Automated micro Loop Flow Reactor technology to measure nutrients in coastal water: state of the art and field application

S. Bodini, L. Sanfilippo, E. Savino and P. Moscetta
Research and Development department
SYSTEA S.p.A.
Anagni (FR), ITALY
info@systea.it

***Abstract* — Nutrient pollution is one of worldwide most diffused, expensive and challenging environmental issues.**

The monitoring of nutrients in coastal areas is a complex task, due to the fact that the available methods reaching the required sensitivity are based on optical wet chemistry measurements. In order to be representative, a reliable water sample collection for further laboratory analysis requires a very strict protocol and sample handling. Because of this, most of the scientists agree in affirming that the best and practical solution is to measure nutrients directly on field.

SYSTEA S.p.A. has been working in the last two decades to improve automated solutions for on-line nutrient monitoring. The micro Loop Flow Reactor (µLFR) technology was presented in 2009 in Oceans conference in Biloxi (USA) and is currently a consolidated technique allowing the field measurement of nutrients in different environmental conditions.

Fast growing urbanization and industrialization of river basins and coastal areas in China are imposing the local Government to improve large water quality monitoring networks; during the last few years, around one hundred in-situ nutrient multi-parameter probes (NPA pro and WIZ) were installed and provided extensive long term data to support the environmental control and related decisions making policy.

The WIZ probe is an advanced real time instrument to measure the nutrient concentrations in aquatic environments. In particular, it is the only portable and submersible equipment able to measure the total concentrations of both phosphorus and nitrogen. The uniqueness of this probe is given by the fact that the unit performing the sample digestion, required for the analysis of both total phosphorus and nitrogen, was downscaled to fit within the probe.

The efficiency of NPA pro and WIZ probes in measuring the concentrations of ammonia, nitrate, phosphate and nitrite was evaluated on field deployments in coastal waters, in China and South Korea, respectively. Importantly, the probe results were found to be highly correlated with laboratory measurements.

The µLFR technology was also applied to the design and manufacturing of µLFA modules, on-line nutrient analyzers integrated in Ferrybox systems for the measurement of ammonia and orthophosphates. In situ results obtained in Ferrybox installations were compared and correlated with laboratory analysis of grab samples.

Finally, a number of practical solutions for the improvement of NPA pro and WIZ probes in terms of instrument robustness, data reproducibility, deployment life and reliability, were, for the first time, presented and discussed.

The Alliance for Coastal Technologies (ACT) is a US partnership of research organizations, water stakeholders and private companies competent in the field of nutrient management which, by means of dedicated market surveys, were able to catalogue the most frequently requested specifications for nutrient analyzers. This record was used as a guide to assess the current performance level of µLFR-based probes and modules with respect to market expectations.

In conclusion, the main factors which facilitated the global diffusion of this technology were lower equipment costs than analogous products and the ability of providing highly experienced timely technical support at the local level.

Keywords — continuous nutrient monitoring; in-situ multi-parameter analyzer; microLFR technology; WIZ probe.

I. INTRODUCTION

Nutrients are chemical substances that are indispensable to plant and animal diet. Inorganic macro-nutrients in water especially include ammonium, nitrate, nitrite, phosphate and silicate ions [1]. Regrettably, an unbalanced release of nutrients into aquatic ecosystems, as in proximity of intensively cultivated areas and large urban settlements, can produce undesirable effects such as toxic algal blooms, anoxic conditions and increases of turbidity and sedimentation. All of these are causes for fish deaths, destruction of aquatic plant beds and coral reefs and consequent loss of biodiversity [2].

Nutrient enrichment seriously affects both freshwater and coastal ecosystems, impairing the use of surface and ground water for drinking water supplies, crop irrigation, recreation activities and aquaculture. Because of this, the definition of in situ nutrient sensing networks became a key issue worldwide [3]. It is now understood that only long term and continuous monitoring campaigns are capable to bring the necessary information to control rapid deterioration of water quality in inland, brackish and coastal waters [4].

Latest developments in fluidic technology provided advanced analytical tools which supported the realization of on-line analyzers and in situ probes for the continuous assessment of nutrient concentrations in the aquatic environment [5, 6].

A wide number of innovative technological solutions for sensing and quantifying nutrient concentration have been reported so far, including microfluidics, microwave technology, amperometry, lab-on-chip technique, fibre optics, ion-sensitive electrodes, synthetic receptors, molecularly imprinted polymers and biosensors [7, 8, 9, 10, 11]. Despite that, standard wet-chemical analysis still offers the most suitable and selective techniques to detect very low concentrations of nutrients in water environments [1, 12].

SYSTEA S.p.A. has been dedicating the last two decades to the development and improvement of cutting-edge automated solutions for on-line nutrient monitoring based on colorimetric and fluorimetric methods. The micro Loop Flow Reactor (μLFR) technology was presented to the international scientific community in 2009 during MTS/IEEE Oceans conference held in Biloxi, MS, USA [13], and is currently a consolidated technique allowing the field measurement of nutrients in the most diversified environmental contexts.

The μLFR tool was the basis for manufacturing a variety of analytical systems including in-situ multi-parameter probes (NPA pro and WIZ) and compact on-line analyzers (μLFA modules) to be integrated in Ferrybox systems [14].

The main goal of this work was thus to introduce the main technical developments of SYSTEA's μLFR technology and present significant results achieved during three long-term field campaigns carried out in China, South Korea and Germany with the NPA Pro probe, the WIZ probe and the μLFA modules, respectively. Final objective of the experimental test was to demonstrate the correlation of field measurements with results obtained with laboratory analysis.

In the United States, a group of resource management organizations, environmental research subjects and private companies founded the Alliance for Coastal Technologies (ACT, see http://www.act-us.info/), a partnership devoted to encouraging and promoting the definition and use of valuable tools for coastal monitoring. Aiming at this, the ACT performed three market surveys (2005, 2006 and 2014) to define suitable requirements, uses and applications and organize an on-line data-base of commercial in-situ nutrient sensors and instruments [15]. These investigations were used as a guideline to evaluate the actual suitability of μLFR-based products to meet the market needs.

In conclusion, low equipment costs and capillary technical support were recognized as essential elements for a successful international diffusion of this line of instruments and, generally, of in-situ nutrient sensors and analyzers.

II. MATERIALS AND METHODS

A. NPA pro and WIZ in situ probes description and field test

As thoroughly described earlier [13], the NPA pro is characterized by a large separated canister to hold reagents and calibrants which is connected to an optical-based analytical unit through a multipole hydraulic connector. The device is usually installed in a closed compartment inside a buoy and is only partially submerged by water.

Differently, the WIZ probe was designed to be completely submerged in water, including the reagent basket that is installed over the top of the analytical section to minimize the distance between the canister and the hydraulic manifold. The laboratory analytical performances of the NPA Pro and WIZ probes are shown in Table I.

TABLE I. NPA PRO AND WIZ TECHNICAL SPECIFICATIONS

Probe	*Parameter*	*NO_3-N*	*NO_2-N*	*PO_4-P*	*NH_3-N*
NPA Pro	Range (mg / L)	0.005-0.6	0.002-0.2	0.003-1	0.003-0.5
	Precision at 100 %	± 1%	± 1%	± 1%	± 2%
	Accuracy at 100 %	± 3%	± 3%	± 1%	± 2%
	Precision at 5 %	± 3%	± 5%	± 2%	± 3%
	Accuracy at 5 %	± 4%	± 4%	± 5%	± 2%
WIZ	Range (mg / L)	0.004-1	0.002-0.25	0.003-1	0.004-0.5
	Precision at 100 %	± 3%	± 2%	± 2%	± 1%
	Accuracy at 100 %	± 2%	± 2%	± 1%	± 1%
	Precision at 5 %	± 3%	± 3%	± 4%	± 3%
	Accuracy at 5 %	± 4%	± 3%	± 4%	± 2%

The disposable filtration device normally used with the NPA pro was hand made and consisted of a plastic syringe containing a foam filter used in home aquariums (Fig 1A), whereas the WIZ filtration apparatus consisted of a 25 μM cut-off syntherized steel filter with copper wire rounded as antifouling protection (Fig. 1B).

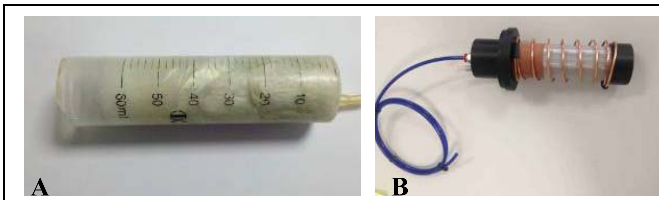

Fig. 1. Syringe type (A) and syntherized steel (B) filters.

Data concerning the NPA pro (Fig. 2) were collected in 2012 by an installation in South China Sea. The integrated coastal buoy system was deployed in a bay of a large island, with an average water depth of 4 - 6 m, a maximum distance from the coast of 2 km and a 2.4 - 3.1 % salinity concentration.

Fig. 2. The NPA pro in situ probe.

The WIZ in-situ probe (Fig. 3A) was deployed between July and November 2012 on a floating platform to monitor oligotrophic coastal waters in Wonmoon Bay, Tongyeong City, South Korea; the average sea bottom depth was 16 - 19 meters.

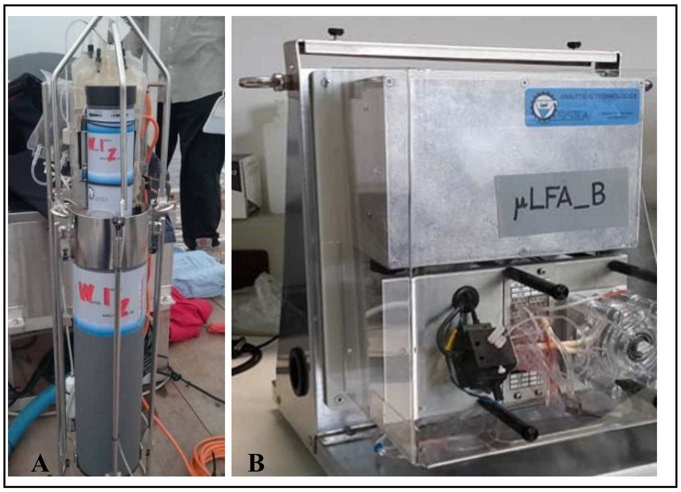

Fig. 3. The WIZ in-situ probe (A) and the μLFA module (B).

B. The μLFA module description and field test

The μLFA module (Fig. 3B) is an autonomous on-line monitoring system performing ammonia and phosphate analysis in fresh and sea water, powered at 12 Vdc. The sequences of steps for orthophosphate measurement can be briefly described as follows (Fig. 4).

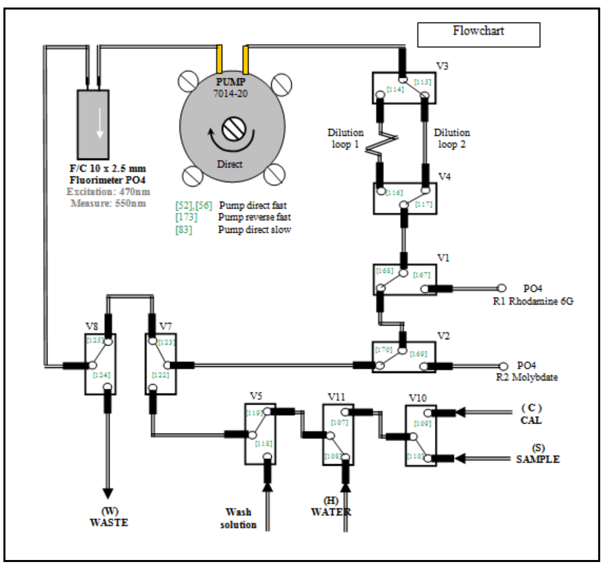

Fig. 4. The μLFA hydraulic circuit for fluorimetric phosphate analysis.

1. The sample is aspirated, introduced in the system and trapped between V3 and V4 valves in such amount directly related to the measurement range of the system, followed by DI water to wash the rest of the circuit.
2. Molybdate and Rhodamine 6G are added and mixed with DI water in the circuit; after this, the starting fluorescence is measured at $\lambda_{exc} = 470$ nm and $\lambda_{emi} = 550$ nm.
3. V3 and V4 valves are opened and the sample is mixed with the starting solution; a fluorescence decrease is observed due to the reaction of Rhodamine 6G with the phosphomolybdate solution.
4. After suitable mixing, the final fluorescence is measured and inversely related to the orthophosphate concentration.
5. The sample is discarded and the circuit is washed with DI water; the system is ready for next analysis.

Under the frame of Trans National Action of Jerico European funded research project (http://www.jerico-fp7.eu/), a laboratory test of the μLFA modules, designed for Ferrybox systems, aiming at unattended nutrient monitoring in seawater, was performed in 2014 in the Institute of Coastal Research of Helmholz Zentrum Geesthacht (Germany).

Next, two independent analytical modules to measure orthophosphate and ammonia, equipped with external filtration through 0.2-μm-pore-size with automatic cleaning, were developed and provided, to be integrated in the existing system layout and local control unit on two testing sites:

(i) a fixed monitoring station at the mouth of Elbe river in Cuxhaven and

(ii) in the Ferrybox Lysbris, in operation on a regular circular route along North Sea based in Moss (Norway) and going weekly to the following harbours: Halden (Norway), Ghent / Zeebrugge (Belgium) and Immingham (England).

The units were externally connected to a data acquisition system and a power supply (12 Vdc, 3 A); the power consumption was 10 W in operation and 4 W in standby.

C. Analytical Methods

Ammonia was measured fluorometrically at 370 nm excitation and 420 nm emission by the orthopthaldialdehyde - sulfite reaction [16]; the method is highly selective and sensitive and matrix effects are negligible.

Orthophosphate was measured in the probes by using acid molybdate solution and ascorbic acid and measuring spectrophotometrically the blue color of the phospho-molybdenum complex formed at 880 nm [1, 5, 9], whereas, in μLFA modules, it was determined by fluorescence quenching of Rhodamine 6G [17].

Nitrate + nitrite were measured using the nitrate UV photo reduction and successive determination of reaction products as nitrites. During sample pretreatment, DTPA and TRIS buffer were added and the mixture was then subjected to UV irradiation in a UV-digester. The digestion step facilitated photo-reduction of nitrates to nitrites; the nitrites formed by the photo-reduction then reacted with *N*-(1-naphtyl)ethylendiamine di-HCl (NED) and sulphanilamide (SAA) in strongly acid medium (HNO_2) to form a pink coloured azo dye measurable at 525 nm [18].

Nitrite was measured spectrophotometrically using NED and SAA as described above.

III. RESULTS

A. NPA Pro and WIZ probes

The first field test performed with the WIZ probe was done in Venice lagoon in 2009, in the frame of WARMER project. Results were compared with those obtained with DPA Pro, a former probe model based on μLFR technology. A highly significant correlation was observed for NH_3-N, NO_3-N and PO_4-P (R^2 = 0.76 – 0.89). Interestingly, the nutrients trends were observed to be associated with the combined effects of water intake from inland rivers and tides [20]. Concurrently, the French Observatoire Océanologique de Banyuls-sur-Mer deployed a WIZ probe to measure nutrients in oligotrophic coastal water conditions, revealing a concentration peak of ammonia and nitrates generated by an influent river after a strong storm [21].

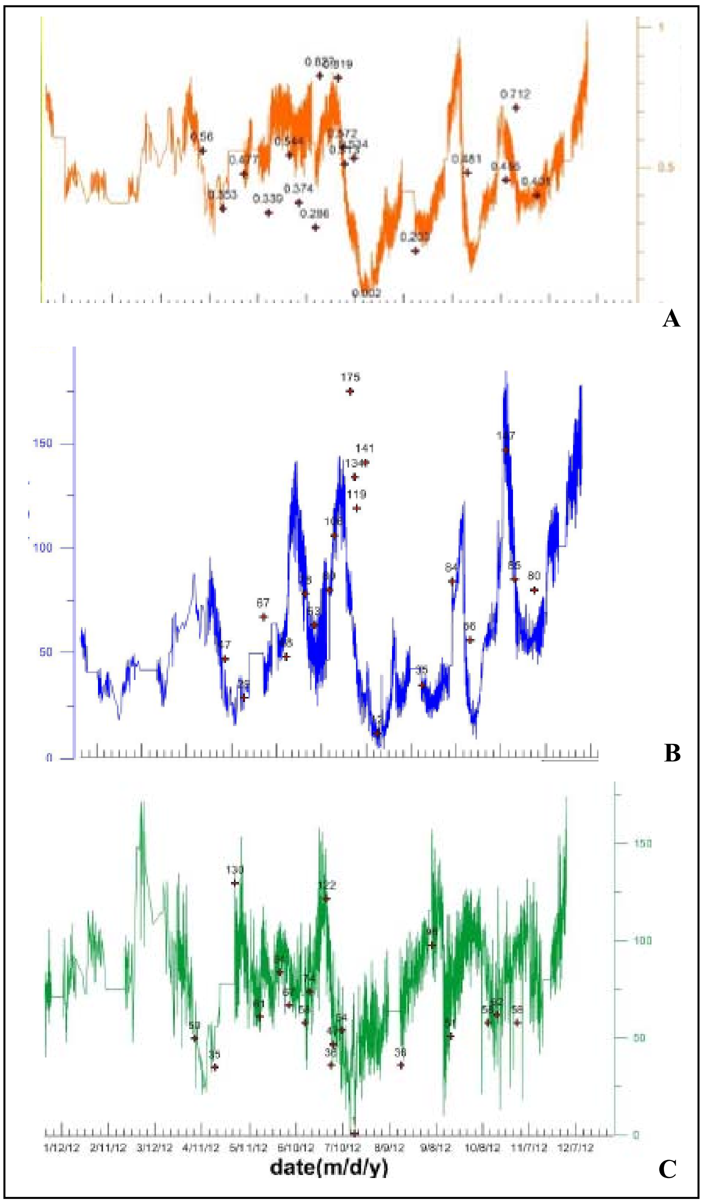

Fig. 5. 2012 yearly graphic trends (μg/L) of NO_3-N (A), NO_2-N (B) and PO_4-P (C), compared with grab samples measured in laboratory (+).

In the last decade, the development priorities of the government of the P.R. China were strongly influenced by the urgent necessity to tackle air and water pollution; this posed the causes of renewed environmental policies which brought to the creation of large water quality monitoring networks. Owing to this, more than one hundred NPA pro and WIZ nutrient probes were installed in China.

Since 2005, an integrated coastal water monitoring network including NPA pro in-situ probes to measure NO_3^- + NO_2^-, NO_2^- and PO_4^{3-} has been installed in South China Sea. In 2012, long term unattended data were collected all over the year and correlated to spot laboratory analysis (Fig. 5).

A WIZ field test was performed in 2012 in the oligotrophic waters of the Korea Strait. Significantly good measurement stability characterized the five-week deployment period (Fig. 6). Most importantly, it was possible to discriminate nutrient concentrations even at levels lower than 10 μg / L. A significant number of field results was related with laboratory measurements (R^2 = 0.98; Mean Percentage Difference = 5 %).

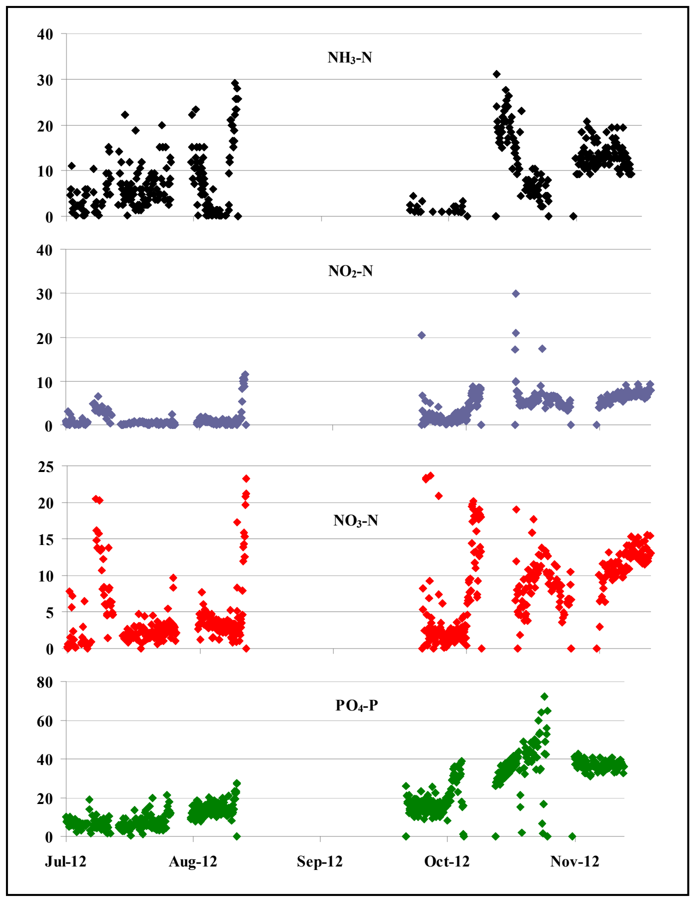

Fig. 6. July to November 2012 graphic trends of NH_3-N, NO_2-N, NO_3-N and PO_4-P (μg/L) for WIZ in-situ probe. A typhoon prevented probe functioning between Aug 24th and Oct 4th.

B. μLFA modules

Two independent μLFA analytical modules, respectively measuring PO_4^{3-} and NH_3, were tested at first in fixed stations (Fig. 7). Successively, the modules were transferred into a

Ferrybox and data were automatically collected during 32 different cruises (an example is shown in Fig. 8). Orthophosphate results obtained during the last cruise (Fig. 9) were compared both with Micromac-1000, an on line analyzer based on LFA technology and with a bench-top laboratory continuous flow analyzer, obtaining a good correlation of 0.95 and 0.89, respectively.

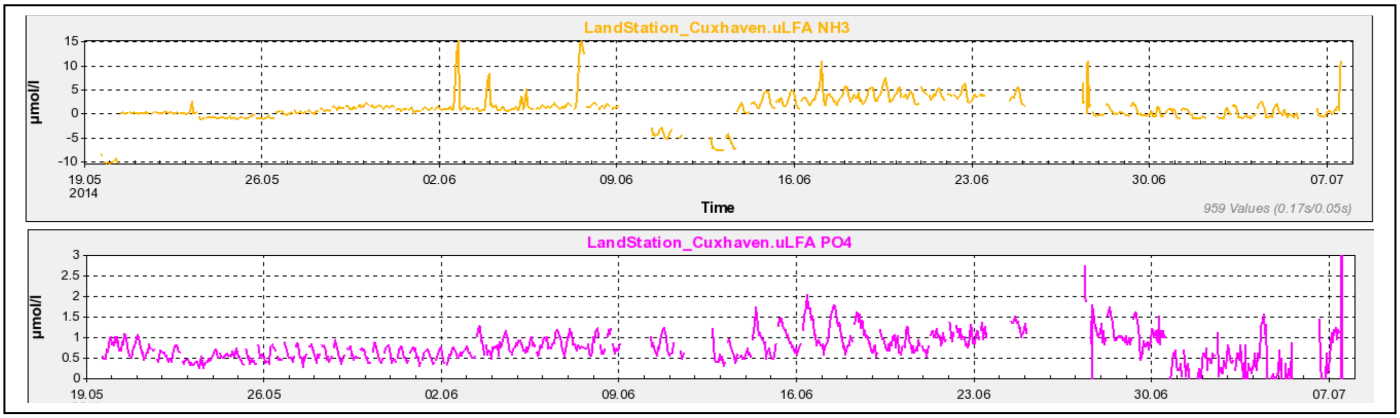

Fig. 7. NH_3 and PO_4 graphic trends (µMol/L) in Cuxhaven fixed station collected between May 19th and July 7th, 2014.

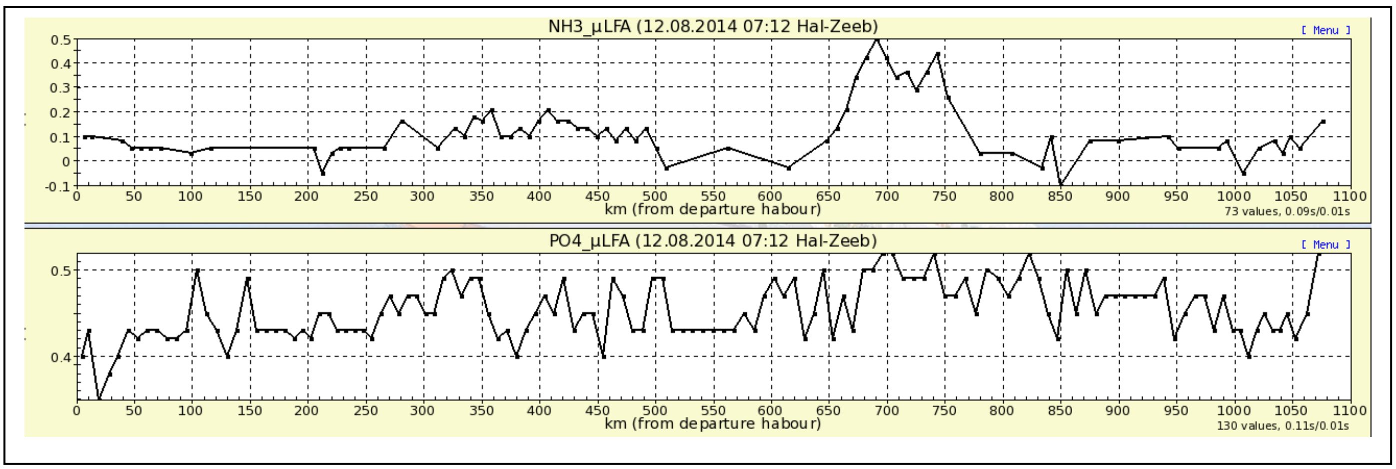

Fig. 8. Data trends of NH_3 and PO_4 concentrations collected during a Ferrybox cruise operating between Halden and Zeebrugge on August 12th, 2014. *x* axis - km from departure harbour; *y* axis - µMol/L

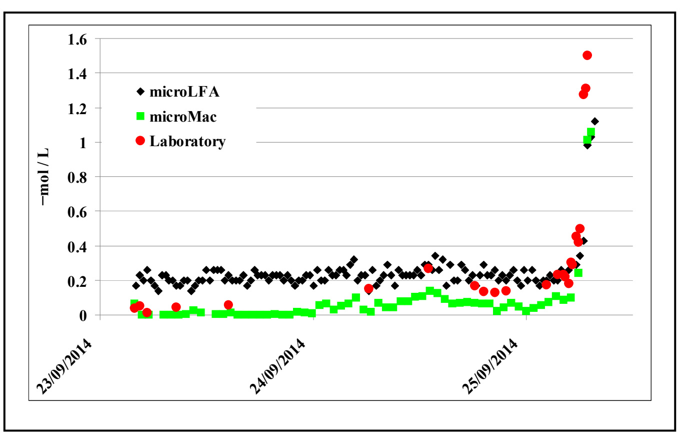

Fig. 9. Ferrybox PO_4 measurements performed with µLFA module (black diamonds), Micromac-1000 (green squares) and with a CFA instrument in laboratory (red dots).

IV. Discussion

Eutrophication has become the primary water quality issue for most of the freshwater and coastal marine ecosystems in the world [22]. Documented episodes of eutrophic coastal zones increased from tens in the 60's to hundreds nowadays [23].

The implementation of suitable analytical tools providing online punctual control of river basins before runoff reaches downstream ecosystems coupled with in situ monitoring of coastal conditions appears to be the most feasible management strategy to improve recipient water quality.

Based on these premises, in a workshop held on September 2014, the US organization ACT started the "Nutrient Sensor Challenge" (http://www.act-us.info/nutrients-challenge/). In this project, the needs of actual and potential users of in situ nutrient sensor systems were identified and listed. The most requested technical sensor characteristics concerned the analytical performance, including accuracy, precision, ranges and detection limits (Table II), followed by deployment life and reliability.

TABLE II. TARGET NUTRIENT SENSOR FEATURES

Criterion	*Nitrate (± Nitrite)*	*Ortho phosphate*
Range	0.005 – 60 mg/L of N	0.005 – 2 mg/L of P
Accuracy	± 5 % or 10 µg/L of N*	± 5 % or 5 µg/L of P*
Precision	± 5 % or 10 µg/L of N*	± 5 % or 5 µg/L of P*
Deployment Length	3 months	

*Difference at upper range from reference value

A. Suitability of NPA Pro and WIZ probes to unattended installation

The intended use for nutrient sensors is on remote unattended platforms for continuous in-situ measurements. The NPA Pro and WIZ probes were designed accordingly. As observed by comparing Table I with Table II, both probes have the potential to fit into the analytical requirements suggested by the ACT. The range of measurement can be extended up to seven times via automated sample pre-dilution step.

Nitrate and nitrite are the most investigated parameters followed by phosphate, ammonia and silicate. For this reason, the probes were designed as multi-channel systems in which up to four methods were included with a full nutrient measurement cycle lasting 38 minutes, lower than the common average interval of one hour; besides, their analytical cycle is completely programmable, allowing customized adaptations to specific applications. A specially designed flow-cell permitted the use of three different wavelengths and two detection methods (double beam colorimetry and fluorimetry).

In particular, the WIZ probe is one of most advanced on line instruments to monitor nutrients in water, since it is the only portable and submersible probe capable to measure both total phosphorus and total nitrogen in fresh and salt water due to a unique sample digester, designed to fit into small spaces.

A significant performance indicator, characterizing nutrient sensors and indicated as central by the majority of the respondents to the ACT survey, is the deployment life, defined as a combination of resistance to biofouling, low power consumption and limited use of reagents. To implement this feature, the hydraulic circuits of WIZ and NPA Pro were provided with fully automated wash function at the end of each analysis that helped to quickly remove the reaction product and reduce internal fouling. Further, the batch operation minimized tubing consumption. As for the WIZ only: (i) a miniaturized hydraulic circuit design allowed significantly lower reagent and DI water consumption (max. 1 L per month) and proportionally reduced waste production, (ii) a detachable reagent canister with reduced size permitted to reduce the area to be refrigerated and (iii) space optimization consented to decrease the power absorption to 3 W in standby mode and 6 W during analysis.

Probe reliability was improved by the use of a loop configuration which supported full removal of air bubbles which are known to notably affect the phosphate determination and the performances of the UV photo-reduction apparatus used in nitrate analysis [18].

B. Suitability of µLFA modules to Ferrybox installation

An original alternative to traditional fixed deployments is the use of ferries and other boats on predetermined routes as platforms for the monitoring of water quality [14].

The use of Ferrybox systems offers several economical and technical advantages among which: a favourable location for the analyzer and, consequently, no need for unpleasant and expensive platforms, absence of energy limitations, easier and cheaper maintenance activities, lower risk of fouling and widely distributed reliable data.

The µLFA modules were designed with hydraulic solutions similar to those developed for the WIZ probe. Thus, these instruments commonly consumed limited amounts of reagents and water and generated lower quantities of waste than commonly expected. In agreement with ACT specifications, great attention was dedicated to facilitate installation, use and maintenance.

Finally, the installation of µLFA nutrient modules on Ferrybox Lybris allowed to successfully investigate ammonia and phosphate dynamics in coastal waters and open sea and provided an invaluable amount of data along the transects.

V. CONCLUSIONS

The µLFR-based WIZ and NPA Pro probes were found suitable for long-term deployment in coastal waters. Results were calculated to be correlated with laboratory measurements.

The µLFA modules provide an innovative alternative to conventional monitoring schemes, since allowing in situ monitoring of wider spaces of fresh and salt water bodies.

A key element for the success of NPA pro and WIZ probes in the international markets was recognized as the continuous and timely technical support provided by manufacturer's product specialists and by suitable local Customer service centres.

Besides that, a wider diffusion of technologies for nutrient determination was observed to be strongly affected by instrument costs, as also highlighted by the ACT survey. The possibility to produce multi-parameter probes and analyzers allowed to reduce costs, compared to traditional instruments, for single parameter analysis.

In view of this, constant technical assistance and ease of calibration and maintenance are the directions towards which commercial strategy and technological development of nutrient technologies shall direct and move.

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