Project	AtlantOS – 633211
Deliverable number	6.3
Deliverable title	Validation of sensor and instrumentation innovations
Description	Validated prototypes of new and enhanced biogeochemical and biological sensors and instruments. Validation will be undertaken in the laboratory, in test scenarios, and by deployment in operational conditions.
Work Package number	6
Work Package title	Cross-cutting issues and emerging networks
Lead beneficiary	NERC
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Comments	

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Stakeholder engagement relating to this task*

WHO are your most important stakeholders?	☒ Private company If yes, is it an SME ☒ or a large company ☐? ☐ National governmental body ☐ International organization ☐ NGO ☒ others Please give the name(s) of the stakeholder(s): Task partners: AWI, DTU, GEOMAR, Ifremer, Kongsberg Marine, NOC, PLOCAN, TELlabs, TU Graz.
WHERE is/are the company(ies) or organization(s) from?	☒ Your own country ☒ Another country in the EU ☐ Another country outside the EU Please name the country(ies): Denmark, France, Germany, Norway, Ireland, UK.
Is this deliverable a success story? If yes, why? **If not, why?**	☒ Yes, because ….. 18 TRLs have been achieved across eight technologies. The developments target EOVs that do not currently have mature autonomous and in situ solutions to call upon and have been highlighted by the international oceanographic community as high impact.
Will this deliverable be used? **If yes, who will use it?** **If not, why will it not be used?**	☒ Yes, by …… Information throughout the deliverable has been drawn into summaries so that review is made easier. Individual sections contain greater detail and contact details for the individual technologies. It is expected the deliverable will be of interest to project partners, sensor and instrumentation providers and users of similar technology (especially those targeting biogeochemical and biology and ecosystem EOVs) within the oceanographic community. Commercialisation of some of the technologies has been secured/being pursued.

Contents

1 Introduction

AtlantOS Task 6.1 was designed to accelerate the Technology Readiness Levels (TRLs) of sensors and instruments to address key gaps. The focus is on priority Essential Ocean Variables (EOVs), hence technology that can deliver pH, pCO_2, DIC, and TA, (fast) oxygen, and nutrients measurements on traditionally challenging autonomous and dynamic observing platforms are targeted.

Enhancing Integrated Atlantic Ocean Observing System capability in biological and (meta)genomic analysis is also of high importance and plans included the development of water and filter (filtrand) sampler technologies and adapting the only oceanic genomic sensor currently at high TRL – the Environmental Sample Processor (MBARI, USA).

The Task 6.1 delivery team also worked closely with the EU funded FP7 "The Oceans of Tomorrow (2013)" projects to provide testing and opportunities for integration with ocean observing networks and platforms to demonstrate greater TRLs than was possible through the original development project.

All prototypes undergo appropriate validation; within the laboratory, in test environments, and by deployment in operational conditions and calibrated/quality controlled using metrology standards.

Owing to funding of the technology development extending beyond the reporting period some technologies described within this report have not undergone final demonstration but are scheduled to do so within the lifetime of the AtlantOS project, this is highlighted for each case.

1.1 Technology Readiness Levels (TRLs)

To track technology development from conception to a final solution TRLs are used. These readiness levels describe criteria and evidence that must be met/collected to justify a development of the technology. The scale runs from one to nine, with one being the formation of an idea with paper study to evaluate feasibility and nine being a fully validated system operated repeatedly in the environment and conditions expected of the technology.

For reference Appendix A includes a summary of TRLs as adapted from NASA guidelines. For a more comprehensive example of TRLs the Air Force Research Laboratory TRL tracker[1] is a checklist that requires only minor modification of final application (e.g. space/flight to marine) to become a complete guide.

TRLs have been used and have been referred to throughout Task 6.1 to gauge the level of development that has occurred.

1.2 Essential Ocean Variables (EOVs)

The Global Ocean Observing System (GOOS) maintains a database of EOVs that have been generated by expert panels[2] to create a framework that focusses community effort on the observation of key parameters whilst also helping to avoid duplication between observing

[1] AFRL TRL tracker

[2] http://www.goosocean.org/index.php?option=com_content&view=article&id=14&Itemid=114

platforms and networks. Each EOV is considered by what variables are required to provide data of sufficient quality (accuracy, temporal/spatial timescales), the methods that are currently used to deliver those readings, the maturity of those methods (concept, pilot or mature) and of the platforms that are and can be used to take the readings from.

The EOVs broadly separate into three categories (Table 1); Physical, Biogeochemistry and Biology and Ecosystems parameters. Typically, Physical parameters are addressed by mature technologies that can operate across all required platforms, Biogeochemistry parameters are addressed by technologies that still require development or are restricted to platforms that can provide ample power and accommodate substantial equipment, Biology and Ecosystem parameters are addressed through laboratory studies and it is only in the last few years that technology that can undertake in situ, autonomous readings has been developed.

The EOVs listed red in Table 1 are those that are tackled by at least one technology developed through AtlantOS Task 6.1 and described in this report. It should be noted that effort has been concentrated on the previously underdeveloped in-situ measurement of Biology and Ecosystems parameters with technologies that target Biogeochemistry parameters aiming to deliver measurements on autonomous and dynamic platforms that have limited space and power.

Table 1 Overview of EOVs.

PHYSICAL	BIOGEOCHEMISTRY	BIOLOGY AND ECOSYSTEMS
Sea state	Oxygen	Phytoplankton biomass and diversity
Ocean surface stress	Nutrients	Zooplankton biomass and diversity
Sea ice	Inorganic carbon	Fish abundance and distribution
Sea surface height	Transient tracers	Marine turtles, birds, mammals abundance and distribution
Sea surface temperature	Particulate matter	Hard coral cover and composition
Subsurface temperature	Nitrous oxide	Seagrass cover
Surface currents	Stable carbon isotopes	Macroalgal canopy cover
Subsurface currents	Dissolved organic carbon	Mangrove cover
Sea surface salinity	Ocean colour	Microbe biomass and diversity
Subsurface salinity		Benthic invertebrate abundance and distribution
Ocean surface heat flux		

2 Development assessment

To synthesise the work that has been undertaken through AtlantOS Task 6.1 each of the developed technologies is presented using the headings detailed in Table 2.

Table 2 Example development assessment summary table

Criteria heading	Description
Motivation for technology innovation.	Why was this technology development undertaken and how does it better meet the needs of end users compared to what is currently possible.
Addressed EOVs	Which EOVs does the technology address directly and indirectly through supporting, derived and sub-variables.
Starting Technology Readiness Level	The TRL that the technology possessed at the start of the AtlantOS project.
Analytical performance targets	The performance measures used to evaluate the technology and what it can achieve.
Method of calibration and performance validation	The manner in which the technology has been evaluated to ensure the analytical performances are verified and validated.
Deployments beyond laboratory studies	Demonstrations of the technology in representative environments.
Final Technology Readiness Level	The expected TRL that the technology will possess at the AtlantOS project.

There are variations on the above for some technology entries, as appropriate, but the intention is to provide an overview whilst also providing detail on specific criteria – such as methods of validation used – for those that wish to learn more about each technology.

3 Technologies

3.1 Technology summaries

Before the main body of this report within this section a summary for each technology developed through AtlantOS Task 6.1 is provided. This is for easy referencing and to provide a snap shot of the generated capability and which EOVs are addressed by a particular technology. Sensor capability (proven or target), instrument dimensions and required resources are all listed when available.

Table 3 is a summary of the pH optode developed through the AtlantOS project, led by KM CONTROS.

Table 3 Technology summary of pH optode

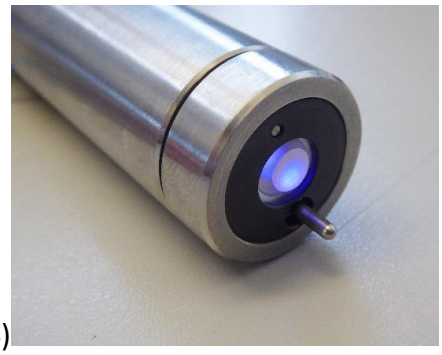
Technology	pH optode (pg 15)
	(A)  (B) 
	Figure 1 (A) Flow-head at top of pressure housing, (B) Excited pH sensing spot.
Parameters	pH of water
Performance	Operational range: 2 - 32 ˚C (target) up to 6000 m depth (target) Accuracy: ± 0.005 pH (target) Response time (t_{63}): < 1 min
Dimensions	Approx. 40 (ø) × 200 mm
EOVs addressed	Inorganic carbon (sub variable) Phytoplankton biomass and diversity (supporting variable) Hard coral cover and composition (supporting variable)
TRL development	TRL 5 at start of AtlantOS TRL 6+ at end of AtlantOS

Table 4 is a summary of the pCO_2 optode developed through the AtlantOS project, led by KM CONTROS.

Table 4 Technology summary of pCO_2 optode.

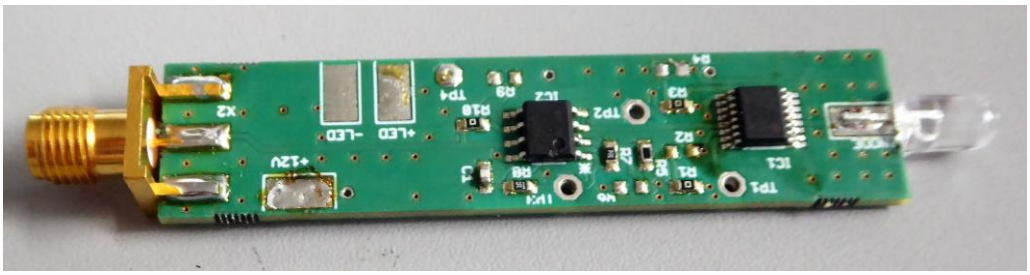
Technology	pCO_2 optode (pg 17)
	
	Figure 2 Electronic board for controlling optics of prototype pCO_2 optode, housing identical to that of pH optode (Figure 1).
Parameters	Partial pressure of CO_2 in water
Performance	Operational range: 100 – 1000 µatm (target)
	Accuracy: ±2 µatm (target)
Dimensions	Approx. 40 (ø) × 200 mm
EOVs addressed	Inorganic carbon (Sub-variable) Stable carbon isotopes (Supporting variable) Phytoplankton biomass and diversity (supporting variable)
TRL development	TRL 5 at start of AtlantOS TRL 6 at end of AtlantOS

Table 5 is a summary of the oxygen optode developed through the AtlantOS project, led by KM CONTROS.

Table 5 Technology summary of O_2 optode

Technology	O_2 optode (pg 18)

Figure 3 Oxygen optode (HydroFlash®)

Parameters	Dissolved oxygen in water	
Performance	Operational range:	0 - 373 µmol/l 2 - 32 ˚C up to 6000 m depth
	Accuracy:	< 1 µmol/l
	Response time (t_{63}):	4 s
Dimensions	23 (ø) × 197 mm (with connector)	
Resources	6 – 32 V	
EOVs addressed	Oxygen (Sub and derived variable) Nutrients (Supporting variable) Inorganic carbon (Supporting variable) Dissolved organic carbon (Supporting variable) Phytoplankton biomass and diversity (Supporting variable)	
TRL development	TRL 7 at start of AtlantOS TRL 9 at end of AtlantOS	

Table 6 is a summary of the total alkalinity sensor developed throughout the AtlantOS project, led by NERC.

Table 6 Technology summary of total alkalinity sensor

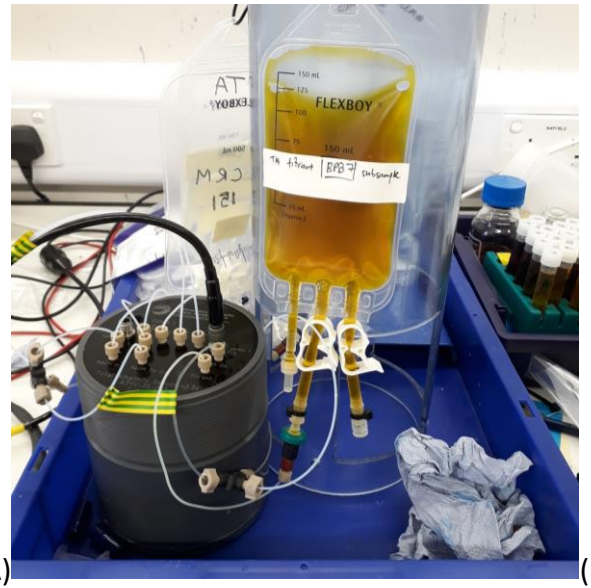
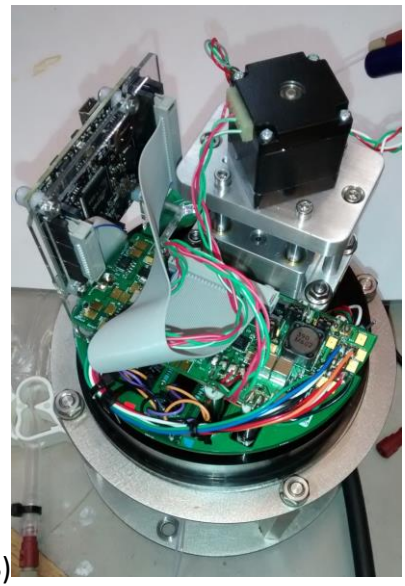
Technology	Total Alkalinity (pg 23)
	 (A)  (B)

Figure 4 (A) Fully integrated total alkalinity system (B) Implemented using standard NOC chemical sensor platform.

Parameters	Total alkalinity in water	
Performance	Accuracy:	2 µmol/kg (target)
	Range:	2000 – 2500 µmol/kg (initial)
		1500 – 3000 µmol/kg (target)
	Data interval:	15 – 30 mins
Dimensions	Approximately 160 ø × 160 mm (without reagents)	
Resources	12 V	
EOVs addressed	Inorganic carbon (Sub-variable)	
	Stable carbon isotopes (Supporting variable)	
	Phytoplankton biomass and diversity (Supporting variable)	
	Hard coral cover and composition (Supporting variable)	
TRL development	TRL 4 at start of AtlantOS	
	TRL 7 at end of AtlantOS	

Table 7 is a summary of the dissolved inorganic carbon sensor developed throughout the AtlantOS project, led by NERC.

Table 7 Technology summary of dissolved inorganic carbon sensor

Technology	Dissolved Inorganic Carbon (pg 25)

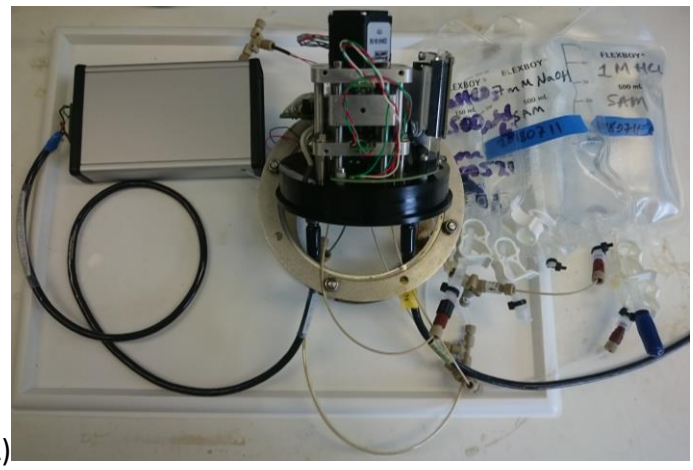
(A)  (B) 

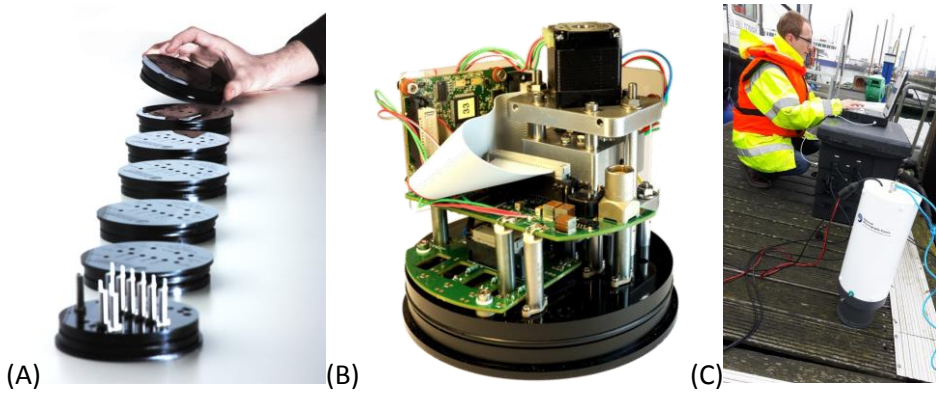
Figure 5 (A) C4D impedance analyser, microfluidic and electronics with reagents (B) accommodated in pressure housing, reagent storage and power.

Parameters	Dissolved inorganic carbon in water	
Performance	Precision:	2 µmol/kg (target)
	Range:	1600 – 2600 µmol/kg
Dimensions	Full deployable system (includes reagents)	
	Approximately 200 ø mm × 560 mm	
Resources	12 V	
EOVs addressed	Inorganic carbon (Sub-variable)	
	Stable carbon isotopes (Sub-variable)	
	Stable carbon isotopes (Supporting variable)	
TRL development	TRL 4 at start of AtlantOS	
	TRL 7 at end of AtlantOS	

Table 8 is a summary of the nutrient sensors developed throughout the AtlantOS project, led by NERC.

Table 8 Technology summary of Nutrient sensors.

Technology	Wet Chemical Nutrients and pH – Lab On Chip (pg 28)



(A) (B) (C)

Figure 6 (A) Microfluidic chips (B) Assembled LoC technology (C) LoC with reagent housing ready for a deployment off a pontoon.

Parameters (1 off)	Nitrite+Nitrate, Phosphate, Silicate or pH
Performance	Dependent on parameter of selected system
Dimensions	Approximately 160 ø × 160 mm (without reagents) Approximately 200 ø mm × 560 mm (with reagents)
Resources	12 V
EOVs addressed	Nutrients (Sub and derived variable) Dissolved Inorganic Carbon (Sub and Supporting variable) Phytoplankton biomass and diversity (Supporting variable) Hard coral cover and composition (Supporting variable)
TRL development	TRL 7 at start of AtlantOS TRL 8/9 at end of AtlantOS

Table 9 is a summary of the Marine Autonomous Plankton Sampler developed throughout the AtlantOS project, led by NERC and TELlabs.

Table 9 Technology summary of Marine Autonomous Plankton Sampler.

Technology	Marine Autonomous Plankton Sampler (MAPS) (pg 32)

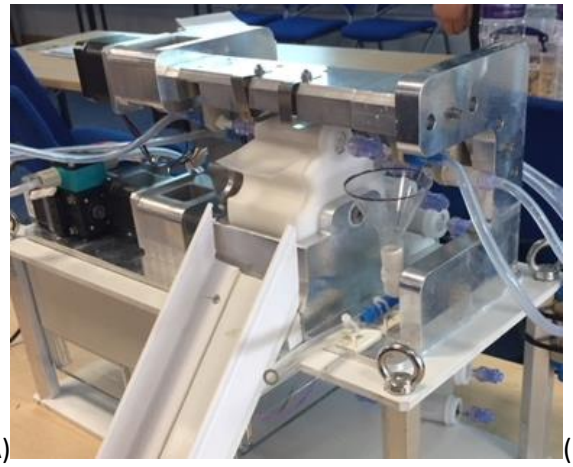
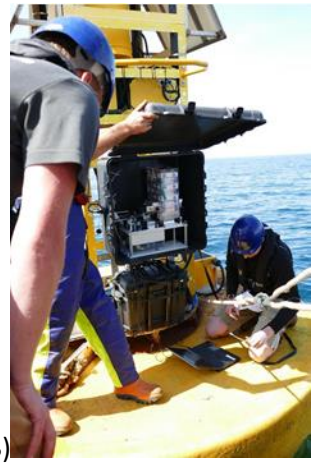
(A)  (B) 

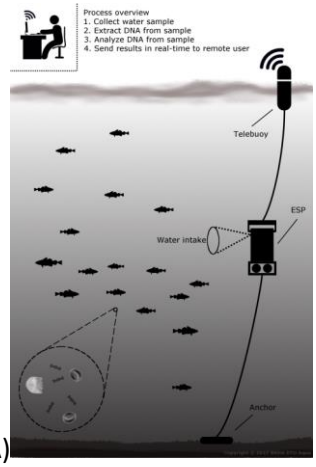
Figure 7 (A) Cartridge MAPS design as used in ships underway system (B) Cartridge MAPS mounted in buoy for unattended long-term deployment.

Parameters	Samples of marine eDNA
Performance	Quantity, quality of DNA and RNA comparable to flash-freezing (target) Composition of DNA and RNA (using next generation sequencing) comparable to flash-freezing (target) Recovery of DNA and RNA amenable to qPCR quantification of known targets
Resources	12 V
EOVs addressed	*Fish abundance and distribution* (sub-variable) *Hard coral cover and composition* (sub-variable) *Phytoplankton biomass and diversity* (derived variable) *Mangrove cover* (derived variable) *Microbe abundance and diversity* (emerging EOV) *Benthic invertebrate abundance and distribution* (emerging EOV)
TRL development	TRL 2 at start of AtlantOS TRL 7 at end of AtlantOS

Table 10 is a summary of the Environmental Sample Processor (ESP) developed throughout the AtlantOS project, led by DTU Aqua.

Table 10 Technology summary of Environmental Sample Processor.

Technology	Environmental Sample Processor (pg 38)

(A) 

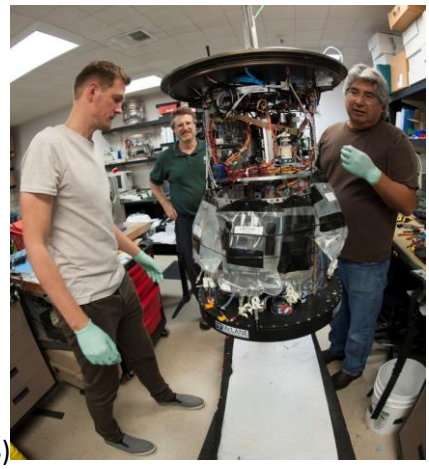
(B) 

Figure 8 (A) Drawing of the 2G ESP in an ocean deployment configuration (B) Final visual inspection of a core 2G ESP before being placed into a waterproof housing.

Parameters	Environmental DNA (eDNA) in water
Performance	66 qPCR samples or 132 archival samples per deployment. Mix of the two types possible (max 44 phases).
Dimensions	Core: 560 ø × 820 mm, Housing: 648 ø × 946 mm
Resources	10 – 16 V
EOVs addressed	Fish abundance and distribution
TRL development	TRL 6 at start of AtlantOS TRL 8 at end of AtlantOS

3.2 pH Optode

Contact: Peer Fietzek (peer.fietzek@km.kongsberg.com).

Motivation for technology innovation

The measurement of pH is critical in understanding the carbonate system and ocean health, especially for corals and crustaceans. Dynamic platforms (e.g. floats, gliders) require sensor solutions that have low power requirements and a small form factor. Placing sensors on such platforms enables long-term and often autonomous long term measurements of the surface and deep ocean. Current solutions either have the low resource requirements or the accuracy required for ocean science. This technology development was furthered to provide a pH sensor that could deliver both.

A focus of development has been the opto-electronics to apply time and frequency domain measurement for the application of a dual reference (f-DLR and t-DLR). Following this the most appropriate sensing dyes can be realised and then assessed.

Addressed EOVs

Inorganic carbon of which pH is a sub-variable. pH is also valuable for the investigation of biogeochemical processes as well as carbonate chemistry and ocean acidification studies. It is listed as a supporting variable for the biogeochemical EOVs of *Phytoplankton biomass and diversity* and h*ard coral cover and composition*.

Starting Technology Readiness Level

The TRL at the start of the AtlantOS project was 5. This was based on the development status and the availability of all principle technical components. Additional components such as the pressure housing, the optical parts and the integrated water temperature measurement features were considered TRL 7 as they are common components with more mature technology (Section 3.4). Figure 9 shows pictures of a pH optode prototype.

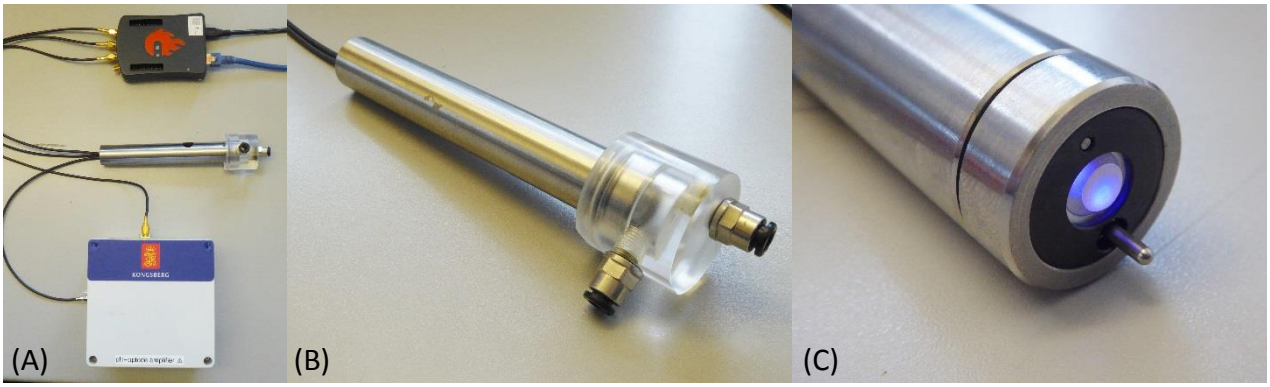


Figure 9 (A) Test setup of the optode prototype incl. function generator and oscilloscope on top, pressure housing with sensing spot in the middle, signal amplifier at bottom. (B) Flow-head at top of the pressure housing. (C) Excited pH sensing spot.

Analytical performance targets

The desired, long-term accuracies for the measurement of pH is ±0.005 pH units. This accuracy is demanding of the optode measuring principle. The following items were used through development as guiding principles:

- Small dimensions, small power consumption and easy integration capability, i.e. electro-mechanical interfacing,
- Fast response time, $t_{63\%}$ < 1min,
- Sufficient signal stability (inclusive of necessary corrections). Degradation of the indicator dye in the sensing membrane and deterioration of the membrane and optical components is expected to cause sensor drift. An assessment and correction requires both a pre-deployment and a post-deployment calibration as well as a count of readings undertaken.
- Adequately characterized temperature and pressure dependence.

Method of calibration and performance validation

Laboratory tests have successfully been conducted on buffer solutions of defined pH value for the determination of the optode's accuracy. The following table lists 2 pH values (Table 11) together with the measuring results obtained by application of the frequency based dual lifetime referencing method (f-DLR; Figure 10).

Table 11 Laboratory results of pH validation

Reference pH	Frequency signal dPhi	Standard deviation of the frequency signal σdPhi	Standard deviation of the signal in pH units σpH
7.0	168.2°	±2.9°	±0.12
9.0	136.2°	±1.9°	±0.13

The time based DLR (t-DLR) was tested as well, but provided less reproducible results.

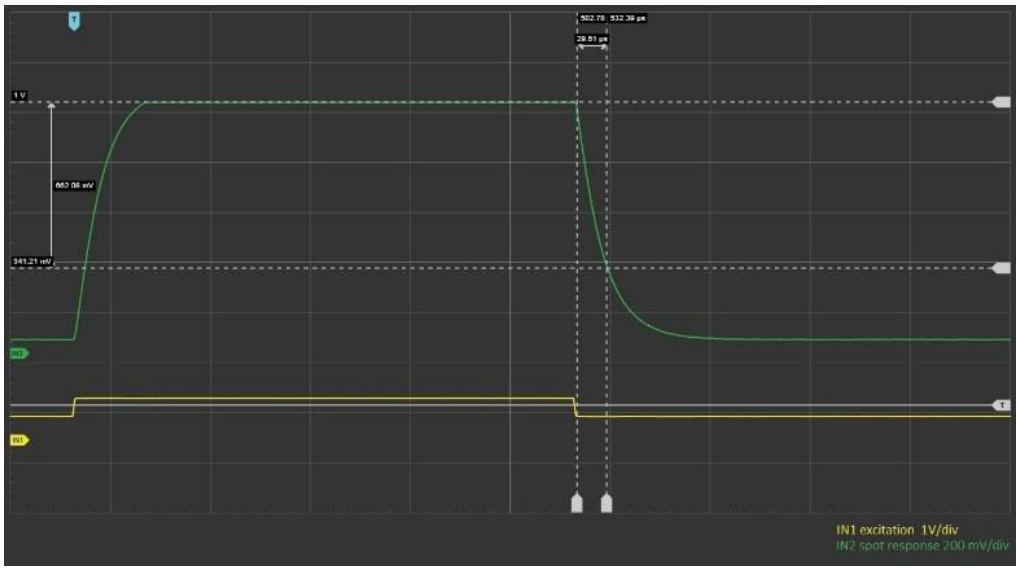


Figure 10 Excitation (yellow) and measurement signal (green) for the t-DLR method.

Final Technology Readiness Level

During the AtlantOS project key internal components for the optodes became unavailable. This prevented greater TRL advancement. However, actions to overcome the supply issue have meant the realisation of universal and comprehensive optoelectronics that enable greater sustainability and flexibility of the technology. It is realistic the final TRL for the pH optodes is 6 (technology demonstrated in relevant environment).

3.3 Partial Pressure of Carbon Dioxide Optode (pCO_2)

Contact: Peer Fietzek (peer.fietzek@km.kongsberg.com).

Motivation for technology innovation

Instrumentation capable of in-situ pCO_2 measurements are extremely sought after for not only long term monitoring on static platforms but also increasingly on dynamic platforms (e.g. floats, gliders) to make regular measurements of the water column. Optodes exhibit many ideal properties (relatively low energy, continuous operation, good data rates, small form factors and low resource requirements) attractive to in-situ and autonomous studies.

pCO_2 as a measurement is highly sought after by the oceanographic community owing to its status as a sub-variable for the study of inorganic carbon and supporting variable to other EOVs. Presently pCO_2 optodes struggle to regularly achieve the accuracy required for oceanographic studies.

This work focuses on the realization and assessment of optode technologies for the measurement of pCO_2. Specifically, universal opto-electronics have been realised and tested. This is a critical development to enable the creation of effective measurement procedures and the advancement of essential sensing dyes for future systems.

Addressed EOVs

Inorganic carbon of which pCO_2 is a sub-variable. pCO_2 is also listed as a supporting variable for the measurement of *stable carbon isotopes* and the biogeochemical EOV of *Phytoplankton biomass and diversity*.

Starting Technology Readiness Level

The TRL at the start of the AtlantOS project was 5. Additional components such as the pressure housing, optical parts and the integrated water temperature measurement features are considered TRL 7 as they are shared with relatively mature oxygen optodes.

Analytical performance targets

The desired, long-term accuracies for the measurement of pCO_2 is ±2 µatm. This accuracy is very demanding of the optode measuring principle. The following items are remaining high level performance targets for the optodes:

- Small dimensions, small power consumption and easy integration capability, i.e. electro-mechanical interfacing,
- Fast response time, $t_{63\%} < 1$min,

- Sufficient signal stability (following necessary corrections). Degradation of the indicator dye in the sensing membrane and deterioration of the membrane and optical components is expected to cause sensor drift.
- Adequately characterized temperature and pressure dependence.

Development status

Procurement of key internal components became impossible during the AtlantOS project. As a consequence, an additional requirement of developing dedicated optoelectronics was placed on to the development schedule. Progress has been made in this area but it has delayed realizing the full TRL development that was initially planned for the pCO_2 optodes.

The optoelectronics will soon reach an advanced enough level that testing of the CO_2 sensitive sensing spots can be fully evaluated. The pCO_2 optode will then be tested in water using the calibration setup used for commercial CO_2 calibrations at KM Contros.

Final Technology Readiness Level

Due to procurement issues experienced through development the TRL by the end of the AtalntOS project will be 6 (technology demonstrated in relevant environment).

3.4 Oxygen Optode (O_2)

Contact: Peer Fietzek (peer.fietzek@km.kongsberg.com).

Motivation for technology innovation

Ocean warming severely impacts oxygen distribution due to a reduction in oxygen solubility and increased stratification in the upper ocean. Models predict a decline of the global oxygen inventory of about 1-7% over the next century and data show a decrease of more than 2% since 1960 (Schmidtko et al., Nature, 2017[3]). To improve the understanding of underlying chemical, biological and physical processes it is crucial to quantify global and regional changes of oxygen. Oxygen Minimum Zones (OMZ) are of high interest because of consistent trends of intensification and spatial expansion exist (e.g., Stramma et al., Science, 2008[4]).

Over the last decade the development of optical based sensors (optodes) for oxygen has enabled routine observations on autonomous platforms with great success (Bittig et al., Front. Mar. Sci., 2018[5]). Profiling platforms have also become increasingly important with users requesting ever

[3] Schmidtko, S., Stramma, L., Visbeck, M., 2017. Decline in global oceanic oxygen content during the past five decades. Nature 542, 335.

[4] Stramma, L., Johnson, G.C., Sprintall, J., Mohrholz, V., 2008. Expanding oxygen-minimum zones in the tropical oceans. Science 320 (5876), 655-658.

[5] Bittig, H.C., Körtzinger, A., Neill, C., Van Ooijen, E., Plant, J.N., Hahn, J., Johnson, K.S., Yang, B., Emerson, S.R., 2018. Oxygen optode sensors: Principle, characterization, calibration, and application in the ocean. Frontiers in Marine Science 4 (429).

better spatial and temporal resolution. There is also constant demand to improve sensor time constants, stability, accuracy and in-situ calibration capabilities.

The novel and fast HydroFlash® O_2 optode (KM Contros GmbH) potentially closes the gap between previous capability and user requirements. The commercially available optode measures oxygen autonomously and in-situ. It shows a temperature-dependent response time ($t_{63\%}$) of about 4 seconds (weak-turbulent flow) and is therefore at least 50% faster when compared to other optical oxygen sensors, e.g. SBE 63 (Sea-Bird Scientific), 4330 (Aanderaa), RINKO (JFE Advantech Co., Ltd.).

Due to its small size and response characteristics, this novel optode could be used on a wide range of autonomous observation platforms including the most challenging floats and gliders. In a changing ocean these energy efficient underwater platforms are likely to record crucial information about the global oxygen budget.

Addressed EOVs

The measurement of oxygen is an EOV and when combined with measurements of temperature, salinity and wind speed derived variables include Apparent Oxygen utilisation (AOU) and air-sea O_2 fluxes. Oxygen is also a supporting variable for the EOVs of Nutrients, Inorganic carbon, Dissolved organic carbon and Phytoplankton biomass and diversity.

Starting Technology Readiness Level

The TRL of the HydroFlash® O_2 optodes at the start of the AtlantOS project was 7. A system prototype had been demonstrated in an operational environment.

Analytical performance targets

The analytical performance targets for oxygen optodes are strongly dependant on the measurement platform and the scientific question. For the most demanding open ocean work the following targets were identified.

- Long-term accuracy of 1 µmol/l
- Capable of in-air measurement for in-situ calibration/drift control
- Characterized temperature and pressure dependence
- Time response of <10 s for slowly profiling platforms (e.g. floats and gliders)
- Minimal size and power requirement
- Easy integration (hardware and software) into existing platforms
- Small biofouling sensitivity

HydroFlash® O_2 optodes operate in oxygen concentration ranges between 0-373 µmol/l, temperature ranges between 2 – 32 °C and depths of up to 6000 m. Laboratory step experiments (weak-turbulent flow) showed a temperature-dependent response time ($t_{63\%}$) of approximately 4 seconds.

Optode calibrations yielded accuracies with RMSE < 1µmol/l. Field tests (CTD performance test, underway measurements) confirmed that oxygen concentrations derived from calibrated optodes

were comparable to wet-chemical results from Winkler titration measurements. Salinity and hydrostatic pressure corrections were applied.

A mooring (120 m depth) of a HydroFlash® O_2 optode operated continuously for 251 days (> 8 months). This used a measurement interval of 10 minutes. Qualitative (quantitative will follow) analysis to an established optode (Aanderaa) showed comparable trends and oceanographic features.

Method of calibration and performance validation

14 HydroFlash® O_2 optodes were calibrated in the laboratory and in the field. Sensor specific characteristics were investigated (setup as described in Bittig et al., 2012[6]), in which they underwent calibrations spanning the typical range of oxygen (0 – 373 µmol/l) and temperature (2 – 32 °C). The calibrations provided high quality results as expressed by an RMSE below 1 µmol/l between the values according to the sensor calibration and the reference measurements (see Figure 11). Calibration polynomials from a selection of the tested optodes were later re-adjusted, as outlined in Bittig et al., Front. Mar. Sci., 2018[7]. Three optodes from this study also underwent further in water calibrations at the manufacturer's premises (KM Contros) for temperature ranges from 5 - 35°C and covering O_2 partial pressure of 0 – 300 mbar. The quality of the calibrations matched that of the earlier work, i.e. an RMSE of ~1 mbar between the calibrated optode values and the reference values as determined through Winkler titration.

Field tests included a reduced calibration (0%/100% at various temperatures in the laboratory and in-situ CTD cast). A focus of the field trials was the utilisation of the optodes in environments with small and large scale oxygen changes, which is required for evaluating accuracy and in the majority of scientific applications. Out of the 14 units thoroughly tested two units failed. All other optodes operated reliably and reproducibly both in the laboratory and the field. The obtained data continues to be analysed for sensor performance and oceanographic studies.

Deployments outside of laboratory validation (or plans of such deployments)

Two HydroFlash® O_2 optodes have been repeatedly used in surface underway (UW) measurements, collectively more than 100 days (Figure 11). Research cruises in 2014 and 2015 served to pre-test, investigate and validate UW and CTD performance. During a zonal transect, crossing the South Atlantic at nominal 34.5°S (R/V Meteor Cruise 133), oxygen concentrations were measured alongside a diversity of other biogeochemical parameters at 5 m depth (pCO_2, DIC/TA, total gas tension, temperature, salinity, chl a/turbidity/phycocyanin). In addition, atmospheric concentrations were recorded and will be compared with underlying physical water mass properties (temperature, salinity, velocity). Wide ranges and strong gradients were observed

[6] Bittig, H.C., Fiedler, B., Steinhoff, T., Kortzinger, A., 2012. A novel electrochemical calibration setup for oxygen sensors and its use for the stability assessment of aanderaa optodes. Limnology and Oceanography-Methods 10, 921-933.

[7] Bittig, H.C., Körtzinger, A., Neill, C., Van Ooijen, E., Plant, J.N., Hahn, J., Johnson, K.S., Yang, B., Emerson, S.R., 2018. Oxygen optode sensors: Principle, characterization, calibration, and application in the ocean. Frontiers in Marine Science 4 (429).

whilst crossing distinctive fronts across the Patagonian Shelf. This underway data continues to be compared with available data for the region to derive air-sea gas exchange, primary production and contribute to process understanding in the South Atlantic.

In cooperation with the Laboratoire d'Océanographie de Villefranche-sur-Mer (LOV), a proof-of-concept float implementation of the optode was successfully deployed and a test profile recorded (Figure 11). The tests revealed a sensitivity of the optodes' sensor spots when exposed to direct solar irradiation at the surface. It is of great interest to conduct further field experiments on floats to implement air-calibrations to improve profiling performance.

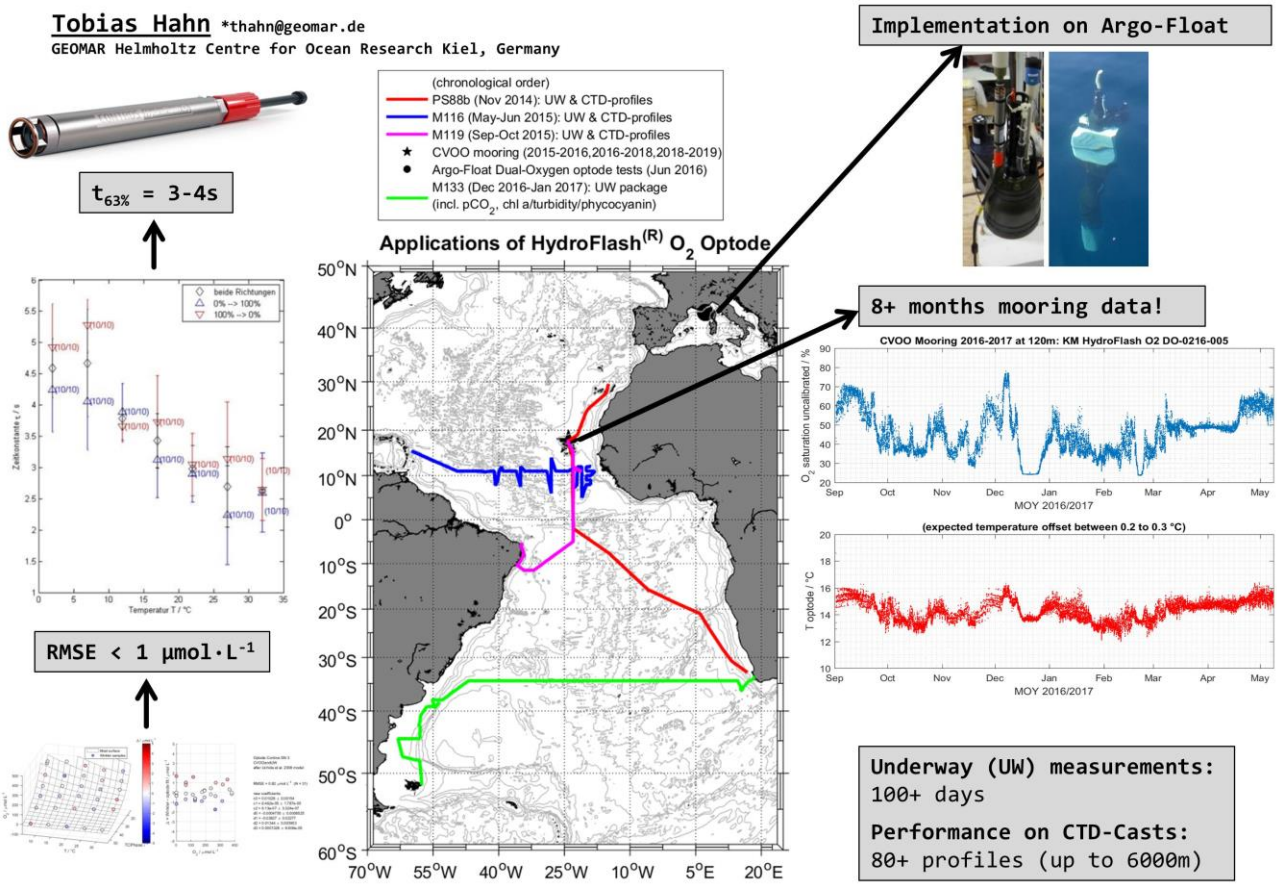


Figure 11 Applications of HydroFlash® O_2 optodes in the Atlantic Ocean

In total, four HydroFlash® O_2 optodes were successfully deployed at the Cape Verde Ocean Observatory mooring for more than a year. The first mooring deployment of two optodes served as a proof-of-concept and data recording issues were encountered, a second deployment (2016 – 2018) provided a data set of 251 days. This duration was limited by the available power supply and the reasonably high measuring frequency of one measurement every 10 mins.

A further two optodes are currently deployed and are scheduled for recovery at the end of 2019. Owing to the low power consumption of the optodes and using a measurement interval interval of one measurement every two hours it is expected data will be collected over the deployment duration of 22 months. Figure 12 shows the installation of the two HydroFlash® O_2 optodes from the 2016-18 deployment in the measuring cage before and after the mooring deployment as well as in the CTD cast for the in-situ calibration procedure.

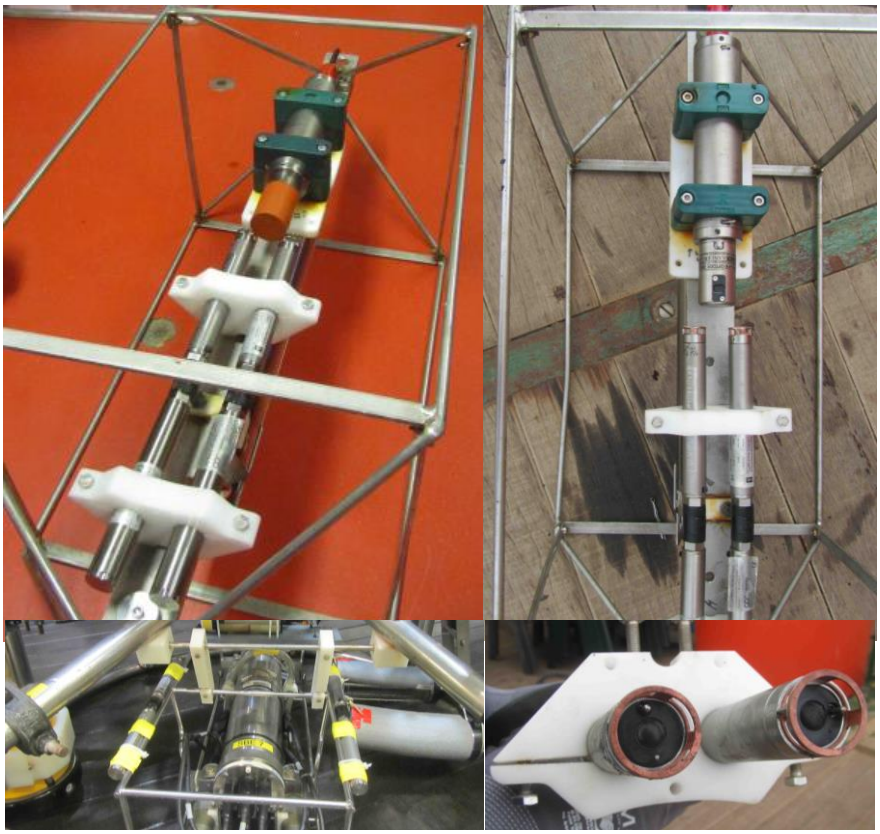


Figure 12 Implementation of two HydroFlash® O_2 optodes at the Cape Verde Ocean Observatory mooring at 120m. Top-left: Measuring cage before the deployment. Bottom-left: CTD calibration setup before the deployment. Top-right and bottom-right: Measuring cage and optode sensor spots after the recovery.

Furthermore, three HydroFlash® O_2 optodes will be installed and used during the Polarstern-cruise PS114 in the North Atlantic (July-August 2018) to obtain surface data and compare three development stages of the optode design during the project.

Final Technology Readiness Level

The final Technology Readiness Level of the HydroFlash® O_2 optodes is TRL 9 (full system proven in operational environment). Identified fields for further investigations beyond the scope of AtlantOS and related to the measuring uncertainty are the influence of high background light levels (e.g. direct solar irradiation) on the measurements and the physical stability of the sensor spots when subjected to mechanical stress.

3.5 Total Alkalinity (TA)

Contact: Allison Schaap (allison.schaap@noc.ac.uk)

Motivation for technology innovation

There is a sparsity of technology capable of undertaking in-situ measurements of total alkalinity in the oceans; the vast majority of measurements are done by sampling that then require laboratory analysis either on a ship or back on shore. Sampling provides the necessary accuracy but observations are tied to the limited deployment of resource intensive ocean going infrastructure.

A handful of autonomous deployable devices have been built and described in literature but they are restricted in their deployable depth, accuracy, and in the number of samples they can do before maintenance is required. A gap exists between what the scientific oceanographic community are requesting for ocean alkalinity measurements and the technology available to them.

Addressed EOVs

Inorganic carbon of which total alkalinity is a sub-variable. It is also a supporting variable for *Stable carbon isotopes*, *Phytoplankton biomass and diversity* and *Hard coral cover and composition*.

Starting Technology Readiness Level

At the start of the AtlantOS project the total alkalinity sensor was at TRL 4. The methodology was a re-development of previous work (Figure 13) but much of the microfluidics, electronics and mechanical housing required limited modification owing to the platform technology approach fostered by the host institution.

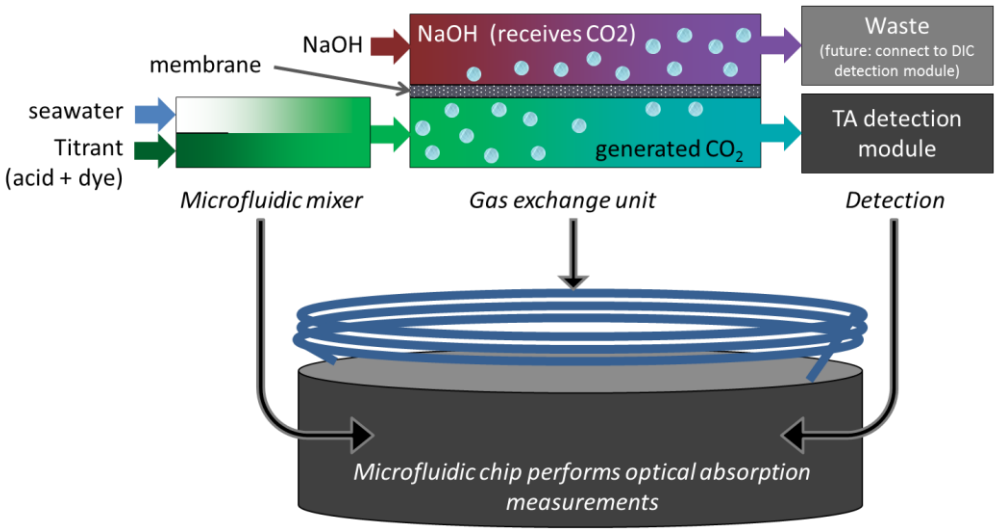


Figure 13 Schematic of system principle of total alkalinity system.

Analytical performance targets

As the technology advances through the TRLs the capability of the device is also scheduled to improve. The current generation of the alkalinity sensor targets an accuracy of 2 µmol/kg and a measurement range of 2000 – 2500 µmol/kg. After the end of the AtlantOS project the operational range will be extended to 1500 – 3000 µmol/kg.

The temporal resolution of the system will be 15 to 30 mins with an endurance of months (dependent on sampling rate and available power).

Method of calibration and performance validation

Sensitivities to temperature were assessed and calibrated for through laboratory testing with a high stability water bath and accurate temperature reference. All other relevant parameters (e.g. Figure 14) were assessed through laboratory and in-field measurements using relevant certified reference materials.

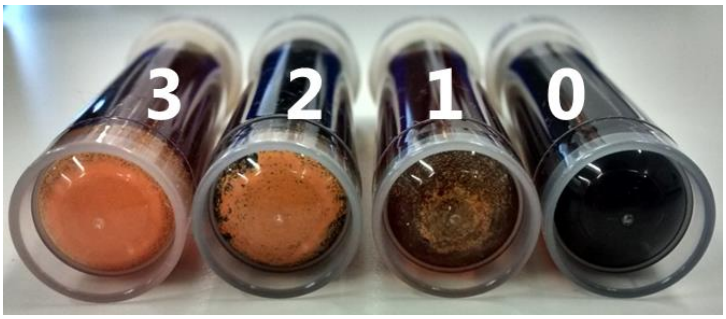


Figure 14 The total alkalinity system uses a pH sensitive dye. A study was required of dye solubility and the effect of surfactants. Note the change in precipitates from test samples 3 to 0.

Performance was validated both in the laboratory and in relevant/in-field operation through measurements against further certified reference materials, laboratory prepared standards and comparison with co-located sampling.

Deployments outside of laboratory validation

The total alkalinity system has been deployed at a dockside in Southampton, UK. It operated continuously and col-located sampling was undertaken throughout the week of testing.

The system is also scheduled to be placed on a lander in the North Sea at the Goldeneye site in co-ordination with another EU project (STEMM-CCS). This testing consists of repeat deployments of a few days at a time over several weeks.

Spring 2019 will see the alkalinity system again placed on a lander at the Goldeneye site but also duplicate units on to various underwater vehicles. This is once again in co-ordination with the STEMM-CCS project.

Final Technology Readiness Level

At the end of the AtlantOS project the total alkalinity sensor will be at TRL 7/8 (prototype demonstrated in an operational environment / qualified through test and demonstration).

3.6 Dissolved Inorganic Carbon (DIC)

Contact: Martin Arundell (m.arundell@noc.ac.uk)

Motivation for technology innovation

Anthropogenic activities have increased the concentration of atmospheric carbon dioxide to levels not seen within the last 400,000 years (Figure 15). The ocean has taken up approximately one third of this carbon dioxide and the resultant change in the marine carbonate system has been a measureable acidification of the ocean. Of the four variables of the marine carbonate system (dissolved inorganic carbon (DIC), pH, total alkalinity and partial pressure of CO_2,) DIC is the only one missing a technical solution to autonomous underway and in situ sensing, despite DIC being one half of the preferred pairs for observing the carbonate system.

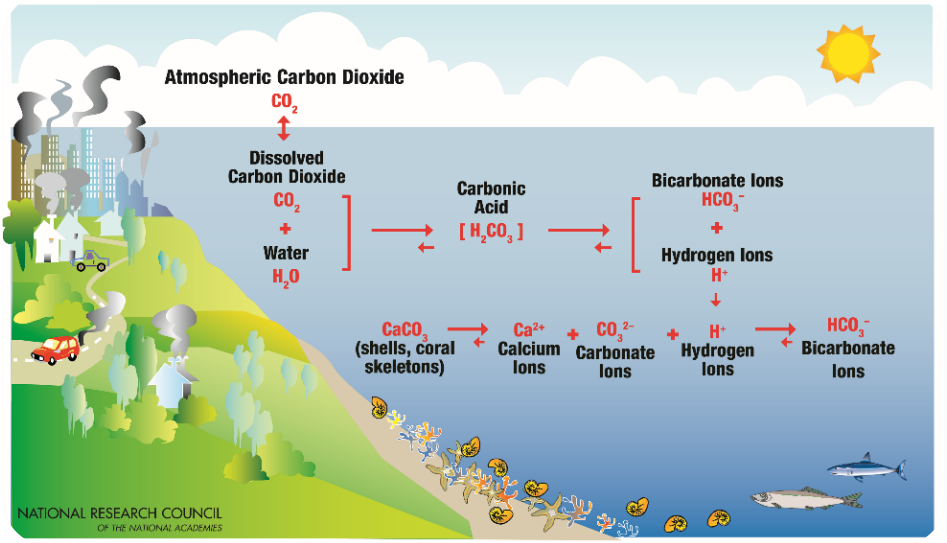


Figure 15 Pathway for atmospheric carbon dioxide to greater acidification of the oceans. Credit: National Research Council of the National Academies.

Addressed EOVs

Inorganic carbon of which DIC is a sub-variable. It is also a sub and supporting variable for *Stable carbon isotopes*.

Starting Technology Readiness Level

At the start of the AtlantOS project the DIC sensor was at TRL 4.

The methodology (Figure 16) has been a major focus of development whilst much of the microfluidics, electronics and mechanical housing required minimal modification owing to the platform technology approach fostered by the host institution.

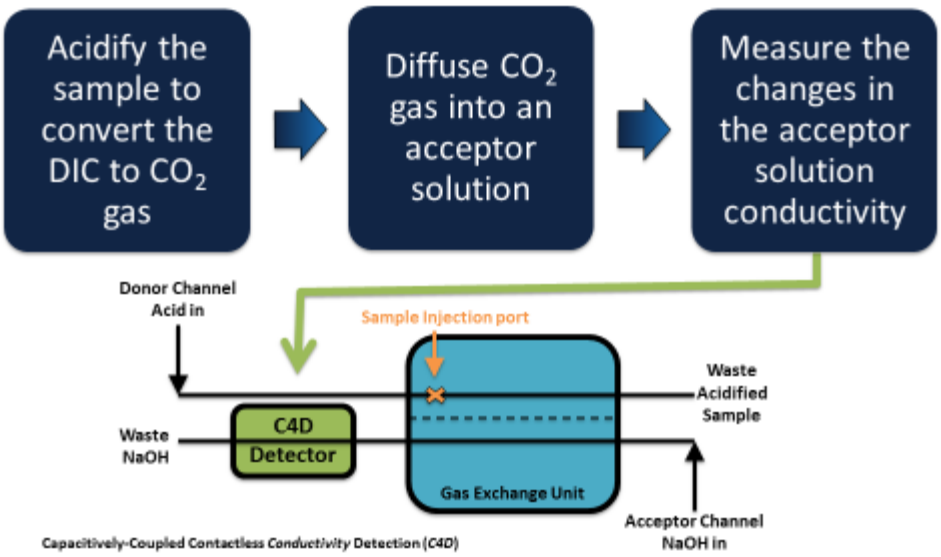


Figure 16 Overview of DIC system operation

Analytical performance targets

The target precision of the system is 2 µmol/kg (with appropriate temperature corrections). This is driven by the requirements stated by the Global Ocean Acidification Observing Network (GOA-ON) to achieve climatically relevant measurements.

The DIC system is calibrated between 1600 and 2600 µM/kg to cover the typical oceanographic values of 1800 to 2200 µM/kg.

Method of calibration and performance validation

Initial development of the DIC sensor was conducted solely in the laboratory using bench top syringe pumps, a ‘tube-in-tube’ membrane gas exchange system and commercial Capacitively Coupled Contactless Conductivity Detector (C4D), Figure 5. Simplex optimisation was used to select flow rates and sample volumes to give the largest signal in the shortest sampling time.

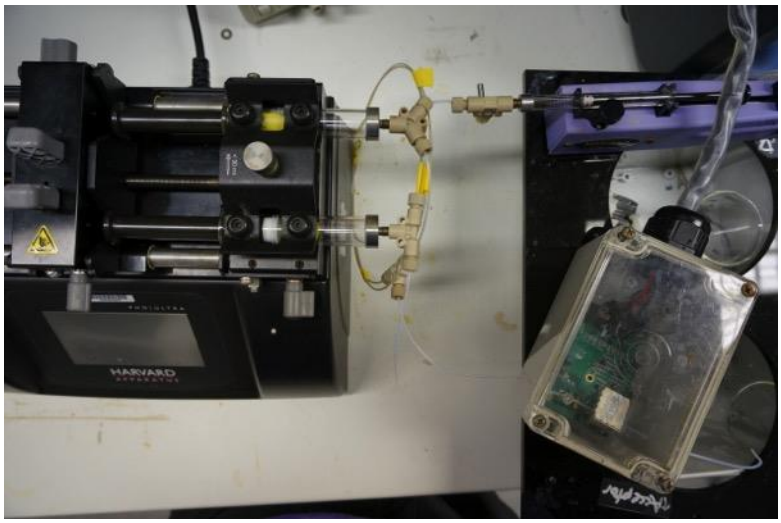
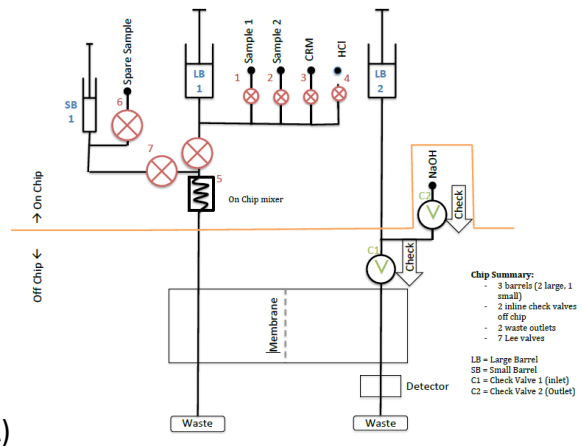


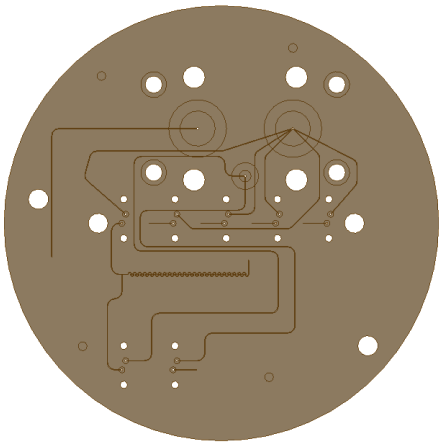
Figure 17 Initial bench top C4D DIC system tested in a simulated environment giving a precision of 22 µmol/kg, showing two syringe pumps on the left, the tube in a tube gas exchange system in the centre, the third syringe and C4D on the right hand side.

Initial calibration of the DIC system used bicarbonate standards between 1000 µmol/kg DIC and 2500 µM/kg which provided a precision of 22 µmol/kg, without temperature correction.

This design was then integrated with the lab-on-chip template developed by the host institution. The schematic (Figure 18 A) shows the system developed with two large syringe barrels, and one small syringe barrel, one for the measurement solution, one for the sample and one for the acid respectively. The PMMA chip (Figure 18 B) also incorporates a micro-mixer to ensure that the acid and sample are thoroughly mixed.



(A)



(B)

Figure 18 (A) Schematic of the DIC lab on a chip, above the orange line shows the features on the chip and below the orange line are modular components situated off chip. (B) Diagram showing the micro-channels, syringe and valve locations on the PMMA chip.

In parallel to the developments supported through AtlantOS a co-running project (STEMM-CCS) is responsible for advances in a bespoke detector and gas exchange system that will be later integrated into the PMMA chip. This second version integrates the planar gas exchange unit and electrodes tailors them for the specific conductivity range required for DIC measurements. A planar gas exchange chip has already been successfully tested with a custom made 4 electrode system (Figure 19) within this parallel body of work, Figure 19.

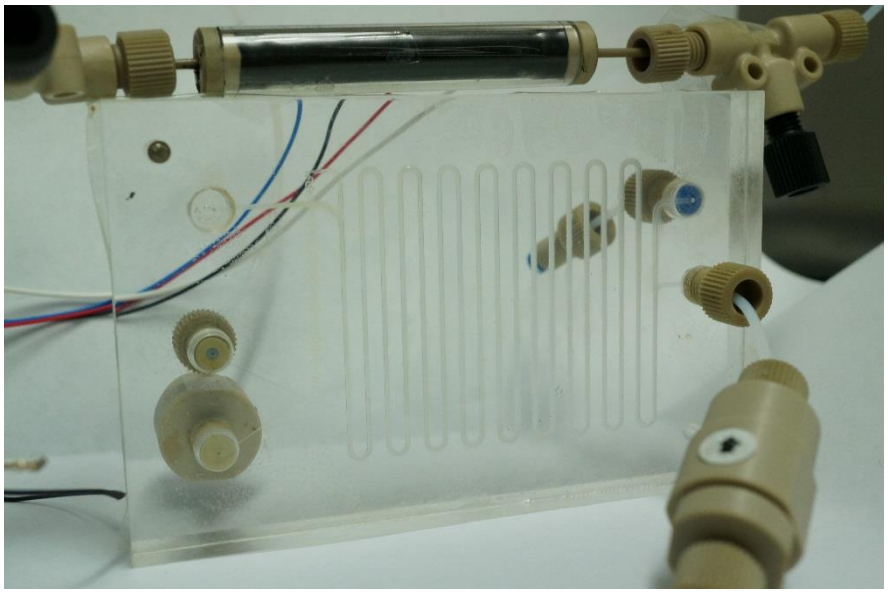


Figure 19 Planar membrane chip (silex 100 µm membrane) with 4 electrode detector and custom made electronics

Before the final fully integrated DIC system is fabricated the version 1 system has been assembled within pressure housing and deployed into a dockside in Southampton, UK, for operation in a relevant environment. Prior to this deployment the system (Figure 5) was calibrated using sodium bi-carbonate (which were also measured by the current internationally accepted standard operating procedure for DIC measurements) and validated using certified reference materials.

Future deployments include being placed as an underway system on a ship and placed on a lander in the North Sea at the Goldeneye site in co-ordination with STEMM-CCS. This testing consists of repeat deployments of a few days at a time over several weeks.

Final Technology Readiness Level

By the end of the AtlantOS project the DIC system has demonstrated a TRL 7 (system prototype demonstrated in an operational environment) and will have started to obtain the evidence to justify a TRL 8 (system qualified through test and demonstration) for the version 1 system. The version 2 system will have reached a TRL of 7.

3.7 Wet Chemical Nutrients and pH

Contact: Matt Mowlem (matm@noc.ac.uk)

Motivation for technology innovation

The measurement of nutrients in the marine environment is well established with recognised methodologies in place for decades, although these are typically from sampling and analysis in a laboratory. Less established are technologies that can deliver in-situ, autonomous measurements to the standard required for scientific study across the pressures experienced and dynamic platforms utilised through oceanographic studies. Microfluidic lab-on-chip sensors are one technology that have recently enjoyed increasing success across fluvial, deep ocean and dynamic platform applications. The technology is such that core components established across a family of sensors enable separate nutrients (and pH) to be targeted by interchanging key components and reagents. Such an approach enables a single technology to address a number of nutrients that in

turn address multiple EOVs. Of the EOVs that are targeted they are recognised as high impact and since the lab-on-chip technology represents a solution for in-situ and autonomous sensing of nutrients it is raising the readiness level of glider and other dynamic platform observations from pilot towards mature.

Focus of technology innovation under the AtlantOS project has been the optimisation of the measurement technique through tightening tolerances on components, refinement of reagent chemistries, thorough analysis of capability and deployments across successively challenging platforms and environments. Operational developments such as 'sleep mode' were also created and trialled during the AtlantOS project (Section 4.1.1).

Addressed EOVs

Nutrients of which nitrate, nitrite, phosphate and silicate are sub variables. pH is also a sub variable for *Dissolved Inorganic Carbon*. Measured parameters are also supporting variables for the EOVs of *Phytoplankton Biomass and Diversity* and *Hard Coral Cover and Composition*.

Starting Technology Readiness Level

At the start of the AtlantOS project most of the lab-on-chip technology was at TRL 7 (System technology prototype demonstrated in an operational environment).

Analytical performance targets

Table 12 is a summary of the analytical performance of the separate types of Lab-on-chip technology. They have been found through dedicated programs of validation against certified standards and checked against references in the field.

Table 12 Performance targets of nutrient lab-on-chip technology

	NO_3+NO_2	**PO_4**	**Si**	**pH**
LOD	20 nM	30 nM	100 nM	n/a
Linear range	0.025 - 1000 µM	0.1 - 40 µM	0.1 - 300 µM	pH 7-9
Accuracy	< 2% in field	< 2% in field	< 2% in field	> 0.004 pH units
Precision	< 2.0 % for concentrations ≥ 3uM	< 2% at 0.5 µM	< 2.0 % for concentrations ≥ 3uM	0.001 pH units
Temporal resolution (measurement / hours)	4 (12 if uncalibrated*)	3 (6 if uncalibrated*)	2.4 (8 if uncalibrated*)	4
Power consumption	1.8 W ave.	2 W ave.	2 W ave.	1.5 W ave.
Sample volume per measurement	320 µL	320 µL	320 µL	550 µL
Reagent volume	300 µL	600 µL	200 µL	3 µL

*calibration standards run alongside sample for accuracy, this can be relaxed to increase sampling rate

Method of calibration and performance validation

Owing to the lab-on-chip technology being used across multiple projects, applications and parameters a thorough calibration and validation procedure was implemented to track performance and guarantee quality prior to use in the field.

Standard operating procedures are now in place for reagent and standard preparation, result recording and quality assurance procedures. It is now regularly the case that a quality assurance report is generated for each lab-on-chip system that includes details on used certified reference materials, limits of detection and calibration curves such as in Figure 20.

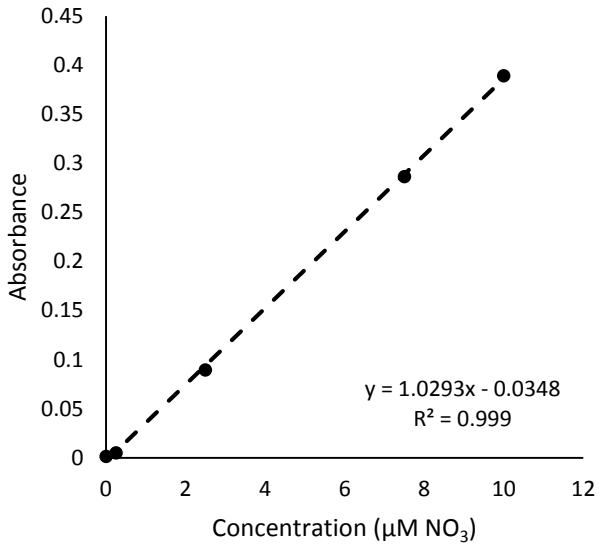


Figure 20 Long Channel Calibration Curve of a Nitrate system

Each systems performance is measured against an appropriate certified reference material and record keeping has facilitated better error analysis and development of numerous protocols, scheduling procedures and preparation check lists.

Deployments outside of laboratory validation

As the lab-on-chip technology was reasonably mature when entering the AtlantOS project there has been the opportunity to offer the technology for a wide range of observations, Table 13**.** Whilst technology development of the core components was limited the optimisation of reagent chemistries, state machine scheduling and appropriate housings for the wide range of deployments has been of vital importance.

Of note is the range of platforms that have been accommodated, namely; fixed shallow and deep landers, profiling floats and gliders. Novel adaptations for operation with a Wave Glider[8] and CEFAS SmartBuoys[9] are included later in Section 4.1.

[8] https://www.liquid-robotics.com/wave-glider/overview/

[9] https://www.cefas.co.uk/cefas-data-hub/smartbuoys/

Table 13 Selection of nutrient lab-on-chip deployments undertaken since the start of the AtlantOS project

	Deployment location	Deployment environment	Ave. temp (°C)	Deployment length	Sampling frequency	Deployment comments	Sensor type	Ref.
1	Southampton Water, U.K.	Estuarine	11	26 days	Hourly		Nitrate+Nitrite	(Beaton et al., 2012)
2	Hampshire Avon, U.K.	Fluvial	5-15	1 year	Hourly	3 monthly reagent change	Nitrate+Nitrite	
2	Fram Strait Fix O^3 mooring, Greenland Sea	Ocean	-2 – 5	1 year	2 per day	-2°C, 80 m deep, unattended deployment	Nitrate+Nitrite	
4	Hampshire Avon, U.K.	Fluvial	10	63 days	Hourly		Phosphate	(Clinton-Bailey et al., 2017)
5	Chesapeake Bay, U.S.A.	Coastal	10-20	3 months	Hourly	Nutrient sensor challenge	Phosphate Nitrate+Nitrite	(Grand et al., 2017)
6	Maumee River, U.S.A.	Fluvial	10-20	1 month	Hourly	Nutrient sensor challenge	Phosphate Nitrate+Nitrite	(Grand et al., 2017)
7	Coconut Island, U.S.A	Coastal	10-20	1 month	Hourly	Nutrient sensor challenge	Phosphate Nitrate+Nitrite	(Grand et al., 2017)
8	Greenland, Denmark	Glacial meltwater	-2 - 2	14 days	Hourly		Nitrate+Nitrite	
9	Steffan Glacier, Chile	Glacial meltwater + Fjord	-2 - 2	25 days	Hourly		Phosphate Nitrate+Nitrite	
10	Mauritanian upwelling region, Western Africa	Ocean, benthic	10-20	5 deployments ranging from 1 day to 2 weeks.	Every 15 minutes	Deployed on a lander at depths of 50 and 100 m.	Nitrate+Nitrite	(Yücel et al., 2015)
11	Celtic Sea	Oceanic	7-15	1 month	Every 5 minutes	Various glider deployments	Nitrate+nitrite	
12	Seychelles, East Africa	Coastal (Marine Park)	20-25	On-going	Every 4 hours	Reagents are changed every 7 weeks; ~25°C; ~15 m deep.	Phosphate Nitrate+Nitrite and pH	
13	Artic Sea	Ocean	-2 - 1	48 hours	Every 10 minutes	Under sea ice (-1.9oC)	pH	
14	Guillmar Fjord, Sweden	Estuarine	5-10	1 month	Hourly		pH	
15	GEOMAR pontoon, Keil, Germany	Estuarine	5-10	2 weeks	Hourly		Phosphate Nitrate+Nitrite, iron and pH	

Final Technology Readiness Level

At the end of the AtlantOS project the separate lab-on-chip nutrient (and pH) sensors will achieve a TRL of 8/9 (System technology qualified through test and demonstration/ successful mission operations).

3.8 Marine Autonomous Plankton Sampler (MAPS)

Contact: Julie Robidart (j.robidart@noc.ac.uk)

Motivation for technology innovation

While many sensors exist for biological monitoring in the ocean, they are either currently restricted in the types of organisms they can monitor or by the need for a trained technician to operate them. The analysis of DNA, which is universal among life on earth, allows us to detect any living organism, from microbes to whales. Since DNA and RNA detection is the primary methodology to distinguish most bacteria on earth its application to marine microorganisms is advanced. The field of environmental DNA, or eDNA, is a newer one, using DNA from sloughed off cellular material from metazoans to allow high-sensitivity detection of rare or cryptic species.

eDNA analytics requires hours of filtration to be undertaken to collect material for analysis. This is principally performed at sea as soon as the waters are collected at site. It is a major benefit to automate this simple process to optimise scientist and ship time.

To address the problem two autosamplers for high spatio- and temporal resolution filtration and preservation of cellular material for later lab-based 'omics' analyses have been developed. The first (Figure 21), is a cartridge system suitable for underway sampling on ships and placing on static floating platforms (e.g. buoys).

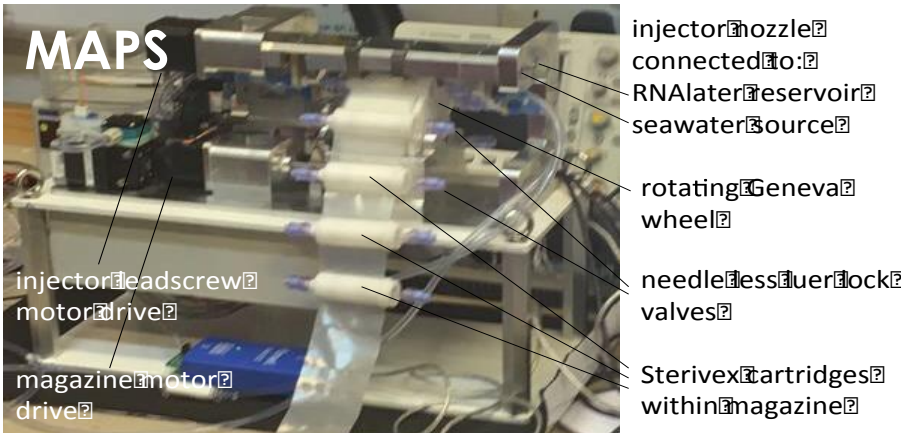


Figure 21 The Marine Autonomous Plankton Sampler. Cartridge sampled hourly from a trace metal clean seawater intake (the TowFish) during 10 days of an Atlantic Explorer cruise 1714 (AE1714). This sampling was coupled to continuous measurements of N_2 fixation rates in collaboration with the Nicolas Cassar Lab (Duke University).

The MAPS cartridge device has been designed to acquire planktonic biomaterial including cells and suspended particulates by pumping seawater through a 0.2 um Sterivex (Millipore) cartridge filter. The collected material is then preserved using RNAlater (Thermo Fisher Scientific) and archived for later lab analyses. MAPS can filter and archive a 4L open ocean (low biomass) sample every 45 minutes.

The second development uses a reel-to-reel design that further increases the number of samples that can be made autonomously and in-situ. The aim for this design is for in-situ sampling on submerged marine platforms (e.g. landers) or even mobile submersible platforms (e.g. crawlers). The development of this design of the technology is led by WP6 partners TELLABS.

Addressed EOVs

Fish abundance and distribution and *Hard coral cover and composition* of which eDNA (and hence MAPS contributes towards) is a sub and derived variable. eDNA is also a derived variable of *Phytoplankton biomass and diversity and Mangrove cover*. It is also of vital importance to the emerging EOVs of *Microbe abundance and diversity* and *Benthic invertebrate abundance and distribution*.

Starting Technology Readiness Level

At the start of the AtlantOS project the two types of MAPS designs (cartridge and reel-to-reel) were at TRL 2 (Technology concept and or application formulated).

Analytical performance targets

The aim of the MAPS technology is that the quantity, quality of DNA and RNA collected is comparable to flash-freezing following traditional pumping and filtration undertaken on research vessels. The composition of collected DNA and RNA (using next generation sequencing) should also be comparable to flash-freezing so that recovered material is amenable to qPCR quantification of known targets.

Method of calibration and performance validation

The cartridge MAPS design has seen the fastest development through the AtlantOS project, quickly becoming a system suitable for underway sampling on a research ship. To validate the technology parallel samples were taken from benchtop filtration by MAPS and flash-freezing (traditional SOPs).

Flash frozen samples and those from the cartridge MAPS system had the DNA / RNA extracted and purified using the same methodologies. cDNA was then synthesized from RNA. Comparisons between flash-frozen and MAPS-collected samples were performed through (1) BioAnalyzer for quality (Quality was high and found to be comparable for DNA and RNA) (2) Qubit for quantification (results in Figure 22) and (3) (RT-)qPCR for transcript and gene quantification, evaluation of inhibitors (results in Figure 23).

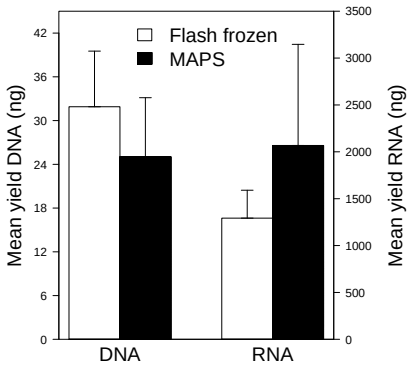


Figure 22 DNA and RNA quantity were evaluated by Qubit on 4 parallel samples. MAPS-preserved samples had comparable DNA and RNA recovery.

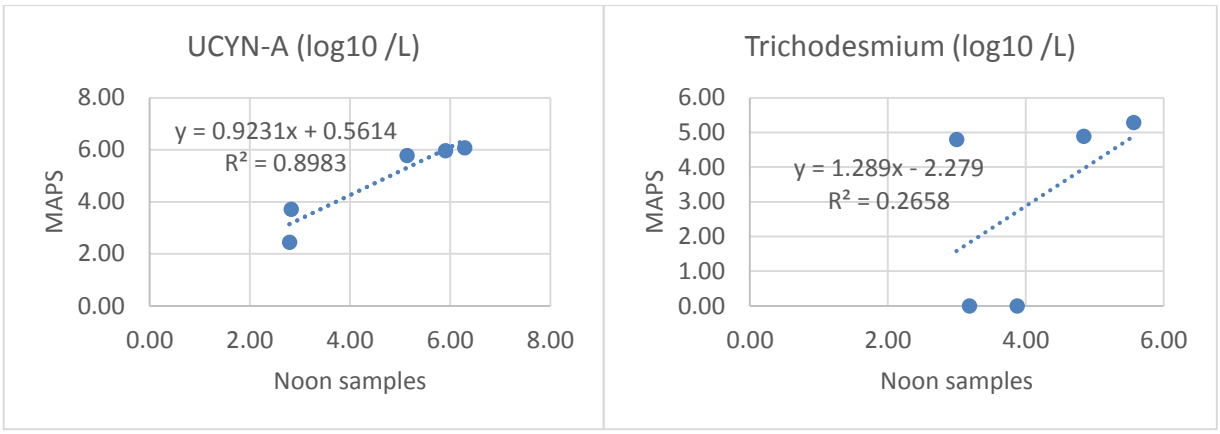


Figure 23 qPCR comparison of UCYN-A and Trichodesmium nifH genes (coding for the nitrogenase enzyme) quantified from MAPS and flash frozen samples. 5 out of 9 flash frozen samples have close matches. The other 4 MAPS samples have not been measured. UCYN-A shows good agreement. We need to note that there are spatial differences between flash frozen and MAPS samples since MAPS samples were collected underway and flash-frozen samples were discrete samples, and that Trichodesmium (a colonial organism) often shows high heterogeneity in parallel samples.

Analysis through MiSeq 16S rRNA, 18S rRNA and Cytochrome oxidase I for sequencing of Bacteria, Eukaryotes and metazoans is scheduled for completion in 2019.

Development of the reel-to-reel MAPS design is less mature and so to date hasn't entered a phase of validation. The design and manufacture of key components and assembly has progressed well. The device is a fully automated sampler which can be submerged. The core design of the pumping, clamping and sealing mechanisms is in its final stages. Once the filter reel design has been finalised, the layout of the sample reel holders and overall size of the system will be determined. The system is designed so it can take a minimum of 200 samples before being returned to the lab.

The image in Figure 24 shows each of the main components in the design. An Arduino Mega 2560 controls the sequence of actuation and timing of the device. A 12 V battery is used to power the device and there is room for another battery to be added in parallel to increase the length of deployment. Two 3D printed reservoirs and two solenoid valves are used to control the flow of water and RNAlater.

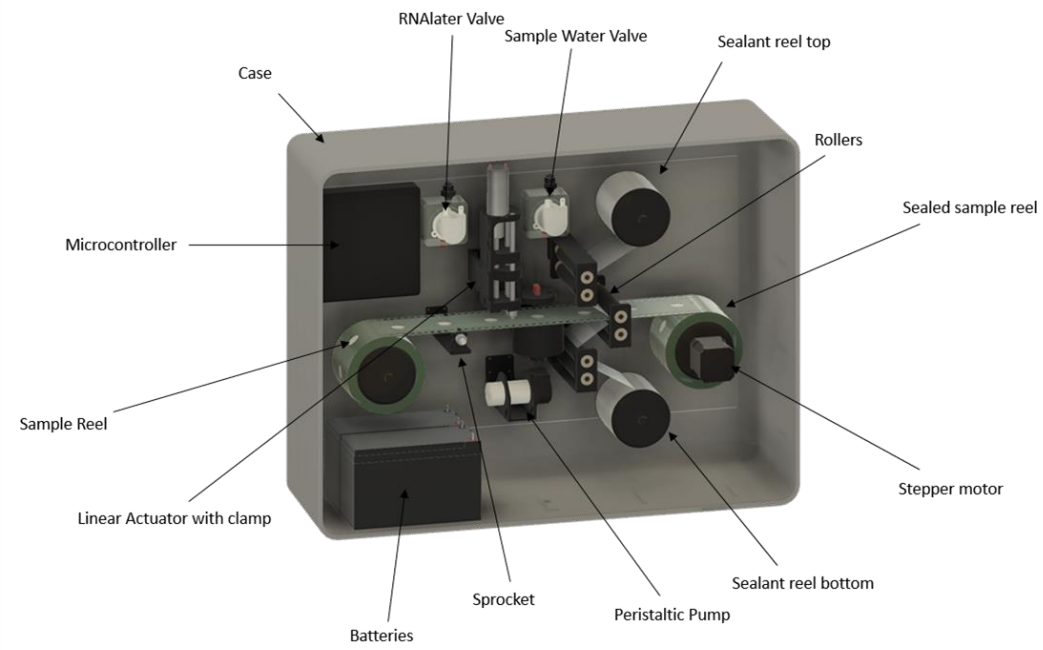


Figure 24 Components in design

Figure 25 and Figure 26 show the assembly of the sample reel. The sample reel is composed of a sheet of PES 0.22 µm membrane and two sheets of plastic. Sprockets are used to keep the sample reel aligned. 3D printed rollers are used to compress the sealant plastic reel to the sample reel.

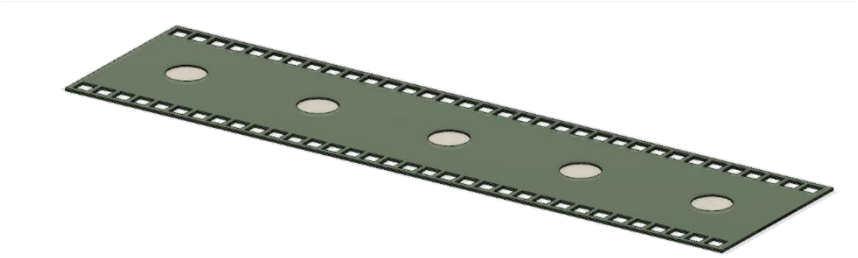


Figure 25 Assembled sample reel

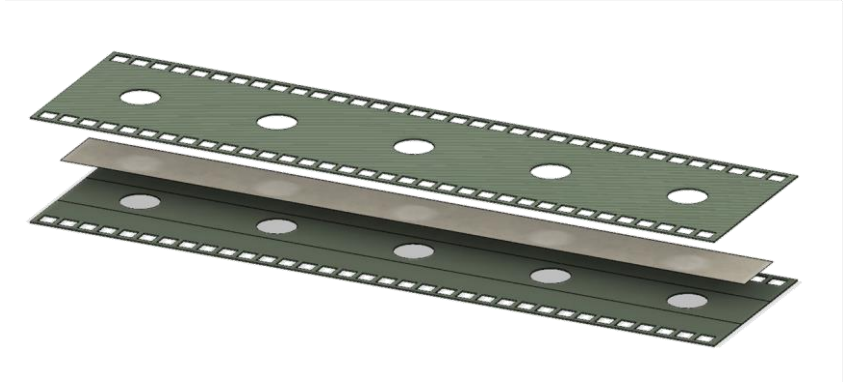


Figure 26 Exploded view of sample view

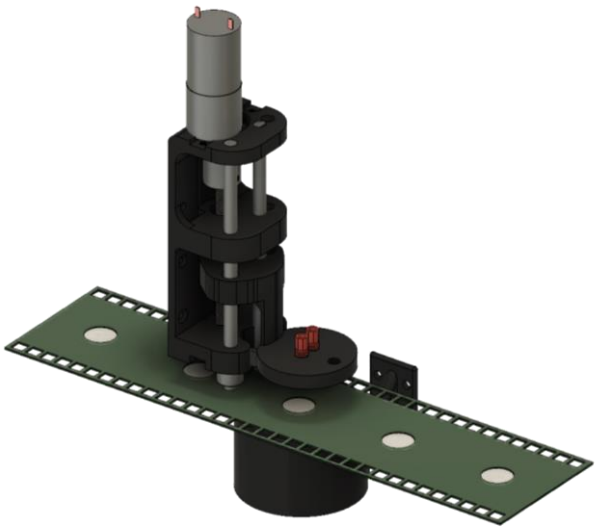


Figure 27 Linear actuator and clamp

The 0.22µm PES membrane is housed in a plastic reel, the linear actuator clamps the reel and seals it. After all the samples have been taken the reel is brought back to the lab where the samples will be analysed.

Before the reel is manufactured all the components are to be tested and sequencing of the electronics finalised.

Figure 28 is the current state of the device (July 2018). All the main components, apart from the sample reel have been manufactured. Initial testing has begun on the electronics and the sequencing.

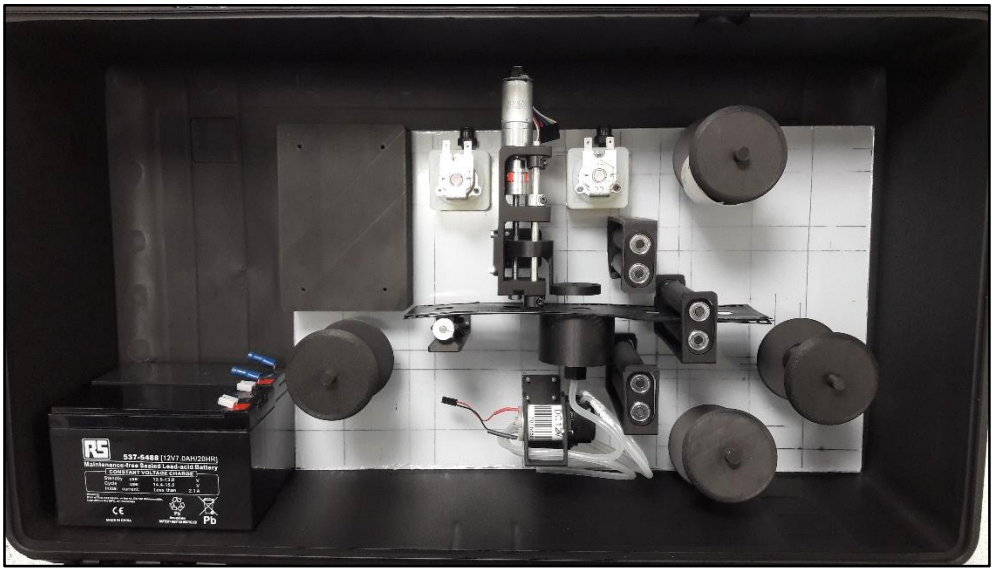


Figure 28 3D printed components assembled

Currently the device is TRL 3-4. As work progresses through prototyping, testing and validation stages the system will reach TRL 6. The schedule for the MAPS reel-to-reel development has submerged testing in the lab early 2019 with field testing by the end of the AtlantOS project.

Deployments outside of laboratory validation

The MAPS cartridge design was deployed as benchtop technology sampling from the underway system during AE1714 throughout August 2017 around Bermuda and NW Atlantic Figure 29. It is from this deployment samples were collected in parallel to the standard flash freezing that have been used for validation.

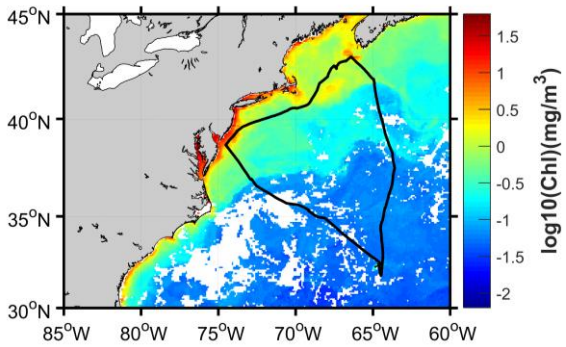


Figure 29 AE1714 Nicolas Cassar - Duke

After returning from AE1714 the MAPS cartridge system was refurbished and placed on the L4 buoy in the Western Channel Observatory May 2018 to sample for 40 days.

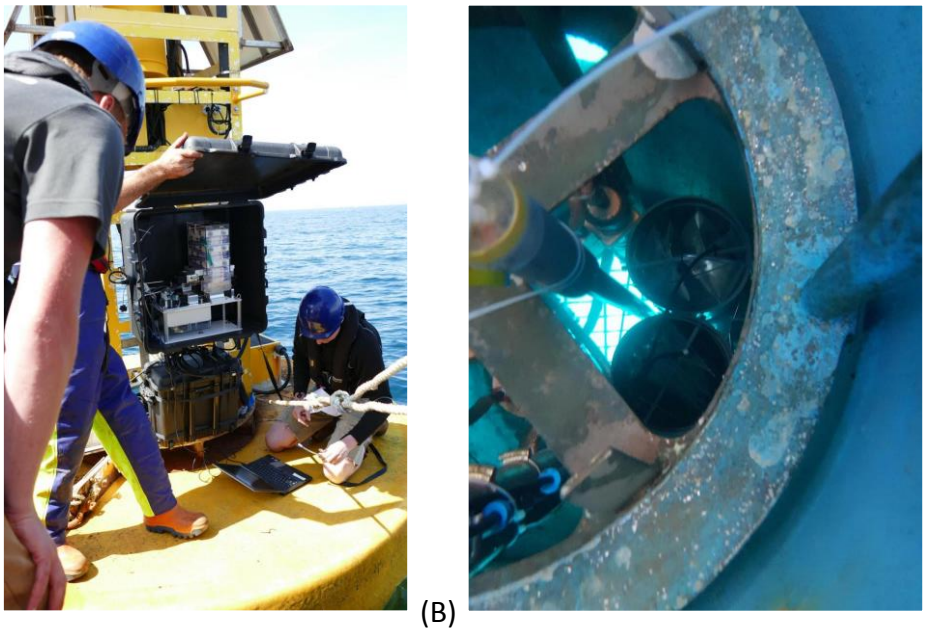


Figure 30 (A) The MAPS was configured with a submersible pump to supply seawater, where it was programmed to filter daily at noon at the Station L4 buoy in the Western Channel Observatory, over the course of 40 days. (B) Nitrate and phosphate Lab-on-a-chip sensors were deployed in the moonpool of the buoy, at the seawater intake.

Results of the latest deployment are pending although further optimisation of the system is expected.

Final Technology Readiness Level

At the end of the AtlantOS project the MAPS cartridge system will reach TRL 7 and the reel-to-reel design will have reached a TRL of 6.

3.9 Environmental Sample Processor (ESP)

Contact: Einar Eg Nielsen (een@aqua.dtu.dk)

Motivation for technology innovation

All aquatic species continuously expel their DNA to the surrounding water. This external DNA (eDNA) can be collected, extracted and analysed providing information on local biodiversity based on captured species-specific DNA sequences. eDNA has revolutionized how we monitor and study aquatic organisms as the methodology has proven to be a sensitive, non-invasive and an easy-to-standardize sampling technique. When coupled with ever-advancing DNA technology eDNA has proven to be cost-competitive when compared to established monitoring approaches and is capable of revealing important temporal patterns of fin-fish migration, community structure and the introduction of invasive species.

However, it is typical that time-consuming and expensive manual sampling of waters at designated sampling sites is required in preparation of eDNA analysis, especially open water time-series. After the water is collected the sample then needs to be processed manually in a laboratory, which is labour intensive and extends the time from sample to result by several weeks.

Reducing the manual labour and improving the time from sample to result drastically enhances the application of eDNA methodology and hence provides an unmatched monitoring tool in relation to adaptive marine management and conservation.

The traditional sampling challenges highlighted above can potentially be alleviated with automation. The so-called “ecogenomic sensors” are automated instruments that can be deployed outside the laboratory and provide *in-situ* collection and DNA analysis of water samples from subsurface without human interaction. One such system is the 2nd Generation Environmental Sample Processor (2G ESP). The instrument is an elaborate submersible electromechanical fluidics system which collects discrete water samples, extracts and analyses DNA through multiple analytical pathways. The 2G ESP offers two-way communication, providing complete user control and enables remote retrieval of data generated in near real-time (a few hours).

The 2G ESP platform offers all the features needed to complete an *in situ* sample-to-result eDNA analysis, the applicability remains to be tested and demonstrated under controlled settings and in the field and it is this activity that has been supported through the AtlantOS project.

Addressed EOVs

Fish abundance and distribution. Monitoring of commercial fish is essential for proper management and conservation of fish stocks and ecosystem function. The eDNA methodology combined with the versatility of remote automated sampling and analysis provides a new paradigm in ocean biodiversity monitoring for fish and other organisms. For example, eDNA is not named as a sub-variable for the EOV, instead the traditional measures of fishing effort and management are stated – such is the novelty of the technique to monitor fish abundance and distribution.

To test the eDNA methodology on the 2G ESP we target a number of fish species of commercial and public interest to investigate the ability of the 2G ESP to provide ecological information such as presence/absence, relative abundance and timing of migration. The target species are a combination of both a pelagic fish species (mackerel) and more stationary benthic fish species (plaice, flounder and eel).

Starting Technology Readiness Level

The core 2G ESP has a broad suite of *in situ* molecular analysis options, but can also collect and chemically preserve the DNA for analyses in a normal molecular laboratory after the deployment of the instrument.

These methods all use DNA extracted directly from the organisms of interest, thus the DNA is in relatively high concentration. However, in order to use the 2G ESP for eDNA analysis, where the DNA from the target organisms is in very low concentration in the water, a method to specifically amplify the target DNA through PCR (Polymerase Chain Reaction) is warranted. In order to do so we added a Micro Fluidic Block (MFB) module to the core 2G ESP (Preston et al., 2011[10]).

This provides an *in situ* quantitative real-time PCR (qPCR) analysis option, as qPCR is one of the most utilized analysis techniques for conventional laboratory based eDNA analysis. The number of samples that the 2G ESP can process per deployment is dependent on the type of analysis conducted. For *in situ* DNA analysis with qPCR it is possible to analyse up to 66 samples per deployment, while if only collects archival samples are made up to 132 samples per deployment can be taken.

At the start of the AtlantOS project the TRL is 7. The core 2G ESP instrument is commercially available and can be purchased from McLane Labs (www.mclanelabs.com), but the additional MFB module is presently not commercially available. The wide usage of the 2G ESP is currently limited by a high purchase and operating cost. Specialist training is also required in order to operate the technology.

The 2G ESP instrument from previous deployments has shown high value for an array of practical monitoring applications, such as; zooplankton detection and distribution (e.g. Harvey et al., 2012[11]) and reporting of public health threats by monitoring harmful algal and bacteria species (e.g. Yamahara et al., 2015[12]). The deployment time of the ESP can be up to six months and has been tested to a depth of 4000 meters; however, the most common deployment depth is shallow at around 20 meters (McQuillan & Robidart, 2017[13]).

[10] Preston, C. M., Harris, A., Ryan, J. P., Roman, B., Marin, R., Jensen, S., … Scholin, C. a. (2011). Underwater application of quantitative PCR on an ocean mooring. *PloS One*, *6*(8), e22522. doi:10.1371/journal.pone.0022522

[11] Harvey, J. B. J., Ryan, J. P., Marin, R., Preston, C. M., Alvarado, N., Scholin, C. A., & Vrijenhoek, R. C. (2012). Robotic sampling, in situ monitoring and molecular detection of marine zooplankton. *Journal of Experimental Marine Biology and Ecology*, *413*, 60–70. doi:10.1016/j.jembe.2011.11.022

[12] Yamahara, K. M., Demir-Hilton, E., Preston, C. M., Marin, R., Pargett, D., Roman, B., … Scholin, C. A. (2015). Simultaneous monitoring of faecal indicators and harmful algae using an in-situ autonomous sensor. *Letters in Applied Microbiology*, *61*(2), 130–138. doi:10.1111/lam.12432

[13] McQuillan, J. S., & Robidart, J. C. (2017). Molecular-biological sensing in aquatic environments: recent developments and emerging capabilities. *Current Opinion in Biotechnology*, *45*, 43–50. doi:10.1016/J.COPBIO.2016.11.022

Analytical performance targets

66 qPCR samples or 132 archival samples per deployment. A mix of the two types of samples is possible within a deployment, although the maximum number of phases is 44.

eDNA analysis performance determined by reagent selection and target species.

Method of calibration and performance validation

Before each deployment the 2G ESP goes through a thorough calibration and maintenance check, which can take up to three months.

For validation of the 2G ESP for eDNA analysis the technology was deployed in the North Sea Oceanarium in their Oceanarium tank (Northern Europe's largest fish tank). This provided a location to test the 2G ESP in a controlled yet semi-natural environment. The system was placed beside the edge of the aquarium tank with a pump pushing water from the tank into the 2G ESP. Since testing of the 2G ESP was performed in an aquarium it was possible to know at all times which species should be detected. Likewise, the species-specific DNA concentration was expected to be reasonably constant with time since the biomass in the tank was static. Another practical advantage was that it allowed easy access to the instrument when challenges occurred during the deployment. The 2G ESP targeted four species, European flounder (Platichthys flesus), European plaice (Pleuronectes platessa), Atlantic mackerel (Scomber scombus) and European eel (Anguilla anguilla), of which three were present in the aquarium (no eel) but occurring in varying numbers and biomass.

Valuable experience was obtained in operating the instrument and knowledge about its limitations for eDNA analysis and how to optimise its performance for future deployments. Figure 31 shows the experimental setup at the aquarium. The only part not shown is the small 12 V pump, which pushed water from the tank and into the 2G ESP. The deployment took place from the end of January until mid-March. Total deployment time was 51 days with 30 sampling days in total. Each day of sampling was composed of one *in situ* eDNA analysis and one or two *in situ* archival sample collections (water collection followed by chemical preservation and on board storage). After recovery of the 2G ESP, the archived samples were processed and analysed with a benchtop qPCR instrument in a laboratory.

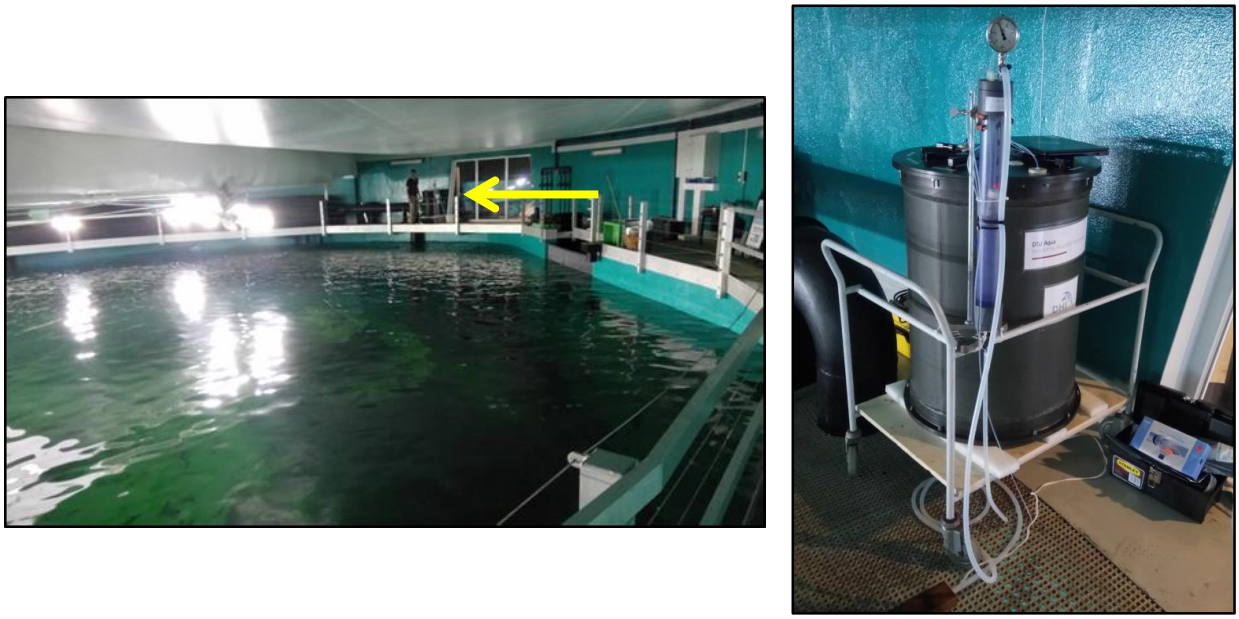


Figure 31 Test in the 4.5 million litre tank in the North Sea Oceanarium. The position of the ESP in relation to the tank is illustrated in the photo (indicated by yellow arrow). Right: The 2G ESP sampling setup in the North Sea Oceanarium. For the deployment, the 2G ESP is inserted into a sealed waterproof metal container, which is the big can in back. On the outside of the can is a "phase-separator", which is the long plastic tube with the pressure gauge. This is connected via tubing to the pump and the 2G ESP. The pump drags water from the tank into the phase separator, the phase separator then builds pressure (1-2 bar) and forces the water into the 2G ESP. After each sampling the phase separator is completely drained, ensuring effective water replacement for the next sample.

Preliminary results from the deployment in the North Sea Oceanarium show the first ever fully automated eDNA based detection of marine fish species in near-real time (Figure 32). Our results demonstrate that the 2G ESP was able to consistently detect and quantify DNA molecules from the most abundant species (Atlantic mackerel) over the study period (Figure 34). The results also indicate that the concentration (likely related to the actual biomass) was fairly constant, which would be expected since the biomass in the tank was static.

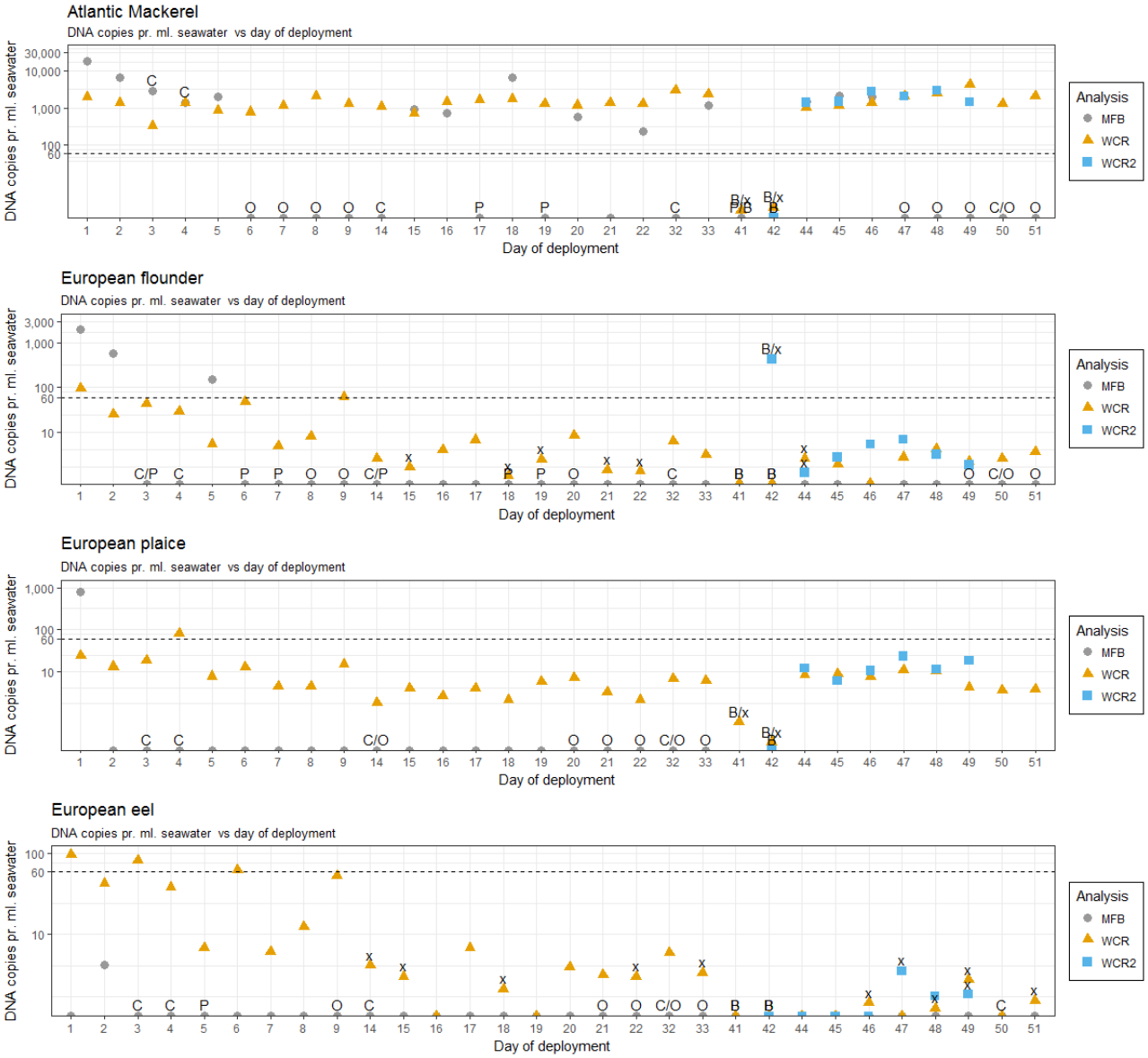


Figure 32 Figures showing preliminary results for the 51 day deployment at the North Sea Oceanarium. Colours and symbols indicate the analytical approach. MFB refers to in situ qPCR DNA analysis by the 2G ESP, where WCR and WCR2 refer to in situ sample archival which were the qPCR analysis was conducted in a conventional laboratory after instrument recovery. WCR samples were collected at 10:00 in the morning, straight after sampling for the in situ qPCR DNA analysis, and WCR2 samples were taken at 8:00 in the evening, subsequent to the in situ analysis. O indicates when a reagent was out of prime (no reaction). P indicates that an increase in fluorescence was observed and the DNA was detected but not quantifiable. x indicates that only 1 or 2 out of three identical reactions amplified. This is only shown for archival samples (WCR and WCR2), which were analysed in the laboratory. B indicates a breakdown of the pump during deployment. C refers to negative control of the entire analysis pathway performed by in situ analysis. The unit for the negative controls are in copies pr. reaction as no water was collected; therefore, these values are artificially inflated.

Archival samples were collected following all *in situ* eDNA analyses providing the opportunity to compare the performance of the 2G ESP with traditional benchtop qPCR instrument analysis. The comparison of the in situ and laboratory based eDNA analyses showed, for most of the samples, good correlation between *in situ* and benchtop results. However, the benchtop measurements were found to be more sensitive for samples with below 10^2 DNA copies/ml. Detection of the less abundant species, European flounder and European plaice, was challenged by several factors, both

related to biological and more technical aspects of the current 2G ESP technology. Firstly, in general we found, for the majority of the sampling days, less than 10^2 DNA copies/ml for both European flounder and European plaice when analysing the archival samples. In comparison we only saw sporadic amplification using the *in situ* analysis. We believe this difference can partly be explained by the time it takes to sample, extract and analyse the DNA *in situ* on the 2G ESP (Figure 33). Time from start of water sampling to qPCR results from the first species took around 4 hours, while the complete analysis for all 4 species took roughly 12.5 hours.

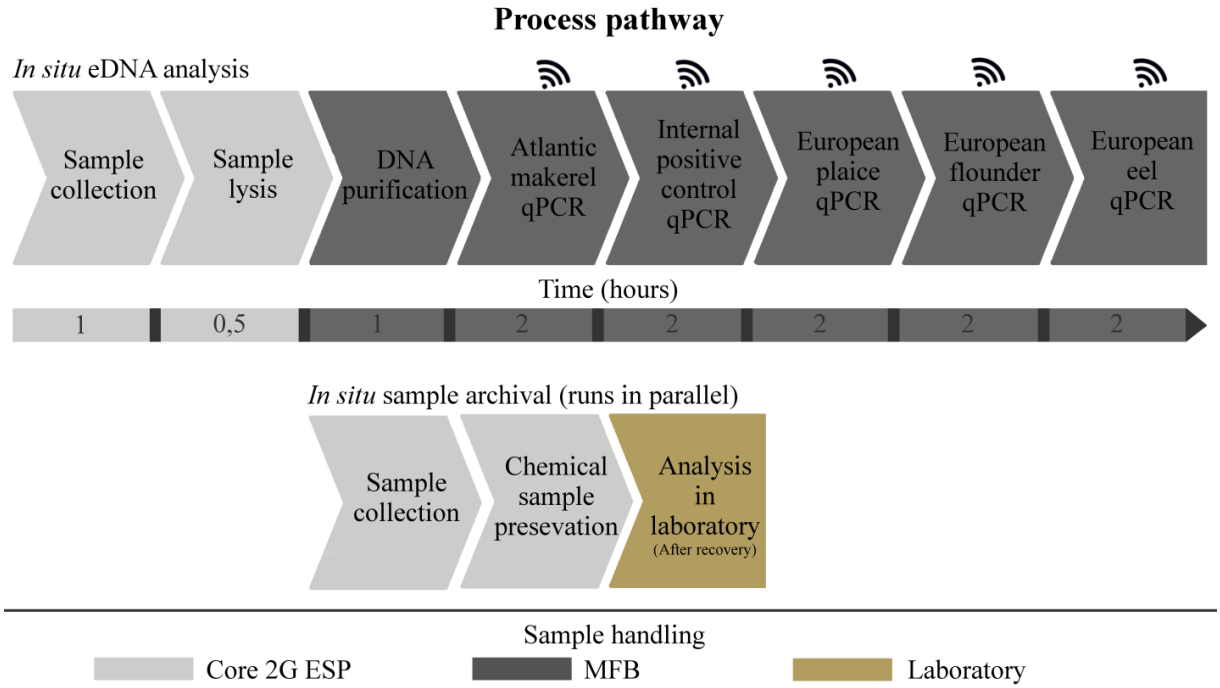


Figure 33 Schematic illustration of the sampling and analytical process during deployment of the 2G ESP in the North Sea Oceanarium. The archival sample processing, starts as soon as the in situ eDNA analysis has completed the usage of the core 2G ESP for processing of the water sample (collection and lysis of sample).

As the qPCR analysis for each species are run sequentially on the 2G ESP, there is up to 10 hours between the first and the last qPCR analysis. DNA degrades over time so low numbers of DNA molecules from a not so abundant species within a sample could degrade within hours and below the level of detection. Secondly, the chemistry for detection of European plaice behaved in a different way than had been observed through laboratory tests and did not appear to work as well in the complex matrix experienced during the deployment. To verify functionality of all detection chemistries they were tested against DNA standards before and after deployment. The detection chemistries are recommended by the manufactures to be held on ice and stored at -20 °C. This is not possible with the 2G ESP setup and the reagents are therefore subject to storage at room temperature for up to half a year, such was the case for this deployment. Despite manufacture recommendations, the chemistries were able to provide an equally capable standard (% efficiency) after deployment, but showed a decrease in sensitivity. Detection assays for European eel, European flounder and European plaice were only sporadically detected with < 6 ×10^2 DNA copies/reaction after deployment.

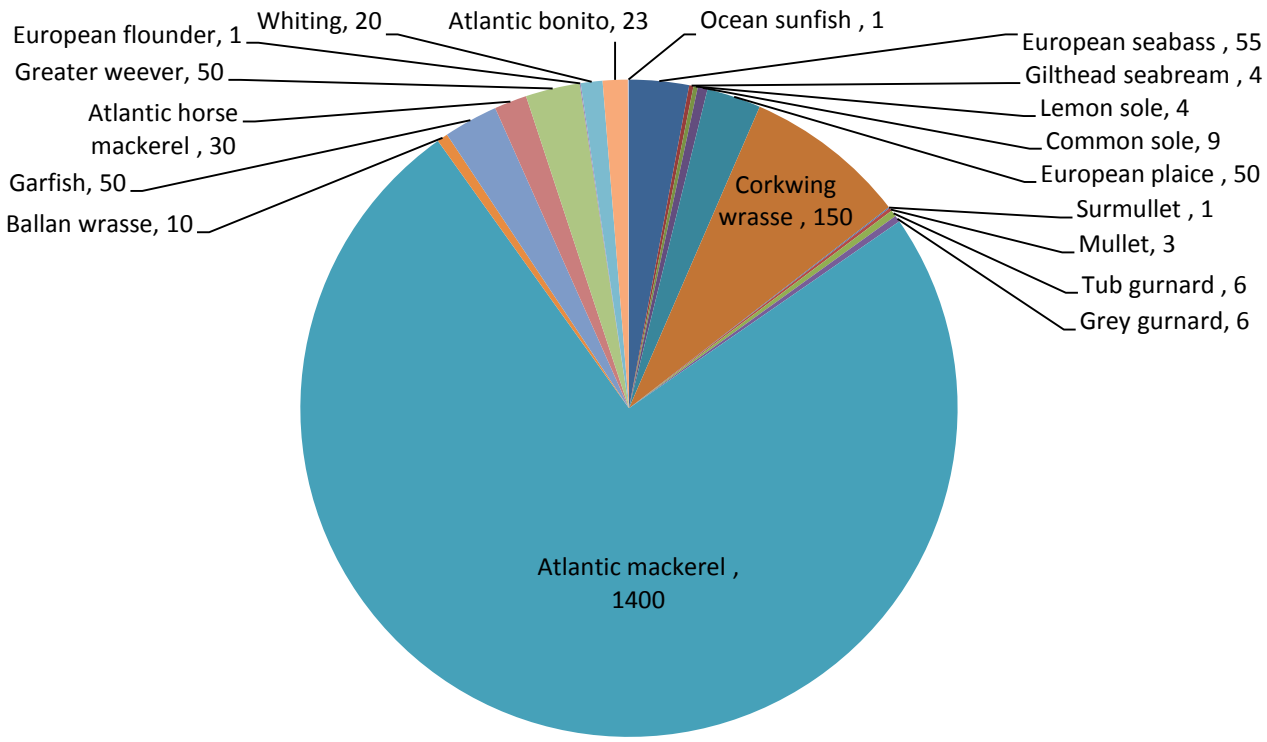


Figure 34 Biodiversity and abundance of fish in the 4.5 million litre tank in the North Sea Oceanarium. The number behind each species refers to an estimated number of individuals. There were also 6 species of shark and 2 ray species in the tank. These are however not included in the chart above.

European eel was detected twice by the *in situ* analysis during the sampling period and almost routinely detected in the archival samples. This is despite eels not being released in the tank. As a potential source of eDNA contamination, we analysed the water intake to the tank. The water from the intake is pulled in from the nearby ocean and during the time of deployment the intake of water is the smaller than any other time of year. Nevertheless, we did find DNA from European eel in the intake water.

Despite the challenges and some technical limitations of the current 2G ESP technology, the results are a good first step and the experience gained allows greater optimisation and improvement of the instrument for future deployments. For instance, changing the schedule to analyse for the expected least abundant species first is likely to mitigate DNA degradation for species detection.

Deployments outside of laboratory validation

As well as the deployment in the North Sea Oceanarium (Hirtshals, Denmark) a second deployment is scheduled in an open water subsurface deployment in the Skagerrak, North Sea, Eastern Atlantic, where the 2G ESP will target Atlantic mackerel (*Scomber scombus*), Garfish (*Belone belone*), *Atlantic bluefin tuna* (*Thunnus thynnus*) and Atlantic bonito (*Sarda sarda*). The aim is to deploy it in open water close to the coast near Lysekil, Sweden, where schools of Bluefin tuna have been observed in previous years

Currently the 2G ESP is in the process being prepared for the “open-water” deployment in August-October 2018 in Skagerrak. The purpose of this deployment is to test the ESP in a completely natural setting and to determine whether the ESP can detect and quantify variation in DNA concentration of species to provide valuable information for tracking the migration of species. The 2G ESP will be set to target four migratory fish species which traditionally have migrated in and out of Skagerrak during the deployment period. Atlantic Bluefin tuna is of special interest as it has been absent from Scandinavian waters for more than 50 years, but large schools have been spotted in the last few years, particularly in Skagerrak from the beginning of September. The migration route and timing of the Bluefin tuna is said to follow the migration of its prey, the Atlantic mackerel and the garfish, which the 2G ESP will also target. It is expected that the ESP will be deployed for 2 months taking 40 samples for *in situ* DNA analysis and 40 archival samples for subsequent analysis in the laboratory after recovery.

Final Technology Readiness Level

By the end of the AtlantOS project the 2G ESP with *in situ* eDNA analysis will have demonstrated a TRL of 8 (System technology qualified through test and demonstration). As previously stated, the core system of the 2G ESP technology is already commercially available so meets a TRL of 9 (System technology qualified through successful mission operations).

4 Technology demonstration of Oceans of Tomorrow technology

An important component of the technology development task was not only the advancement of technology named in the call but also providing existing work from previous EU funded projects the opportunity for further deployments to demonstrate greater TRLs. In particular, the Oceans of Tomorrow projects; NeXOS, SenseOcean, EnviGuard, BRAVOO, SCHeMA and, COMMONSENSE.

In September 2017 an invitation was extended to the Oceans of Tomorrow projects that had or were soon to end to offer possible deployments on drifting profiling floats, fixed estuarine and port dock deployments, pressure testing facilities and fixed mooring in the Atlantic Ocean. The Task 6.1 delivery team would co-ordinate with projects to ensure access, integration and required resources were available.

A number of deployments and projects did take up the invitation. Most required minimal support to help facilitate final deployments as part of the parent Oceans of Tomorrow project (e.g. transport costs, integration effort) and have been reported in the final reports of those projects.

The deployments that are described herein benefitted in a significant way from AtlantOS support, be that through funding of materials, labour or peripherals (e.g. reference materials) and provided demonstrable evidence for a TRL greater than previously obtained. Within each deployment overview the motivation for the deployment, performance targets, method of validation are all discussed.

4.1 Deployment of lab-on-chip nutrient sensors on Waveglider and SmartBuoys

Purpose of deployments is to assess the reliability of the lab-on-chip sensors (Section 3.7) for given nutrient parameter in locations with constantly changing external environments (e.g. salinity, temperature, sediment/turbidity, biofouling) that could affect quality of data and to determine at what limits the validity of the data is compromised.

4.1.1 Deployment of OTE lab-on-chip nitrate sensors on Cefas SmartBuoys

Motivation for deployment

Cefas have deployed and maintained SmartBuoy autonomous moorings in various coastal & North Sea locations since 2000. These platforms are part of the UK National Marine Monitoring Programme; which collects data for use in policy decisions and to fulfil monitoring requirements for the EU OSPAR (eutrophication) convention and the UK Water Framework Directive. A nutrient time series has been maintained in a region of freshwater influence in the tidal area of the Thames estuary outflow for nearly 20 years using a combination of autosamplers and an obsolete Nas3x nitrate sensor. However, current technology is no longer commercially available and requires replacement to ensure continuation of the time series. The deployments aim to test and validate the OTE lab-on-chip sensors as a potential replacement for the existing SmartBuoy nutrient sensors (Figure 35) and includes the first demonstration of the ‘sleep mode function’ for reducing power consumption, which is critical for the deployment duration on SmartBuoys (Figure 36).

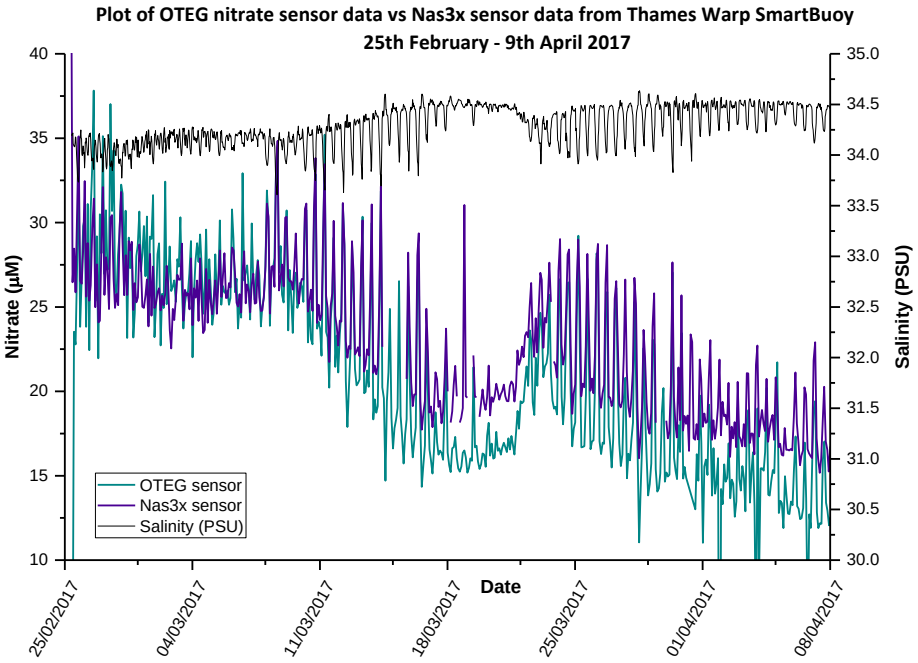


Figure 35 Comparison with Nas3x (pilot deployment to test integration, 6 weeks of data – no sleep mode. Difference between the two values is attributed to reagent degradation in the Nas3x due to the sensor design requiring higher Griess concentrations than the OTE sensor)

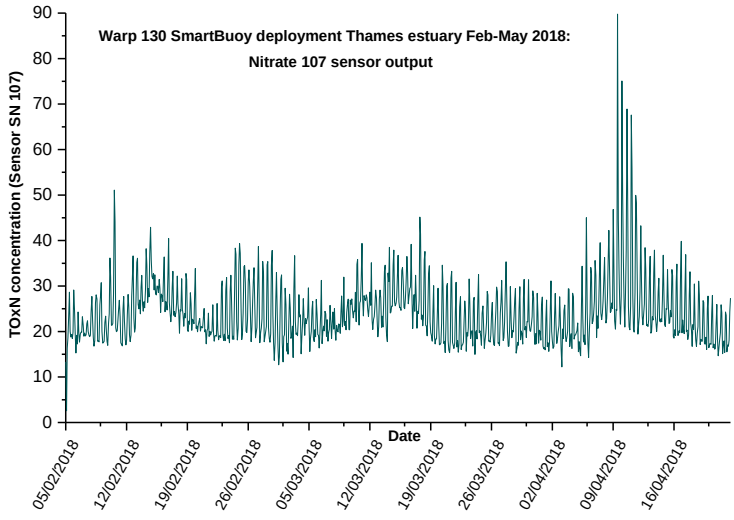


Figure 36 Full 3-month dataset following development of sleep mode

Analytical performance targets

Validation must equal or exceed that of the existing sensors. The sensor aims to measure at 2 hourly intervals for a 3-month period; although the current technologies rarely achieve this. Power consumption is a key factor. Nitrate range is variable on a tidal cycle (salinity range 20-35 PSU); but is usually within the 0-40 µM range. Hardware robustness and data recovery is a critical parameter with the existing technology rarely completing the deployment. Figure 37 shows the recovery and deployment of the tested technology on separate occasions.

Method of calibration and performance validation

Various calibration methods were trialled; key being a five standard benchtop calibration on recovery; reproducibility on a standard before and after deployment, and reproducibility and accuracy on a KANSO CRM pre- and post-deployment. A Nas3x (correlation between two sensor datasets Pearson's coefficient = 0.9, R^2 = 0.8, with offset and increasing deviation over time attributed to Nas3x reagent degradation) and an *in-situ* autosampler (for later onshore laboratory analysis) was run alongside for assessing performance. Hardware robustness and data recovery was compared with historical time series data from 121 previous technology deployments; as the sensor is located 30 km offshore, close to the surface and experiences shipping and wave action (flat to >2 m height) as well as high sediment loads (5-65 mg/L) and biofouling.

(A)  (B) 

Figure 37 (A) Recovery of biofouled LoC sensor on SmartBuoy Feb 2017 (B) LoC (copper) & Nas3x (black/yellow) sensors pre-deployment on SmartBuoy Feb-May 2018

4.1.2 Deployment of lab-on-chip nutrient sensors on Ferrybox underway outflow

Motivation for technology innovation

Surface nutrient measurements are of particular interest for automation since the current methodology requires time consuming and expensive ship-based rosette sampling, followed by sample preservation and subsequent laboratory analysis. The Ferrybox/Ships of Opportunity initiatives[14] aimed to increase sampling frequency and reduce research operating costs by exploiting movement of existing, often non-research vessels in areas of scientific interest by installing automated monitoring systems on ships. This presents different challenges to a true *in-situ* deployment due to the sensor operating in a different temperature environment to the water mass sampled. A pilot study was undertaken to compare the higher frequency sensor output to traditional sampling methods; which would be further evidence of the operational performance and measurement validity of the sensor output (Figure 38). The data is also used to examine different calibration methods that might improve correlation between sensor output and traditional nutrient sampling methods across changing environmental conditions. Salinity (31.5-35.5 PSU), sea temperature and turbidity change as the ship travels through different areas including a mixture of offshore coastal and estuarine locations. The real-time sensor output was

[14] https://www.ferrybox.com

also used to target plankton net sampling during the May 2018 cruise as the presence of blooms corresponds with areas of nutrient depletion.

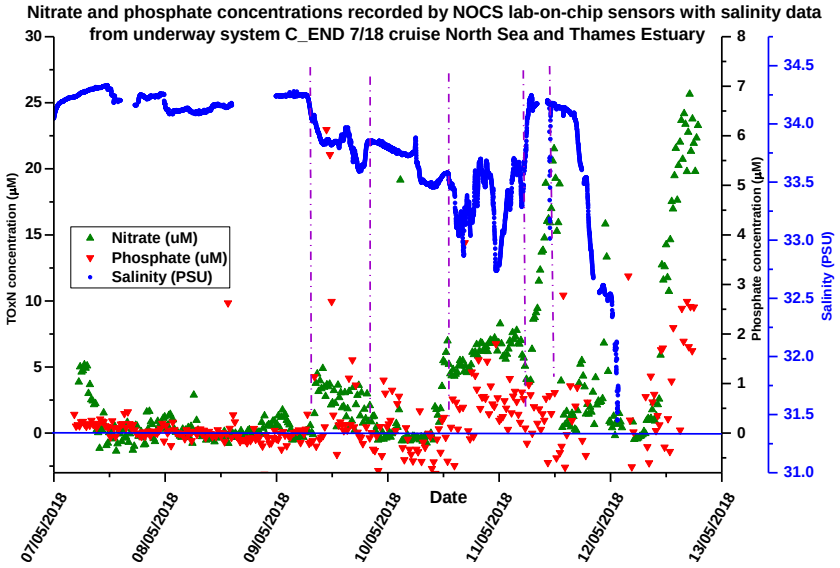


Figure 38 Nitrate and phosphate data as recorded by lab-on-chip technology alongside salinity from deployment in the Thames estuary

Analytical performance targets

Various calibration methods were trialled; key being a 5 standard benchtop calibration on recovery; reproducibility on a standard before and after deployment, and reproducibility and accuracy on a KANSO CRM pre- and post-deployment. Reference sampling using a combination of rosette sampling and laboratory analysis of the underway system outflow was run alongside all deployments for comparison (approx. Pearson's coefficient on uncorrected raw sensor output vs reference sampling = 0.9, R^2 = 0.8 for deployment shown below).

There's some additional optical correction and salinity cross reference testing – lab based but on real reference samples alongside this, and also some basic correlations between temperature offsets and the variation in the standard curve due to the temperature dependence of the reaction kinetics.

Deployments outside of laboratory validation

Three deployments (November, February, and May) to show seasonal variation in the North Sea were undertaken (Figure 39).

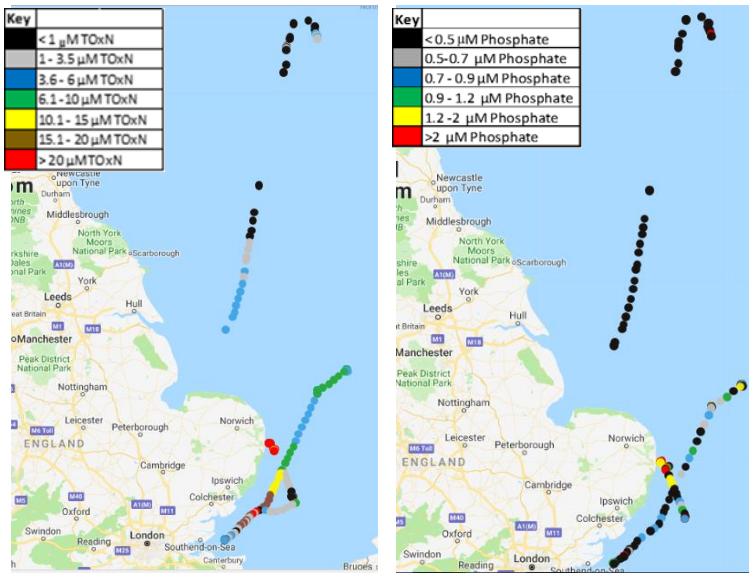


Figure 39 Summary of the nitrate and phosphate concentrations experienced during three deployments on Ferrybox system

Figure 40 and Figure 41 give greater detail of the nitrate readings throughout the February 2018 deployment. Broadly there is good agreement between the device under test against the existing methodology.

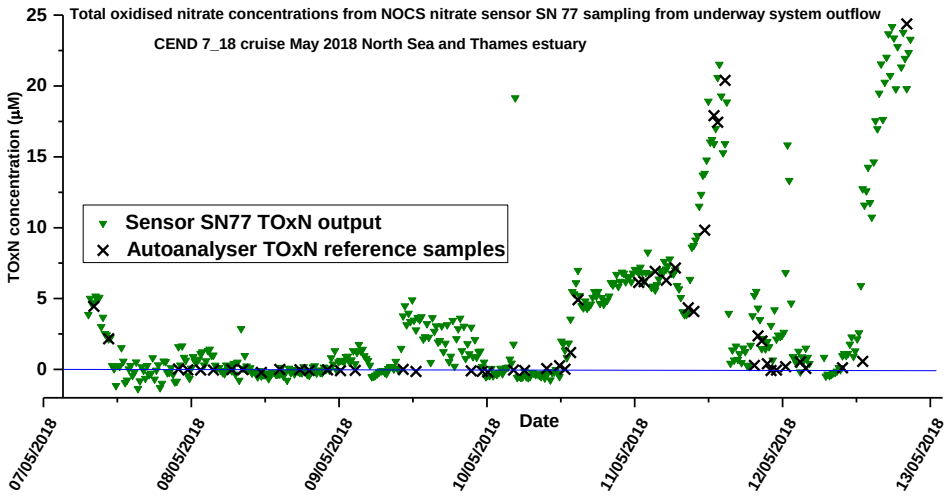


Figure 40 Uncorrected sensor output from Ferrybox deployment (Feb 2018, North Sea)

It is likely that with further development integrating the lab-on-chip technology to a Ferrybox system that reliable nutrient data can be collected autonomously and remotely available in near real time.

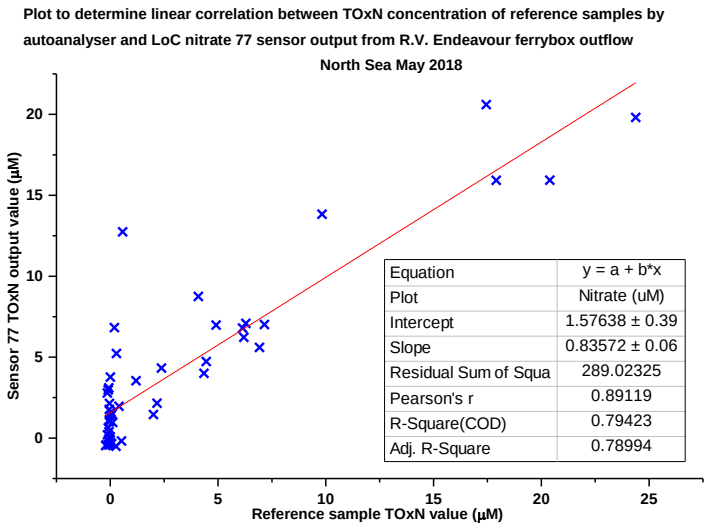


Figure 41 Spatial nitrate variation from nitrate sensor on deployment (Feb 2018, North Sea)

4.2 Deployment and validation of *AUTOFIM* from EnviGuard project

Contact: Felix Janssen (felix.janssen@awi.de)

The Oceans of Tomorrow project Enviguard[15] resulted in an automated filtration system AUTOFIM. AtlantOS WP6 partners AWI and Ribocon were able to use the developed technology (TRL 4/5) and demonstrate and validate its use through support from the AtlantOS and other co-running projects. The work informed the partners contribution to WP3 of AtlantOS and helped secure a TRL of 8/9 for the AUTOFIM technology.

Motivation for the technology

Marine ecosystems will be affected by global change in multiple ways including shifts in temperature, stratification, circulation, and sea ice coverage, as well as nutrient input, oxygen content, and ocean acidification. Information on biodiversity, biogeography, and functions of photosynthetic marine protists with adequate temporal and spatial resolution is urgently needed to better understand the consequences of environmental change for marine ecosystems. AUTOFIM ('AUTOmated FIltration system for marine Microbes') is a remotely-controlled, automated system for installation on ships or fixed monitoring platforms. AUTOFIM is coupled to the ship's pump system and performs recurrent filtration of particles from pre-set volumes of seawater (5L max. volume) to disc filters (Figure 42). Twelve Filters are stored in a filter wheel that can be easily exchanged concurrently with a simple rinsing step and a brief system check (typically 1-2 times per week at standard operation). Operation of the device does not require special expertise and all necessary steps can be performed by lay persons. All steps related to the filtration process, including application of Lysis Buffer are carried out automatically. Metadata (time, position, filtration information) are automatically stored.

[15] http://www.enviguard.net/

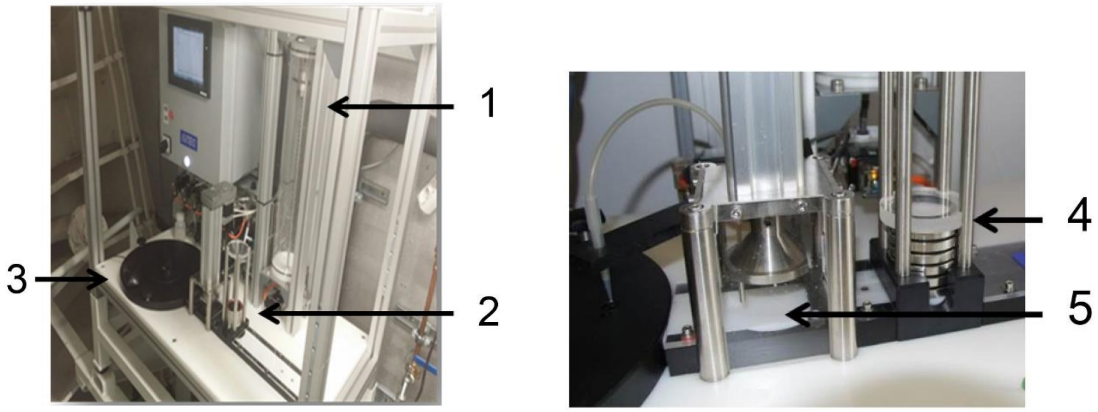


Figure 42 AUTOFIM installed on board RV POLARSTERN with close-up of the filtration-module (lower panel). (1) Sample reservoir, (2) filtration-module, (3) archive for preserved filters, (4) filter stack, (5) filtration cap; from Metfies et al. (2016), modified.

Samples are typically preserved for later laboratory analyses but may also be directly subjected to molecular surveillance of key species aboard the ship *via* automated biosensor systems or quantitative polymerase chain reaction (PCR) (not part of the AUTOFIM installation). Preserved samples are typically analyzed in the shore lab by molecular fingerprinting methods for a quick overview of differences in protist community structure while the latest next generation sequencing (NGS) technologies are used to generate a detailed analysis of taxonomic protist community composition.

The technology represents a key part of the 'microbial component' of the FRAM (FRontiers in Arctic marine Monitoring) observatory infrastructure (for a general description of FRAM see Soltwedel et al., 2013[16]). Molecular ('omics') tools have already been applied in previous studies at the LTER observatory HAUSGARTEN (e.g., Soltwedel et al., 2016[17]), one of the long term time series continued and extended by means of the FRAM infrastructure (see Soltwedel et al., 2005[18] for a general description of HAUSGARTEN). AUTOFIM underway sampling in FRAM allows for cost-effective genomic observations in the surface mixed layer during the expedition season in the Arctic summer with a unique areal coverage. Observations obtained in consecutive years address inter-annual variability and long term changes in phytoplankton / microbial communities. AUTOFIM observations in FRAM are combined with analyses of water and particle samples obtained year-round by means of moored automated samplers to resolve seasonal and vertical patterns of microbial communities. Furthermore, analyses of legacy particle trap samples carried out with AtlantOS support within WP3 are currently added to reconstruct long-term baseline conditions and connections between communities and biogeochemical processes.

[16] Soltwedel, T., Schauer, U., Boebel, O., Nöthig, E. M., Bracher, A., Metfies, K., ... & Klages, M. (2013) FRAM-FRontiers in Arctic marine Monitoring Visions for permanent observations in a gateway to the Arctic Ocean. In OCEANS-Bergen, 2013 MTS/IEEE (pp. 1-7). IEEE

[17] Soltwedel, T., Bauerfeind, E., Bergmann, M., Bracher, A., Budaeva, N., Busch, K., ... & Jacob, M. (2016) Natural variability or anthropogenically-induced variation? Insights from 15 years of multidisciplinary observations at the arctic marine LTER site HAUSGARTEN. Ecological Indicators, 65: 89-102

[18] Soltwedel, T., Bauerfeind, E., Bergmann, M., Budaeva, N., Hoste, E., Jaeckisch, N., ... & Quéric, N. V. (2005) HAUSGARTEN: multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean, Oceanography, (3).

Addressed EOVs

Phytoplankton biomass and diversity and the closely associated emerging variable of *Microbe biomass and diversity*. AUTOFIM also delivers observations that serve as supporting variables for EOVs that address particulate and dissolved organic matter.

From the start, FRAM included molecular-based ('omic') approaches to biological observations as they are now championed by the G7 augmented observatory initiative. The focus of underway observations with AUTOFIM lies on eukaryotic primary producers / unicellular algae. These are combined with investigations of bacteria and archaea communities in samples typically collected with CTD and moored samplers to study connections between community patterns of prokaryotic and eukaryotic communities and their functions with a focus on organic matter cycling in the changing Arctic.

Technology Readiness Level at the start of AtlantOS (April 2015)

AUTOFIM's development is a combined activity of the Helmholtz Association Young Investigators Group PLANKTOSENS at the Alfred Wegener Institute in Bremerhaven (AWI) and the local SME iSiTECH that took place in the project Enviguard (EU-FP7 #614057). The development is based on preceding studies carried out by PLANKTOSENS with support by the Coastal Observing System for Northern and Arctic Seas (COSYNA). Enviguard started only 1.5 years earlier than AtlantOS. At the start of AtlantOS, AUTOFIM was at a TRL between 4 ('technology validated in lab') and 5 ('technology validated in relevant environment').

Analytical performance targets

The separation of suspended particles and organisms from natural waters by filtration through disc filters is a well-established technique performed routinely for scientific studies and for monitoring purposes, e.g., to address levels of suspended matter, contaminants, or pathogens. Hence, AUTOFIM is based on proven scientific concepts and the development focused on automation to facilitate cost-effective processing of large numbers of samples while reducing the need for trained scientific personnel. Therefore, the performance targets were mainly associated with failure-free long-term operation. Key target was the unattended sample processing for batches of 12 filters (i.e., until reloading of the 12 compartment filter wheel). Failure-free operation addressed targets such as (1) exact and wrinkle-free positioning of the disc filters, (2) accurate and precise sample and preservative volume, (3) proper sealing of the filter module during filtration. These performance targets where carefully observed during testing in the lab and during test runs on board voluntary ships.

Performance tests regarding the molecular analysis of protist communities from environmental samples were carried out during the development of the molecular observation strategy for marine areas in mid to high latitudes (e.g., Kilias et al., 2015[19], Wolf et al., 2013[20], Wollschläger et

[19] Kilias, E. S., Wolf, C., Metfies, K. (2015) Characterizing variability in marine protist communities via ARISA fingerprints – a method evaluation, Limnol. Oceanogr.-Meth. 13: 74–80, doi:10.1002/lom3.10008

[20] Wolf, C., Frickenhaus, S., Kilias, E. S., Peeken, I., Metfies, K. (2013) Regional variability in eukaryotic protist communities in the Amundsen Sea, Antarct. Sci., 25: 741–751, doi: 10.1017/S0954102013000229

al., 2014[21]). Hence also in this respect, AUTOFIM could build on existing knowledge. The specific analytical performance target for the new sampling technology could therefore focus on the scientific relevance of AUTOFIM results in the context of the multi-instrument observation strategy suggested for the microbial observatory in the Arctic (Metfies et al., 2016[22]). After validation of the integrity of the DNA extracted AUTOFIM samples, it was tested if AUTOFIM samples, collected at a single depth (i.e., the depth of the ship's pump system intake at approx. 10 m below surface) were representative of the full euphotic zone where the main planktonic primary production takes place.

Method of calibration and performance validation

For initial validation of the technical performance, bench-top versions of AUTOFIM were installed on Helgoland for time-series sampling connected to the established long-term observations of unicellular algae carried out as part of the Helgoland Roads time series (Wiltshire et al., 2010[23]).

The analytical performance indicators were assessed during a comprehensive assessment of the suggested molecular observation strategy for the Arctic that was carried out in the LTER HAUSGARTEN area in 2014 and 2015 and applied a large suite of molecular techniques, including; molecular fingerprinting, rRNA-based biosensors, and quantitative PCR, as well as NGS (Metfies et al., 2016[24]). The analytical performance was fully validated: the community structure, assessed with Automated Ribosomal Intergenic Spacer Analysis (ARISA) showed no significant differences to those obtained by manual filtration of CTD samples taken in different layers of the euphotic zone down to 50 m water depth.

With contributions from AtlantOS, calibration and performance validation is extended by comparing microscopic counts of planktonic algae with community composition information based on NGS 18S gene sequencing with Ilumina. The comparison focuses on the optimisation of the molecular analysis protocol with special emphasis on the effects of different primer pairs used for the amplification of environmental DNA. This study is currently in preparation for publication and will be submitted within the lifetime of AtlantOS. As far as possible, the validation includes a quantitative evaluation based on a comparison of 18S gene abundance and microscopic counts. This is, however, limited due to the highly variable ribosomal DNA copy numbers in eukaryotic algae. Future work with AUTOFIM and with other FRAM microbial observatory components will further profit from activities of AtlantOS partners AWI and Ribocon that are gathering microbial observatories within the 'Global Omics Observatory Network' (GLOMICON) to better coordinate

[21] Wollschläger, J., Nicolaus, A., Wiltshire, K. H., and Metfies, K.: Assessment of North Sea phytoplankton via molecular sensing: a method evaluation, J. Plankton Res., 36, 695–708, doi:10.1093/plankt/fbu003, 2014

[22] Metfies K., Schroeder, F. Hessel, J., Wollschläger, J., Micheller, S., Wolf, C., Kilias, E., Sprong, P., Neuhaus, S., Frickenhaus, S., Petersen, W. (2016) High-resolution monitoring of marine protists based on an observation strategy integrating automated on-board filtration and molecular analyses, Ocean Science 12: 1237–1247

[23] Wiltshire, K. H., Kraberg, A., Bartsch, I., Boersma, M., Franke, H. D., Freund, J., ... & Wichels, A. (2010). Helgoland roads, North Sea: 45 years of change. Estuaries and Coasts, 33(2): 295-310

[24] Metfies, K., Bauerfeind, E., Wolf, C., Sprong, P., Frickenhaus, S., Kaleschke, L., Nicolaus, A., Nöthig, E. M. (2017) Protist Communities in Moored Long-Term Sediment Traps (Fram Strait, Arctic)–Preservation with Mercury Chloride Allows for PCR-Based Molecular Genetic Analyses, Frontiers in Marine Sciences 4: 301, doi: 10.3389/fmars.2017.00301

multiregional, long-term, and omically enhanced observation activities. Current activities connected to AtlantOS focus on paving the path for an agreement of best practices by assessing differences in the currently applied methodologies and their effects on the biological information that is collected. These activities include the collection of site-specific best practices from community members and the exchange of environmental samples and mock community DNA extracts for analysis with the different analytical and bioinformatics pipelines and feed into deliverables D6.4 and D6.5.

Deployments outside of laboratory validation

Starting in 2014, an AUTOFIM prototype was installed in a container on the North Sea shore in the German Bight near Cuxhaven for a period of rigorous testing. This served to validate the technical performance of the sampler during operation under field-like conditions and represented the final step before test operations in the target area during the Arctic summer campaigns of the Research Icebreaker POLARSTERN in 2014 and 2015 (PS85 and 96; Metfies et al., 2016; see also above).

Final Technology Readiness Level

Development of AUTOFIM was completed within the lifetime of the Enviguard project. After test operation during RV POLARSTERN campaigns in 2014 and 2015 (see above) routine operation was carried out during three consecutive field campaigns to the target area in Fram Strait (the 2018 season is ongoing at the time of writing). AUTOFIM is now an established component of the suite of sensors and samplers for underway observations installed on board RV POLARSTERN. AUTOFIM has reached a readiness between TRL 8 (‘system complete and qualified’) to 9 (‘actual system proven in operational environment’). The system is so far only used by academia and commercialisation is pending. Future installation also on voluntary ships, however, may happen in the near future as more studies address the applicability of molecular observations as part of environmental monitoring programs and start to create demand for such data also in governmental bodies (e.g., Leese et al., 2016[25] and 2018[26]).

[25] Leese, F., Altermatt, F., Bouchez, A., Ekrem, T., Hering, D., Meissner, K., ... & Steinke, D. (2016). DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe, Research Ideas and Outcomes (2): e11321, doi: 10.3897/rio.2.e11321

[26] Leese, F., Bouchez, A., Abarenkov, K., Altermatt, F., Borja, Á., Bruce, K., ... & Duarte, S. (2018). Why we need sustainable networks bridging countries, disciplines, cultures and generations for aquatic biomonitoring 2.0: a perspective derived from the DNAqua-Net COST action. In: Advances in Ecological Research 58, p.63-99, Academic Press.

4.3 AtlantOS WP6 Task 6.3: Shared Infrastructure

Contact: Eric Delory (eric.delory@plocan.eu)

As part of WP6 Task 6.3 led by Eric Delory of PLOCAN is a demonstration of best practices for shared infrastructure. This demonstration is an opportunity for Oceans of Tomorrow technologies to not only demonstrate greater effectiveness at a greater TRL but also to work within the full infrastructure required to undertake, transmit and disseminate data. Task 6.3 is not required to report until later in the AtlantOS project but outline plans are included here as there is significant overlap with the aims of Task 6.1.

4.3.1 Invitation by Task 6.3

At the Oceanology International 2018 exhibition a workshop was held to engage with the community to further demonstrate, in the field, new interoperability tools that have been developed and field-tested for ocean sensor and real-time data sharing, based on Open Geospatial Consortium (OGC) standards[27]. These are available open-source and now require less engineering time than in the past. A session was specifically dedicated to the training of workshop participants interested in an interoperability experiment, in the framework of AtlantOS, and in collaboration with other initiatives like EMSO ERIC, Seadatanet, EMDONet, ODIP, and ENVRIPlus. The workshop was open to ocean scientists, engineers and technicians dealing with in-situ sensor and observing systems, from academia or industry. Participants were offered presentations and demonstration of the latest interoperability technologies, and the opportunity to participate in a joint field experiment.

Presentations of the workshop are available on-line and include links and contacts for the tools made available to the community for the implementation of OGC Sensor Web Enablement and OGC PUCK software solutions.

Results of each mission will be presented at the AtlantOS final General Assembly, UNESCO headquarters, Paris during the last week of March 2019.

4.3.2 Planned demonstrations

The best practices of shared infrastructure demonstration will be conducted at the PLOCAN site in Gran Canaria (Figure 43). The test site has excellent resources available to participants and provides all that is necessary to evaluate the separate connection schemes, data flows and parameters expected from the separate technologies.

[27] https://www.atlantos-h2020.eu/events/training-workshop-interoperability-technologies-for-sharing-ocean-instruments-and-real-time-data/

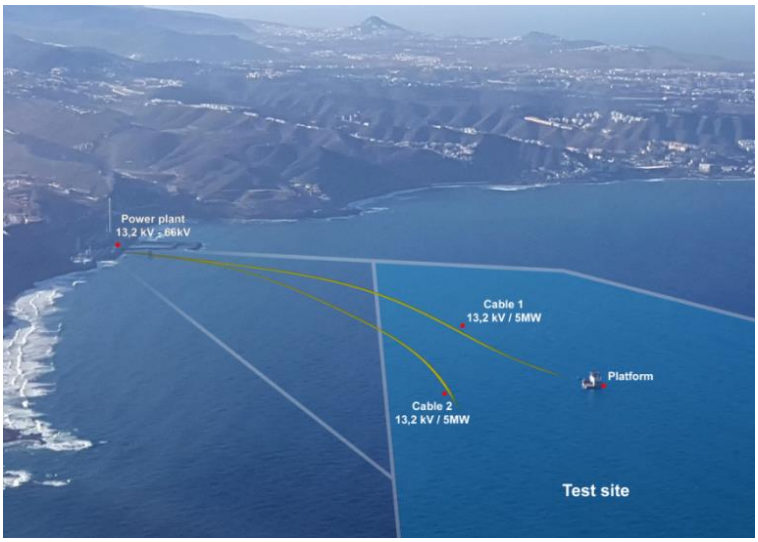


Figure 43 Test area at PLOCAN, Gran Canaria.

The marine platform planned for the interoperability work is PLOCANs wave glider SV2 (Figure 44). The program of whether sensors will be mounted simultaneously or separately to span different times/locations is to be finalised pending partner submissions of requirements.

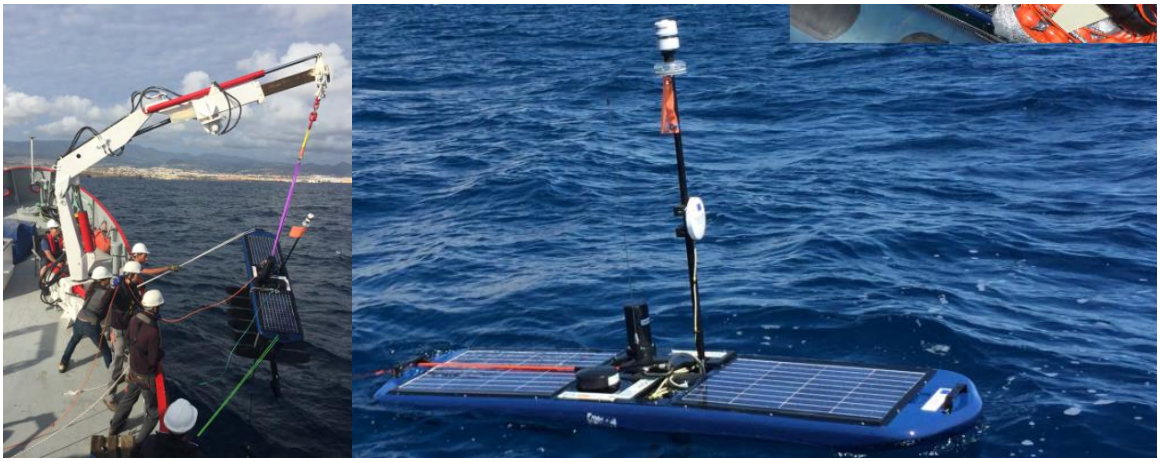


Figure 44 Wave glider platform planned for use during interoperability studies

The planned work will enact all of the best practice for shared infrastructure identified throughout AtlantOS Task 6.3. There are also close links with Task 6.4 (Best practice on observing systems) with the management of data and operations also planned to be demonstrated.

4.3.3 Technologies in study

Planned activities are:

- Deployment of the PLOCAN NeXOS sensor A1 (acoustic - TRL 7, Figure 45 A) and TriOS NeXOS O1 (matrixflu TRL 7) on waveglider SV2 with OGC SWE data flow and PUCK interoperability (TRL7).
- Deployment of three Turner Design fluorometers (est TRL 9, Figure 45 B) from waveglider SV2 with OGC SWE data flow and PUCK interoperability (TRL7). This study will separately look at the variables of turbidity, Ch-a and of refined fuels.
- Deployment of the EMSO EGIM module (Figure 45 C) with standard sensor package at Taliarte harbour, on PLOCAN test site mooring and from an electro-optical cable from the PLOCAN platform.
- To be confirmed. Deployment of a Lab-on-chip nutrient sensor.

(A) 

(B) 

(C) 

Figure 45 (A) NeXOS A1 sensor (B) Turner Designs Fluorometer (C) EMSO Generic Instrument Module.

Contextual data will be collected through existing and well established sensors (TRL 9). Including; Temperature, Conductivity, pressure - SEABIRD SBE37-SIP, Pressure - SEABIRD SBE 54 Tsunami, Dissolved O2, temperature - AADI-3005214831 DW4831, Turbidity - Wetlabs NTUrdt, Ocean currents, Compass and tilt meter - Teledyne Workhorse monitor ADCP 300 KHz and Passive acoustics, Compass and tilt meter - OceanSonics icListen SB60L-ETH.

5 Conclusion

AtlantOS Task 6.1 has been responsible for the progress of eight technologies that address key EOVs and extend the ability to observe those variables on an increasing number of dynamic and resource restricted platforms. Figure 46 provides a summary of the TRL advances of the technologies, ordered by final demonstrated TRL.

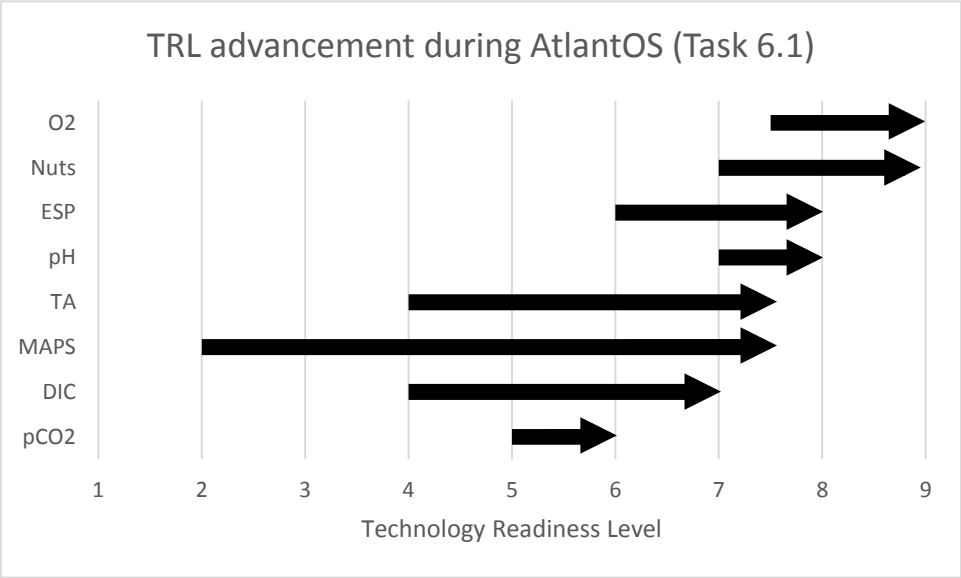


Figure 46 TRL advancement through AtlantOS Task 6.1.

TRLs in Figure 46 are not restricted to integer values since the evidence that has been collected through development justifies part recognition of TRL advancement.

Across Task 6.1 partners have achieved a total of 18 TRL advancements. As described in Table 1 the developments have all targeted Biogeochemical and Biology and Ecosystems EOVs which to date do not typically have mature technologies that can be called upon to undertake autonomous and in situ measurements. As a consequence, the work undertaken throughout Task 6.1 represents a substantial high impact improvement on what existed beforehand as it is now more feasible to undertake studies into phenomena which simply did not have the tools to make necessary measurements or suffered from not being able operate with dynamic and resource restricted platforms.

Furthermore, collaboration between Task 6.1 and previously funded projects from the EU's H2020 FP7 program has enabled the demonstration of sensor and instrumentation capability beyond what was previously supported. Such an association prevents technology development stalling, which is critical in maintaining cost effective progression by preventing the need to rebuild teams, materials and expertise.

A number of partners throughout Task 6.1 include SMEs and it is always welcome to see academia and private industry working together to deliver extra capability to the oceanographic community. Indeed, some technologies developed as part of Task 6.1 are already available commercially and for others it is being considered as the higher TRLs are met. This represents capable technology becoming widely and sustainably available, a legacy that will exist beyond the AtlantOS project.

6 Appendix A

Technology readiness levels, adapted from NASA[28].

TRL	Description	Example / Notes
1	Basic principles of technology observed and reported	Evidence in the literature or from experiment indicates that a measurable response to the target parameter(s) is observed
2	Technology concept and or application formulated	Requirements of the application / market formally recorded, concept design(s) documented
3	Analytical and laboratory studies to validate analytical predictions	The analytical element (e.g. assay plus absorption cell) has been tested and performance evaluated vs design expectations
4	Component and / or basic sub-system technology valid in a lab environment	Benchtop system (e.g. labview control, benchtop pumps, simple chip) performance validated in the lab
5	Component and / or basic sub-system technology valid in a relevant environment	Components of the technology, or subsystems validated in a relevant environment (e.g. pressure pot, or dockside tests of elements of the system)
6	System / sub-system technology model or prototype demo in relevant environment	Prototype demonstrated in pressure pot or dockside
7	System technology prototype demonstrated in an operational environment	Prototype demonstrated in target deployment (e.g. in a river, mooring, glider etc.)
8	System technology qualified through test and demonstration	Performance in final environment validated through repeated testing and deployment
9	System technology qualified through successful mission operations	Technology has delivered data to science in the target environment on more than a handful of occasions

[28] https://esto.nasa.gov/files/trl_definitions.pdf