode and one multiparametric portable

probe by Hanna Instruments were deployed in a tank (800x500x500 mm) furnished by an Haake N2 immersion circulator and two aerators. One CTD system was provide by HCMR and the other from CNR. The Aanderaa Instruments AS Optode model 3975 was used as a reference for the real-time data acquired by three Sea-Bird Electronics, Inc., model SBE43 dissolved oxygen sensors.

The two SBE43 oxygen sensors from CNR were tested together connecting the SBE43 s/n 2050 to the auxiliary output 0,1 of the SBE16 plus s/n 4489 and the SBE43 s/n 2281 to the auxiliary output 2,3 of the SBE16 plus s/n 4489, respectively. One pump SBE 5T configured with a delay before sampling of 15 seconds have been used for the experiment. The two oxygen probes were measuring the same flux by means of an Y tube connector that allows the flowing of the water into the sensors.





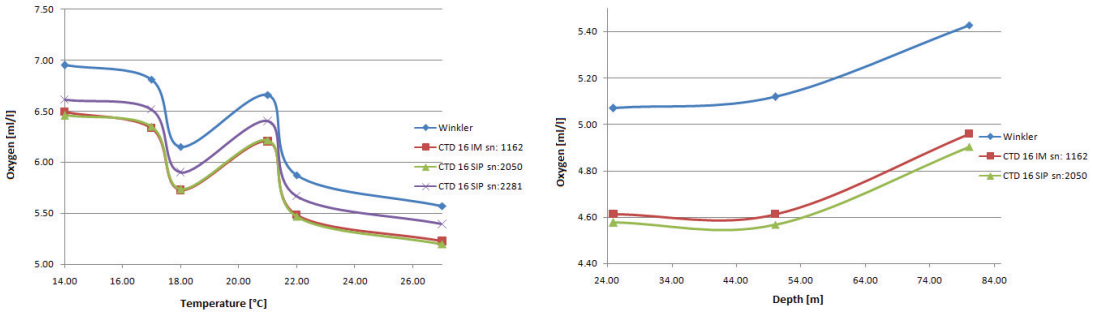
The two SBE43 installed on the SBE16 ready for the oxygen calibration and the experimental set-up.

The experimental protocol consisted in an evaluation of the performance across a range of water in controlled laboratory conditions. Winkler chemical tritation samples for dissolved oxygen were used as the reference standard for evaluating performance characteristics. The temperature and the oxygen concentration in the water tank (in µM) was constantly monitored by the Aanderaa Optode sensor. Once the temperature reached the desired value and the dissolved oxygen showed a good stability, the simultaneous acquisitions of the two SBE43 sensors by CNR and the SBE43 by HCMR started.

Five calibration points (at 14°C – 17.7 °C – 20.2 °C) have been chosen and three samples for each point have been used for the Winkler analysis.

During the one day cruise onboard the R/V Philia, carried out the last day of the experiment, it was possible to validate the laboratory calibration by means of the acquisition of water samples at 20 m, 50 m and 80 m depth that were analyzed following the Winkler tritation method.

The results show an overestimation of the values obtained by using the Winkler tritation method with respect to the ones obtained by the sensors for both the laboratory tests and the in-situ samples.



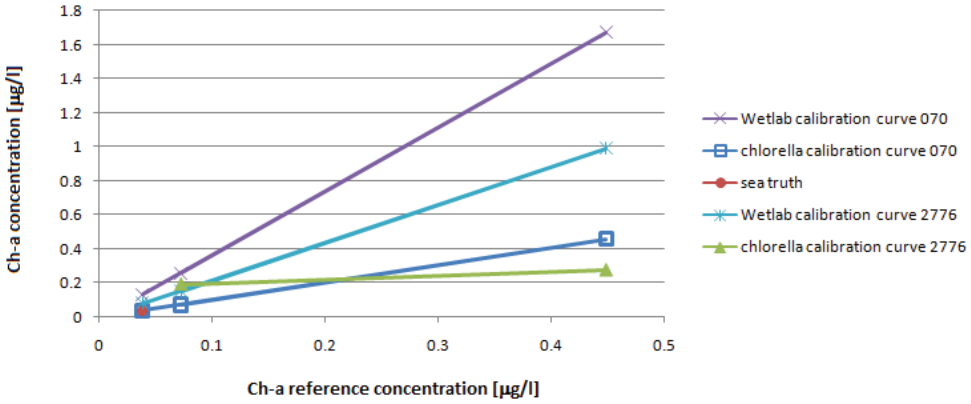
Oxygen calibration: laboratory (left) and field (right) test

Two separate experimental sessions were performed for the fluorescence sensors: the first one was carried out on the fourth day, and it was based on the calibration by means of two reference concentration of chlorella culture of respectively 0.072 µg/l and 0.448 µg/l since we were interested to a minimum and a maximum point of calibration for the Ligurian seawater. The second session lasted all the fifth day in the meantime of one day cruise onboard the R/V Philia thus the calibration was made only on the sensors not used during the cruise and several reference concentration of uranine solution were considered.

The first experiment allowed to obtain a new calibration curve for the sensors and a new scale factor that is about 3 or 5 times lower than the value from the characterization datasheet of the sensor, whereas the blank values are very similar to the ones provided by the manufacturer.

The calibration made by means of uranine solution shows a very good linearity, but high values if we used the obtained curve to compare the results with the in-situ data, and this behavior is probably due to the fact the uranine is a derivate product and its fluorescence characteristic differ from real culture.

The new curve were validated by a direct comparison with the in-situ data acquired during the one-day cruise onboard the R/V Philia and show good preliminary results. The three water samples acquired at 20, 50 and 80 m depth had the same chl-a concentration, thus only one reference point at sea has been evaluated.



Fluorescence calibration: calibration curves and validation at sea

Both the calibration exercises show a high variability of the acquired signal for all the analyzed instruments, thus, in order to obtain signals as stable as possible, great accuracy in the experimental setup coupled with long periods of acquisition are necessary to obtain good results.

The need to spend more time than expected for the chl-a calibration extended the schedule and was not possible to perform the turbidity calibration that was postponed and performed several weeks by the HCMR team.

During the preparation for the turbidity calibration experiment, unfortunately, the HCMR team noticed that the rotor of the fluorometer s/n FLNTUS 070 immediately after the power up started rotating without stopping and through a deeper analysis they found water inside the sensor. The sensor was used during the on-day cruise on board R/V Philia and probably during the ascending phase of the cast or the washing procedures water flowed inside the sensor causing irreparable damage to the electronics.

The turbidity experiment was performed only in laboratory for blank and three points of reference solution based on Turbidity 50 NTU Calibration Standard by Fluka diluted in dionized water producing the concentrations of 2.5, 1.25 and 0.625 NTU. For the blank measurements the method suggested by WetLab was used covering both the LED and detector whereas the lower dark values was recorded with black tape only on the detector, and the sensor inside deionized water.

The turbidity calibration set-up.

The results show blank values very close to those provided by the WetLab characterization sheet for all the analyzed instruments (sn 2776, 594, 616, 625, 649, 653), a linear response and also a very good agreement with the scale factor provided by WetLab calibration.
The performed exercises point out that:

- the calibration results obtained using reference standard are in complete agreement with those provided by the manufacturer, thus the proposed methodology can be considered valid and accurate;
- the calibration results obtained using in-situ observations didn't show a proper match with those provided by the manufacturer underlying the need to perform an in-situ calibration especially for chl-a measurements because the fluorescence signal emitted by real culture is very different from those produced by derivate product.