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

2nd Call of Proposals
14 January – 27 March, 2013

A) General Information

Proposal reference number(1)	CALL_2_4
Project Acronym (ID) (2)	FITO MicroLFA
Title of the project(3)	Field Test Of MicroLFA nutrients monitoring device for Ferrybox systems
Host Research Infrastructure(4)	COSYNA_1 and COSYNA_2
Starting date - End date(5)	COSYNA_1 (Ferrybox Lysbris): From 16 July 2014 to 25 September 2014 (this is the period the SYSTEA µLFA NH_3 and PO_4 units were installed on Lysbris) COSYNA_2 (Cuxhaven station site): From 9 May 2014 to 4 July 2014 (this is the period the SYSTEA µLFA NH_3 and PO_4 units were installed in Cuxhaven) An additional µLFA PO_4 unit was installed again in Cuxhaven station from 6 August to 22 September 2014.
Name of Principal Investigator(6) **Home Laboratory** **E-mail address** **Telephone**	Dr. Luca Sanfilippo SYSTEA S.p.a., Via Paduni, 2A 03012 Anagni (FR), Italy luca.sanfilippo@systea.it +39-0775-776058
Additional users (7)	Enrico Savino, SYSTEA S.p.a

B) Project objectives (max. 250 words) (8)

The proposed TNA project was aiming to test in operative conditions a new line of product specifically developed by SYSTEA S.p.A. to be extensively used in Ferrybox systems for unattended nutrients monitoring in sea and surface water.

The proposed field tests were performed in the facilities of Institute of Coastal Research / KOI of Helmholz Zentrum Geesthacht (HZG), partner of Jerico project.

Two kind of field tests were performed:

- a first field test was performed in the Cuxhaven fixed monitoring station at the Elbe river mouth, to measure PO_4 and NH_3
- a second field test was performed in the Ferrybox Lysbris managed by HZG, in operation on a regular route along North Sea.

Two independent analytical modules to measure PO_4 and NH_3 were provided and integrated in the existing system layout and local control unit on both sites; a third unit to measure PO_4 was dispatched, to change the first one for the second experiment on board of Lysbris. The first PO_4 unit module was later installed again in Cuxhaven station.

On both sites a comparison between existing instruments manufactured by SYSTEA and in use from several years by HZG were performed too.

SYSTEA provided the microLFA units already prepared to be installed and operated unattended.

HZG allowed SYSTEA to install those units on both sides and provided the technical support during the field experiments.

C) Main achievements and difficulties encountered (max. 250 words) [9]

Several weeks of unattended measurement on both NH_3 and PO_4 parameters were collected in both sites; the data results were elaborated by HZG and technically commented.

The installation and operation inside the Ferrybox Lysbris was difficult to be performed, due to the fact of limited space available, but the compactness of the units to be tested allowed the integration and use on the running system.

D) Dissemination of the results [10]

An oral presentation with slides was performed by the SYSTEA technician in charge E. Savino during the last Ferrybox meeting in Tallin on 8-9 September 2014.

A slide presentation describing the experiment results is going to be done by L. Sanfilippo on the Jerico Science day on 28-29 April 2015.

E) Use of the Infrastructure/Installation [11]

	In situ	By remote
Nr. of Users involved	1	1
Access units (days/months/etc)	day	day
In situ stay day / Remote Access duration	Lysbris: 7 Cuxhaven: 5	Lysbris: 71 Cuxhaven: 56 + 47 [*]

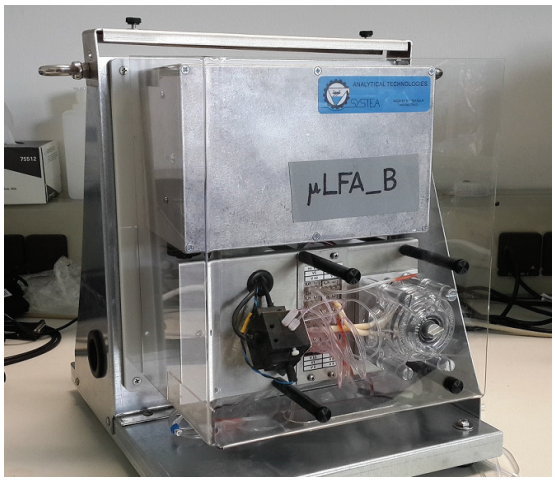
(*) µLFA PO_4 only

F) User project scientific field

Main field [12]	Earth Sciences & Environment
Scientific description [13]	Marine Science/Oceanography

H) Technical and Scientific preliminary Outcomes (max. 2 pages) [14]

The MicroLFA is an on-line monitoring system that performs the analyses of PO_4 or NH_3 in water or seawater; it is 12 Vdc battery operated, controlled by a remote computer but autonomous when put in monitoring mode. The system makes the analyses with fluorimetric methods for both parameters: the ammonia is analyzed by the reaction between OPA and NH_3 in slightly alkaline medium, with a preservative reagent, the excitation is done at 375 nm and the reading at 460 nm. For the PO_4, the system uses the reaction where phosphomolybdate decreases the fluorescence of rhodamine 6G in slightly acidic environment, the decrease of fluorescence is proportional to the PO_4 concentration, excitation is done at 460-470 nm and the reading at 540-550 nm.



The two systems are connected to the computer by two RS-232 serial ports by a connector, in which there is also the connection for the external power supply (12 Vdc, 3 A max), the normal power consumption is 10 W when the system is operating and about 4 W with systems in standby.

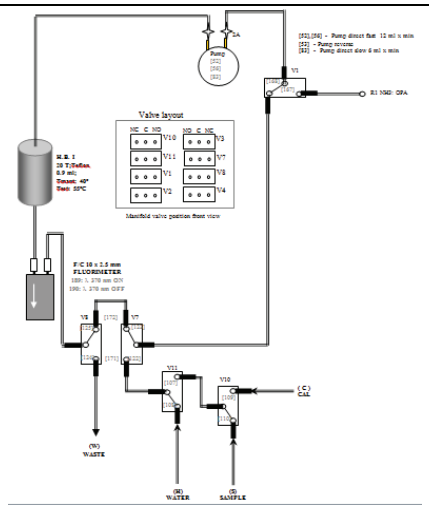
Sample Turbidity

The turbidity of the sample is eliminated by external filtration at 0.2 µm (available on both testing sites) and the zeroing of the colorimeter for both the PO_4 and NH_3 reading is performed before each analysis. For NH_3 the start fluorescence is measured together with the system zeroing, but it is not possible for the PO_4 measurement, for this the filtration is suggested for turbid samples.

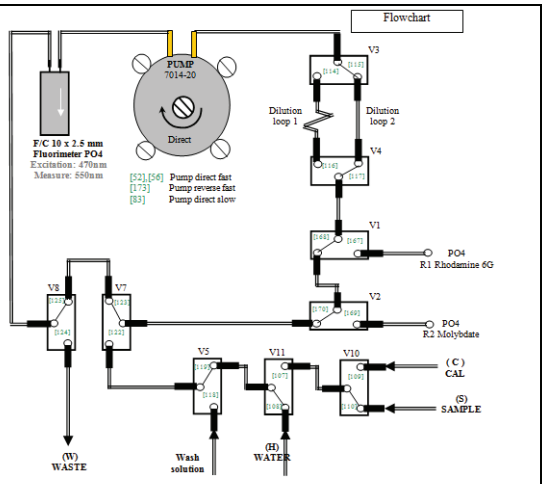
Calibration

Both methods can be calibrated in lab or in situ, using calibrant solutions stored near the systems. The linearity of the two systems are at about 6 micromol/L PO_4 (for the high sensitive range) and about 30 micromol/L NH_3. Both system uses DI water as calibrant for the zeroing of the calibration curve; salinity is not influencing the measurements for normal seawater (up to 35 g/l salt). A range of 10 micromol/L PO_4 is also available on the second method of the system.

Hydraulic circuits and operating description



µLFA Ammonia



µLFA Orthophosphate

Analytical sequence description for NH_3

The following steps describe the principal of operation of the NH_3 system:

1. When the analysis starts, the circuit is opened and the sample is aspirated by the system internal pump and fills all the circuit, the fluorimetric mode is started
2. the system is closed
3. the start fluorescence is taken
4. the OPA reagent is aspirated and the sample is mixed with it throughout all the circuit
5. the system stops: the solution inside the heating bath is warmed up to 55°C to speed up the reaction for a set time
6. after reaction is completed, the product of reaction is sent to flow-cell and the final fluorescence, proportional to the NH_3 concentration, is measured
7. the circuit is opened and the system is washed by the aspiration of sample.

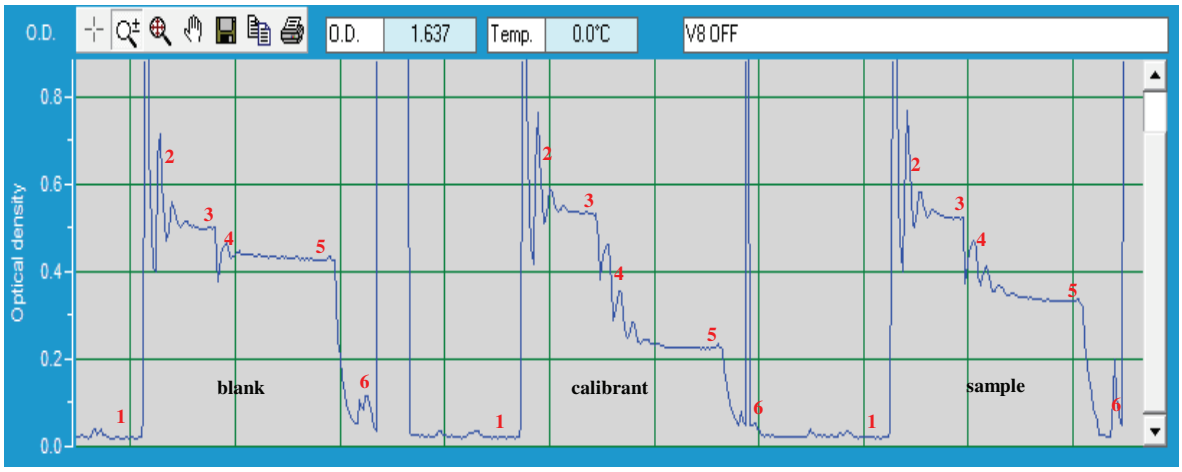
Analytical sequence description for PO_4

The following steps describe the principal of operation of the PO_4 system:

1. When the analysis starts, the circuit is opened and the sample is aspirated by the system internal pump and trapped between two sampling valves, the fluorimetric mode is started: the amount of sample trapped in the valves is related to the range of the system, being long for low range and short for high range
2. the sample valves are reset, the aspiration of water allows the washing of all the sample from the circuit
3. the system is closed and the dosing of the two reagents, molybdate and rhodamine 6G is performed, the reagents are mixed with DI water
4. after proper mixing, the start fluorescence of rhodamine is taken (OD start)
5. the sample is mixed with the fluorescent solution and produces a decrease in fluorescence due to the PO4-molybdate solution
6. the final fluorescence is measured and the decrease is correlated to the PO_4 concentration
7. the circuit is opened and the water flushes the product of reaction, to leave the system as clean as possible for next measurement.

PO_4 peak graph description

The following is a description of the peaks of PO_4 as it appears on the graph during the analysis.



The sequences of steps can be briefly described as follows:

1. the sample is aspirated and put into the tube between V3 and V4, then water is aspirated to wash away the sample: no detection of fluorescence is visible at this time
2. the water is added of the two reagents and the fluorescence of rhodamine 6G can be visualized on the graph: at the end of this phase there is stability of the solution
3. the starting fluorescence is taken
4. the V3 and V4 are opened and the sample is mixed with the reagents, a mixing phase is clearly visible: note that for blank the decrease is due to the slight dilution introduced by the volume of the sample loop
5. after a proper mixing time the stability is reached, the final fluorescence is taken: the resulting fluorescence (F end – F start) is proportional to the PO_4 concentration
6. the system is washed by DI water and there is again no fluorescence, the system is ready for next analysis.

Note that in this system the start fluorescence is taken with water and reagents to avoid difficult management of the dosing of the fluorescent reagent rhodamine 6G, that would lead to erratic calculation of the final fluorescence: for this, the sample is trapped before this measure and then mixed after the start F is checked; in this way the starting fluorescence is not relevant, but the delta can be linked to the PO_4 wherever the fluorescence starts from.

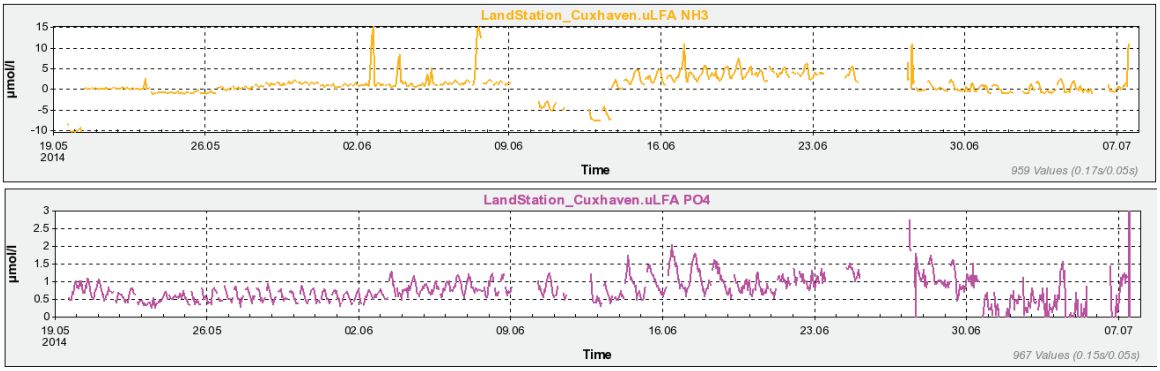
In the examples above, the blank decrease is minimum if compared with the calibrant (maximum deflection of fluorescence) and the sample (mid deflection).

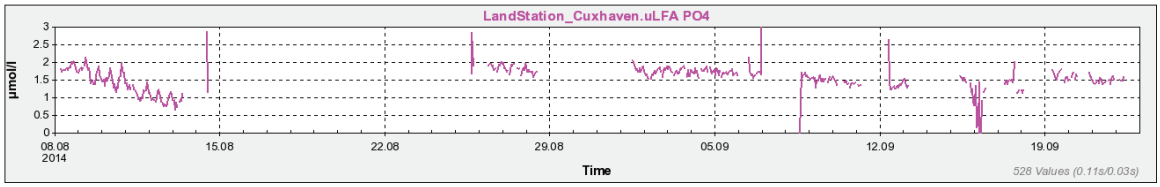
Note that these principle of operation brings to have the calibration curve that is with both the calibrant and the blank negatives, the blanks being in the range -0.1 to -0.2 and the calibrants in the range -0.6 to -0.8.

The start fluorescence is checked regularly to ensure that the system is working properly, and should be around 0.6 to 1 fluorescence.

Field experiment n.1 – Cuxhaven monitoring station

NH_3 and PO_4 measurement data were automatically collected in Cuxhaven fixed monitoring station from 19 May to 07 July 2014; a further set of PO_4 monitoring data were also collected between 9 August and 22 September 2014. Please refer to the related graphic trends reported in the next page.

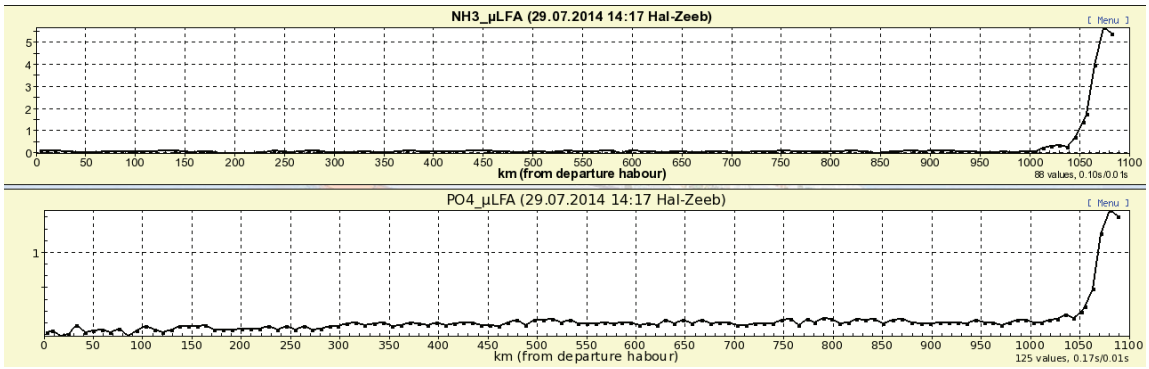


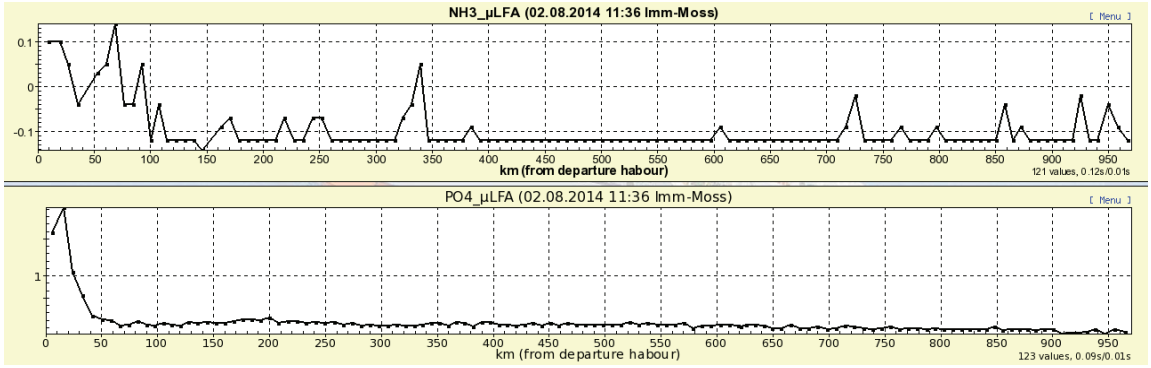


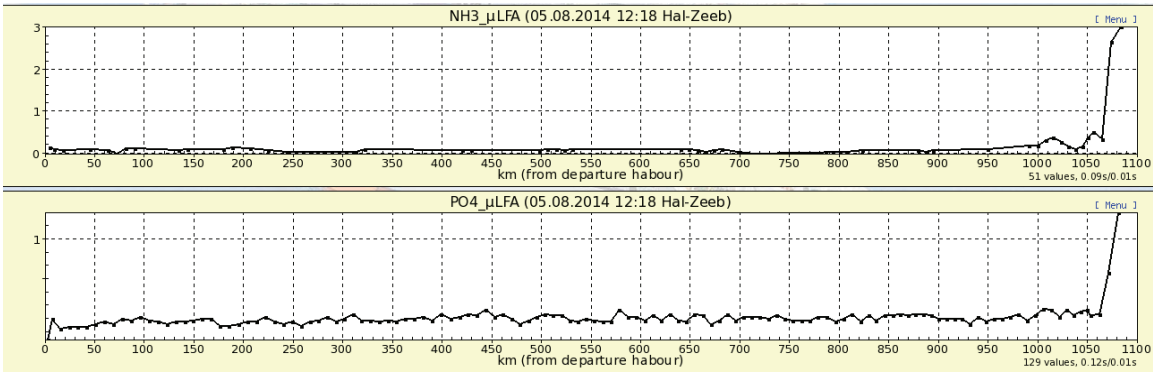
Field experiment n.2 – Lysbris Ferrybox

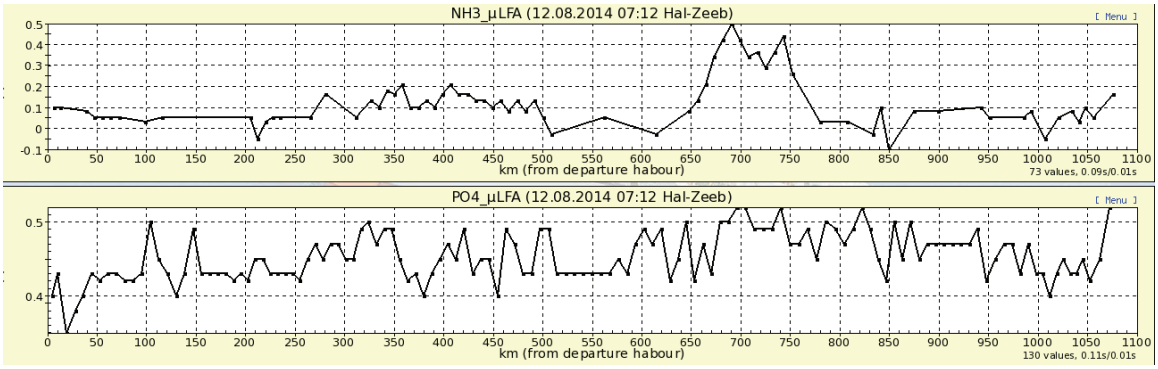
NH_3 and PO_4 measurement data were automatically collected during n.31 Lysbris Ferrybox cruises from 28 July to 23 September 2014.

Here follows example of collected data trend in µMol/L.









From the last week of September the two modules to measure NH_3 and PO_4 installed in Lysbris Ferrybox and the additional PO_4 unit that was mounted in Cuxhaven station were tested again in HZG laboratory, in order to verify their measurement performances, including some comparison tests between the modules and the data measured by their laboratory AutoAnalyzer.

On 14 November HZG issued a technical report summarizing the verification tests performed during the field campaigns and later in their laboratory.