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Deliverable number	D3.17
Deliverable title	OceanSITES Innovation Report
Description	Innovation and improvement report on the extension of capabilities to measure emerging EOVs including metagenomics across different observational platforms with links to MicroB3 best practice.
Work Package number	WP3
Work Package title	Enhancement of autonomous observing networks
Lead beneficiary	GEOMAR
Lead author	Arne Körtzinger (GEOMAR)
Contributors	Pier Luigi Buttigieg, Felix Janssen, Katja Metfies, Ian Salter, Frank Wenzhöfer (AWI), Tobias Steinhoff, Björn Fiedler, Tobias Hahn, Katharina Seelmann, Anna Canning (GEOMAR), Richard Lampitt (NERC), Patricia López, Marimar Villagarcia (PLOCAN), Jörg Peplies (Ribocon)
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Stakeholder engagement <u>relating to this task</u>*

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): National and international organizations in the field of fixed-point observations, ocean observation in general, standardization of metrology and data, environmental data management and access (e.g., GOOS, (Int), OceanSITES (Int), Marine BON (Int), Genomics Standards Consortium (Int), Biodiversity Information Standards / TDWG (Int), Global Biodiversity Information Facility / GBIF (Int), Ocean Biogeographic Information System / OBIS (Int), KDM (DE); National authorities in charge of implementing international obligations and treaties (e.g., MSFD), e.g., state and federal environmental agencies in case of Germany; Institutes and observation networks, projects, and that address emerging EOVs (e.g., NEON (US), NOAA / Earth microbiome Project, NOC / PAP (UK), Continuous Plankton Recorder / SAHOS (UK, Int), LTER Hausgarten / FRAM (DE), Genomic Observatories Network (Int), DNAquaNet (EU), Tara Oceans (Int), MBARI (US), Agriculture and Agri-Foods Canada (CA), Genome Canada (CA))
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): Potentially all countries engaging in ocean observation to link and address societal and scientific needs and fulfil international obligations to assess ocean health and implement environmental protection (see also list above for specific countries). As many organizations engaged have a global mandate, much of progress summarized here includes considerations to generalize progress made to developing countries.

Is this deliverable a success story? If yes, why? **If not, why?**	☒ Yes, because it describes major steps towards enhancing both technological and community-based capacities for long-term, automated, ocean time series observations. These steps contribute to building crucial capacities to provide observations to multiple stakeholders through mechanisms such as the GOOS EOVs. ☐ No, because …..
Will this deliverable be used? **If yes, who will use it?** **If not, why will it not be used?**	☒ Yes, by 1) scientists and organizations in need of an overview of state of the art methods for time series observations with fixed-point observatories and 2) stakeholders in emerging biological observation networks, particularly those focused on microbial indicators. For details, individual, peer reviewed publications are probably more relevant ☐ No, because …..

NOTE: This information is being collected for the following purposes:

1. To make a list of all companies/organizations with which AtlantOS partners have had contact. This is important to demonstrate the extent of industry and public-sector collaboration in the observational community. Please note that we will only publish one aggregated list of companies and not mention specific partnerships.
2. To better report success stories from the AtlantOS community on how observing delivers concrete value to society.

*For ideas about relations with stakeholders you are invited to consult D10.5 Best Practices in Stakeholder Engagement, Data Dissemination and Exploitation.

Summary

The need to cover established and emerging Essential Ocean Variables (EOVs) as defined by the Global Ocean Observing System (GOOS) calls fir the development and refinement of the available sensors and samplers, specifically for biogeochemical and biology/ecosystem observations. For several of these EOVs as well as for microplastics as a relatively novel variable of particular societal concern, technological progress has been made as part of AtlantOS. This involves the samplers and sensors and the platforms to use them from as such as well as the required methodologies for obtaining relevant and well-validated results and disseminate data according to the FAIR principles.

For biological observations, a main focus was on automated sampling of particles and water samples. While active, pump-based samplers for particles in the water column have been available for many years, it turned out that they were not yet fully mature for operational sampling of zooplankton, microorganisms (e.g., bacteria, archaea, phytoplankton and other eukaryotic unicellular organisms), and microplastics. AtlantOS partners joined forces with manufacturers to overcome limitations with respect to quantitative filtering without leakage, avoidance of plastic contamination and the option for preservation with appropriate agents. Technical solutions were identified and partly tested but could not in all cases be fully implemented in the time frame of the project. Technologies for automated water sampling proved to be more mature and samplers could already be successfully included in observation programs. For both water and particle samples only very few manufacturers offer off-the-shelf solutions which slows down innovation and adaption to user's needs and may impede successful implementation of appropriate instruments on a larger scale.

Particle traps are well-established and operational passive samplers of sinking particles that are widely used for phytoplankton and particulate matter observations based on microscopic sorting and chemical analyses. Using legacy samples collected in the Arctic it could be demonstrated that the same samples can also be used for omics-based observations allowing to address the emerging EOV 'Microbe biomass and diversity' and also contributing to the 'Phytoplankton biomass and diversity' EOV. Applied to legacy samples also from other sites, this holds the potential to assess past microbial communities of the Atlantic that could serve as a baseline for comparisons to recent communities that are subject to global change.

Significant progress was achieved in building capacities for the implementation of omics-based observations of marine organisms into recent and future observation programs. The feasibility of samplers and different preservation agents was tested and a comparison of different methods for omics-based investigations of microbial communities was conducted. The Global Omics Observatory Network (GLOMICON) was established to better connect the institutes and initiatives that are active in the field. As part of GLOMICON, solutions were implemented that allow for a registration of omics observatories and for the sharing of protocols and bioinformatics code. Irrespective of these achievements, major steps still need to be taken to consolidate and standardize approaches in this rapidly evolving field and to establish operational and well-integrated omics-observations as part of an Atlantic Ocean Observation System.

For biogeochemical observations, the focus was placed on sensors for oxygen and marine CO_2 system parameters (pCO_2, total alkalinity) and their readiness for integration into classical as well as emerging biogeochemical observation platforms.

For oxygen, the situation is very favourable as the oxygen optode technology and the best practices routines developed around it can be considered fully operational. There are no obstacles for the

integration of oxygen optodes into the full range of autonomous ocean observation platforms (mooring, drifter, glider, wave gliders, floats, voluntary observing ship etc.).

For marine CO_2 system parameters, work carried out in AtlantOS focussed the CO_2 partial pressure (pCO_2) and total alkalinity (TA). With respect to pCO_2 it can be stated, that the membrane-equilibration sensors with NDIR detection have clearly matured to a level that they can be used routinely on a range of platforms (mooring, wave glider, voluntary observing ship) with an accuracy of ~1% under well-constrained operation conditions and with rigorous data processing routines. Major limitations still exist, however, for this sensor technology on moving platforms (long sensor response time) and platforms with stringent payload and energy limitations (float, glider etc.). In contrast, the pCO_2 (as well as pH) optode technology, in which significant hopes lie, has not been forthcoming and existing products still do not meet the quality requirements for open ocean applications. For TA, our intensive testing both in the laboratory and in the field has led to significant improvement of the commercially available system, which now can be considered operational. It allows high-quality autonomous bench-top measurements (e.g., on voluntary observing ships). Ideas for a submersible version of the system are in early stages and would need significant design and testing efforts.

With respect to the possibilities of oxygen and carbon measurements from novel autonomous observation platforms, our work in AltantOS has shown very promising applications on profiling Argo floats, submersible winch systems with upper ocean profilers as well as wave gliders. On all these platforms, we were able to successfully implement oxygen and carbon measurements for high-quality observations.

1. Introduction

AtlantOS Task 3.2 ‘OceanSITES’ represents the network of fixed point biogeochemical observatories in AtlantOS and addresses major observational gaps in terms of variables central to the MSFD. Two major foci were placed on the possibility to obtain time series data on:

(1) Biological EOVs such as zooplankton, phytoplankton, particles and microbial biomass and diversity to assess the structure and function of the biological communities, and
(2) Biogeochemical EOVs such as carbonate system parameters, the dissolved organic carbon or fluorescence-derived ocean colour to assess pressures such as ocean acidification or pollution by microplastics and other hydrocarbons.

In the following, we provide a short status report of the work carried out under Task 3.2 and the observation readiness level achieved with respect to the various quantities to be observed:

2. Biological EOV Innovations

Zooplankton

Authors: Marimar Villagarcia[1], Richard Lampitt[2]

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[2] National Oceanography Centre, Southampton, UK

As no biological samplers were included in the mooring at ESTOC (European Station for Timeseries Ocean Canary islands) and PLOCAN did not have experience with them, the project provided funds to purchase a zooplankton sampler, a phytoplankton sampler and a sediment trap to include these devices. When the project started, PLOCAN searched in the market for a zooplankton sampler to be included in the mooring located at the ESTOC station. Only one that was suitable for deployment in an oceanic mooring was found on the market (Time-Series Zooplankton Sampler, ZPS 6-50, McLane Research Laboratories Inc., East Falmouth/MA, USA). Just after AtlantOS began, it was becoming clear that there were significant engineering problems associated with the McLane ZPS and eventually the company announced that these faults were fundamental and could not be rectified. They were then removed from the market by McLane. NOC engineers then developed ideas to modify the ZPS so that it would be able to collect zooplankton quantitatively and effectively. This was done in consultation with McLane and a Non-Disclosure Agreement was signed with the company so that these ideas could be implemented and marketed by the company. Unfortunately, funding for the NOC engineer was insufficient to take this through to completion and the development stalled.

With this experience in mind, purchase of the system was not thought to be wise and no zooplankton sampler was therefore acquired. A second sediment trap was purchased instead.

Phytoplankton

Authors: Patricia López[1], Marimar Villagarcia[1]

[1] Oceanic Platform of the Canary Islands (PLOCAN), Canary Islands, Spain

A Phytoplankton Sampler (PPS, McLane Inc.) was purchased and some testing has taken place. The instrument may require certain modifications to be deployed again, following the collaboration between McLane and AWI in relation to the preservatives used. One of the tests was made to check the retention capacity for microplastic analysis on stain steel filters, and for doing this two filter holders were placed together (Fig. 1). The device was recovered but the filters have not been analysed yet. Concerning in situ monitoring employing CTD-rosette systems, PLOCAN has opened the cruises to an associated team from the University of Las Palmas of Gran Canaria (ULPGC) to start testing a methodology for sampling microplastics at ESTOC by means of filtering seawater at certain depths.

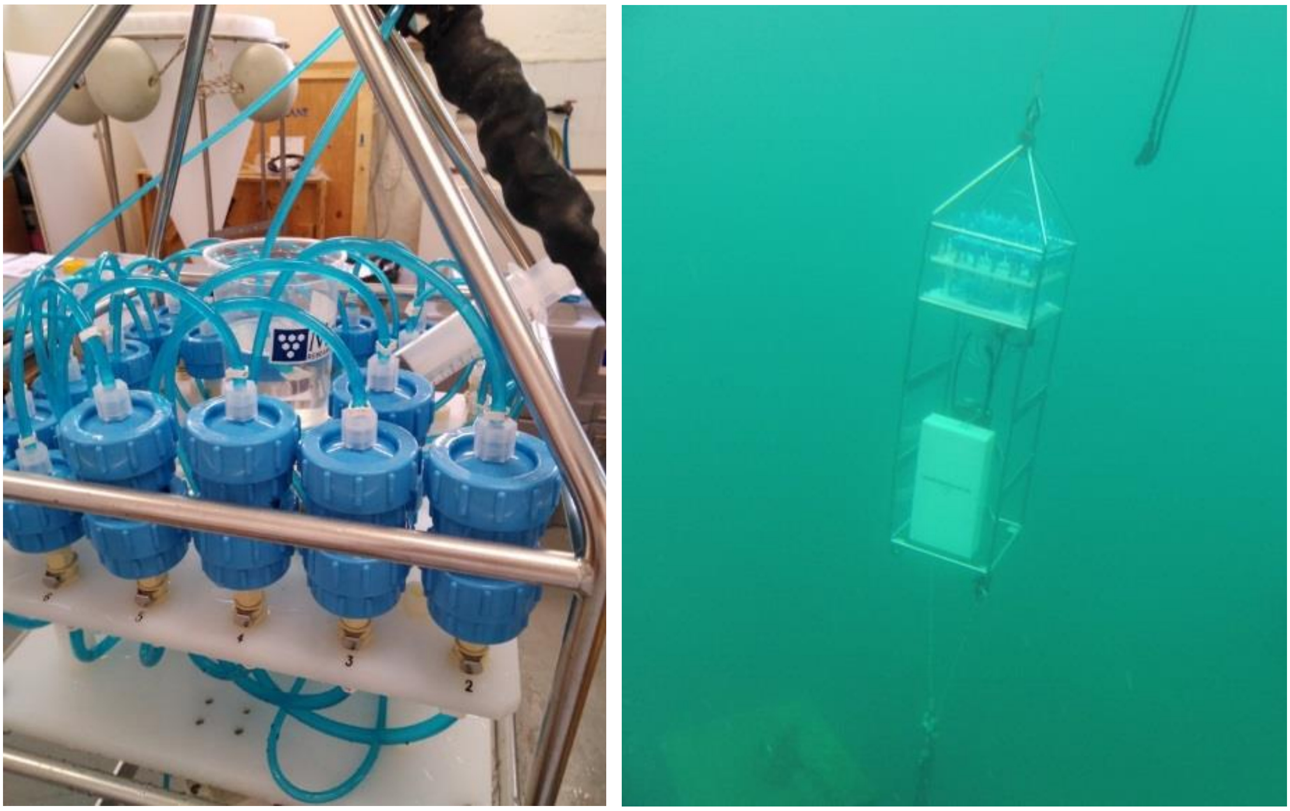

Fig. 1. Detailed set up of two filters for microplastic sampling (left) which were deployed by PLOCAN (right) (Fotos: M. Villagarcia, PLOCAN).

Particles

Authors: Patricia López[1], Marimar Villagarcia[1]

[1] Oceanic Platform of the Canary Islands (PLOCAN), Canary Islands, Spain

PLOCAN did not have any sediment traps in the oceanic ESTOC mooring when AtlantOS started, hence the work done was mainly inclusive. Two sediment traps (Parflux Mark 78H-21, McLane Inc.) including a microcat each were purchased within the project and deployed at the ESTOC mooring. The first deployment (1500 m) gave as a result the contamination of the samples due to small fish entering the system (Fig. 2). Further deployments have taken place since then, and currently one is deployed since spring 2018 and it is expected to be recovered at the end of February 2019 during the next cruise maintenance to ESTOC. The other one could not be recovered in spring 2018 because the mooring releaser did not open, and a new acoustic unit is going to be used in February 2019 to try to release it. Approaches to observations of microbial communities based on the innovation suggested by other partners and in collaboration with them (e.g. based on AWI findings) seem to be the next step to include multi-omic observations at ESTOC. PLOCAN is always open to the testing of new sensors/devices and updates/adaptations of already-existing ones at ESTOC, as well as those necessary to do in the nearby harbour, the coastal test site, the oceanic platform and/or laboratories.

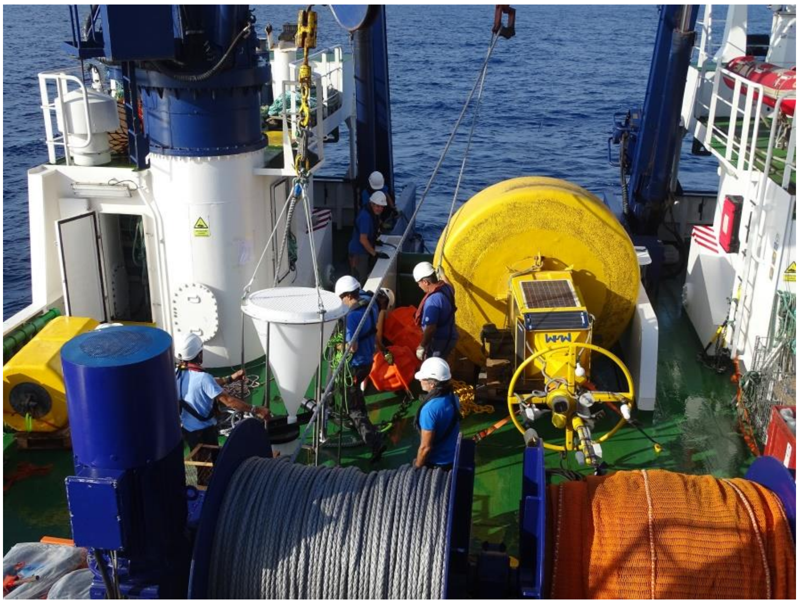

Fig. 2. First deployment of McLane Parflux Trips Mark 78H-21 at ESTOC (left). Instrument after recovery with contamination by small fishes (Fotos: M. Villagarcia, PLOCAN).

Multi-omic observations with a focus on microbial diversity

Authors: Pier Luigi Buttigieg[1], Felix Janssen[1], Katja Metfies[1], Jörg Peplies[2], Ian Salter[1], Frank Wenzhöfer[1]

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Introduction

The GOOS Biology and Ecology Panel has previously recognised the need to account for microbial life when reporting on the ocean's essential ecology (Miloslavich; Bax et al., submitted). Bacteria, archaea, fungi, and viruses are central to the every marine ecosystem - from volcanic vents on the deep seafloor to tropical coral reefs and the ice-covered waters of the high latitudes - and are the central link between the biosphere and geosphere (Moran, 2015). Investigations and assessments of the biomass and diversity of marine microbes have shown great promise in the creation of bioindicators for a wide range of phenomena, and also in developing our basic understanding of how marine ecosystems function and respond to stressors. Global efforts to provide long-term solutions for monitoring microbes are rapidly increasing in their readiness, however, these lack an overarching framework and coordination body to align activity, methods, and information products to address basin-scale and global needs. Further, large expanses of the surface ocean and almost all of the deep ocean are not observed over time, and no baselines are available to detect key environmental change. These themes are explored by Buttigieg et al. (2018) in their commentary on the status of long-term marine microbial monitoring; here, they noted some ~70 marine observatories engaged in microbial observation. In addition to an underdeveloped framework, the following key gaps exist (1) the lack of field-tested, autonomous technologies to extend the reach of omics sampling to environments such as the Arctic and deep sea, (2) a lack of FAIR omics data and metadata products and standards that are tuned to the needs of omics observatories and their scientific and societal missions, (3) a lack of cyberinfrastructure dedicated to observatory-grade activities and associated dissemination points for distributed and linked data, (4) a scientific evaluation of the significance of omics-based observations of microbial communities for time-series observations of ecosystems, and (5) a lack of intercalibration between observatories using rapidly evolving omics technology.

Activities in task 3.2 to build capacities for omics-based observations were mainly carried out by AWI, Ribocon, and NOC and focused on approaches for a monitoring of microbial communities in the Atlantic Ocean and beyond. Activities includes the assessment, optimization, and development of technologies for sampling and sample preservation, the demonstration of the feasibility of omics approaches for time-series observations of microbial communities and their use to address connections to biogeochemical processes and element cycling, a comparison of existing methodologies for lab- and bioinformatics analyses, as well as the implementation of data management and analysis procedures to improve data accessibility and integration. Further, these partners have created a Global Omics Observatory Network (GLOMICON) to fill the need of a global coordination framework to integrate activities in this emerging field. This activity will be enhanced by the AWI partner’s new role as the lead of the emerging Microbial EOV (which heavily relies on omics) on behalf of GOOS.

Building capacities for in situ time series sampling for omics analyses

Omics-based assessments are so far the sole possibility that allows for unattended time-series observations of biological communities, e.g., in the context of OceanSITES installations. The first applications of sequencing for ocean sciences only happened relatively recently. However, several studies have demonstrated the applicability for investigations of the oceanic microbiome, i.e. communities of unicellular pro- and eukaryotes that often cannot be identified based on their morphology. Similar technologies also proved powerful to discriminate multicellular organisms and can even be used to detect the recent presence of organisms in a given environment based on traces of extracellular DNA (eDNA) they left behind. A recent proof of concept study has demonstrated that eDNA techniques can be used to map the spatial distribution of fish biomass in complex oceanographic regimes (Salter et al., in prep. a). As omics methods are only very recently used in the context of ocean observation, appropriate sampling technologies are either not available yet or missing validation of their feasibility in the context of omic-based observations. This also applies to preservation agents that are able to preserve omics samples for the period of autonomous collection of samples. Instruments that may be used for time-series sampling of solutes and particles are typically either passive funnel- or cylinder-type particle traps or active samplers for water or suspended particulates and include off-the-shelf instruments as well as new developments.

Available water and particle samplers

Commercially available water and particle samplers have been deployed at the Long-Term Ecological Research (LTER) observatory HAUSGARTEN, an OceanSITES site, that AWI runs in Fram Strait since almost twenty years. All instruments have been previously used for time-series studies of dissolved and particulate constituents and have thus a high technology readiness level. However, the use of these samplers for DNA-based time-series observations of biological communities is new to science and activities in AtlantOS aimed to raise the TRL as defined by the Framework for ocean observing1 from Concept to Pilot level. Water samplers from two different manufacturers (Greeneyes 'Aqua Monitor'2 and McLane 'RAS-500'3) were deployed in the lower part of the mixed surface layer for concurrent observations of microbial communities and nutrients (Fig. 3).

Fig. 3. Concurrent deployment of the RAS-500 water sampler and the PPS particle sampler during POLARSTERN cruise PS 99 in Fram Strait (image: I. Salter, AWI)

After one year of repeated sampling in plastic bags, DNA was successfully extracted from samples obtained by both types of samplers and used for amplicon sequencing with a focus on eukaryotic unicellular algae and prokaryotes. A publication focusing on the succession of microbial communities and the connection to the availability of nutrients measured in the samples and by 'lab on a chip' nutrient analyzers is currently under preparation (Salter et al., in prep. b) and will provide a proof of principle for omics-based time series investigations of protists and bacteria and a showcase of what results are to be expected. An off-the-shelf particle sampler (McLane 'PPS'[4]) required some modifications to allow for highly concentrated, negatively buoyant preservatives. After the manufacturer carried out these customizations in close collaboration with AWI scientists, also the PPS has been successfully used to collect microbial biomass for several periods of one year. In comparison with the water sample the larger volume of water being filtered (up to 10 L as compared to 500 or max. 2000 mL for the RAS-500 and the Aqua Monitor, respectively) allows to collect more biomass per sample and is particularly useful for observations of protist communities while the pore size of the filters excludes quantitative sampling of prokaryotes.

Particle traps collect sinking particles in series of sample cups that are automatically exchanged over the time-course of the deployment. Particle traps are available from different manufacturers and represent a mature technology that is established for decades. While the typical focus of particle traps is on vertical export of particles, attempts to use them for omics-base microbial community studies are just starting. Based on initial studies that demonstrated the feasibility of DNA extraction

[1] http://www.oceanobs09.net/foo/FOO_Report.pdf

[2] http://gescience.com/wp-content/uploads/2017/02/aquamon.pdf

[3] http://mclanelabs.com/wp-content/uploads/McLane-RAS-500-Datasheet_0.pdf

[4] https://mclanelabs.com/wp-content/uploads/2018/01/McLane-PPS-Datasheet.pdf

from preserved particle trap samples (Fontanez, et al. 2015[5]; Metfies et al., 2017), investigations by AWI in AtlantOS focused on the demonstration of particle trap time-series observations of microbial communities (see next section).

Emerging sampler and in situ analyzer technologies

New technologies for omics-based time-series observations are currently developed with contributions by AtlantOS partners. NOC is developing the ‘Marine Autonomous Plankton Sampler’ (MAPS) that allows to collect large numbers of particulate samples by filtering water through sterile filter cartridges (see AtlantOS D6.3[6]). The system is designed to be installed on moored or towed platforms and will allow to increase sampling frequency as compared to existing instruments. At the end of AtlantOS, different MAPS types are expected to reach TRL 6-7.

AWI started to develop a time series sediment sampler that shall be attached to benthic crawlers. A first design concept and a preliminary design has recently been developed (Fig. 4). Once finalized, the sampler will autonomously collect and preserve small aliquots of the upper layer of deep-sea sediments for periods of up to one year that will be used for analyses of microbial communities and meiofauna after retrieval of the benthic crawler. The development of the automated sediment sampler is not directly associated with AtlantOS and currently at TRL 1-2.

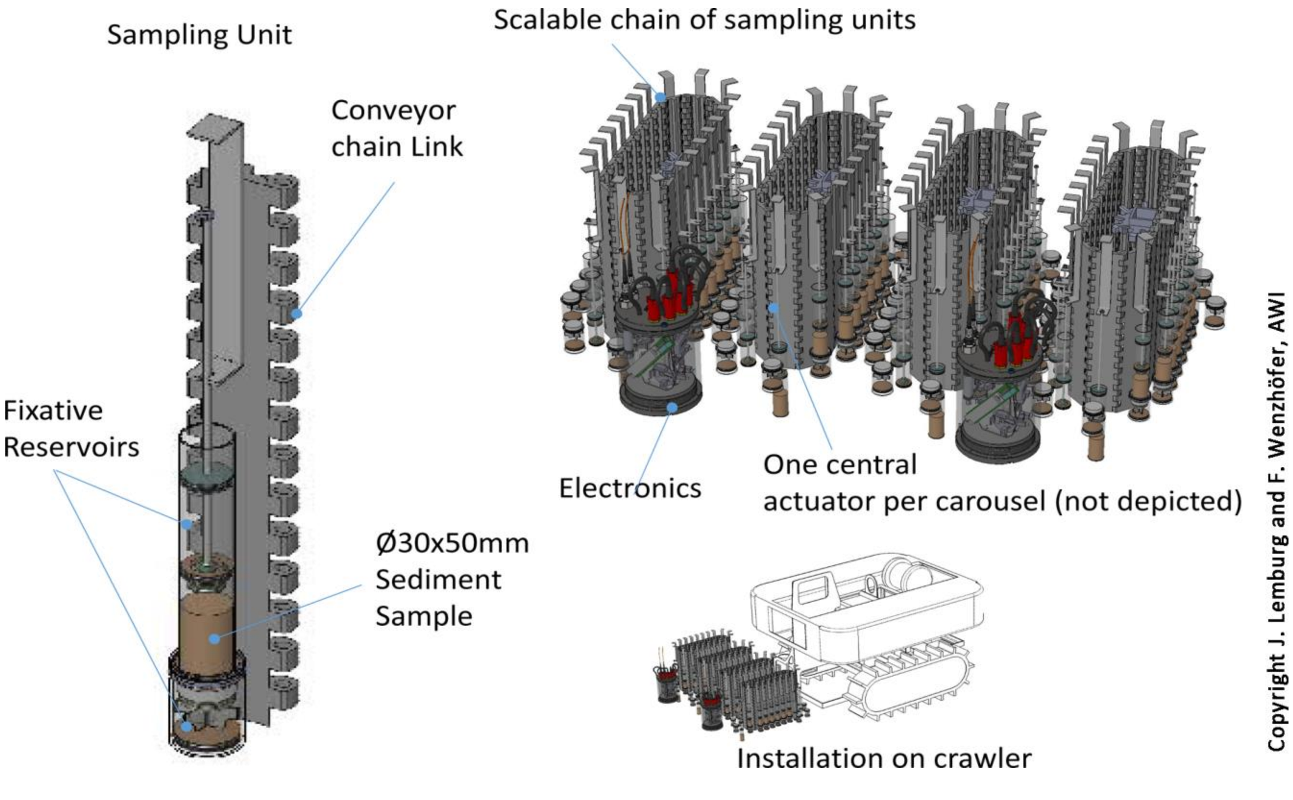

Fig. 4. Design concept of the sediment sampler currently developed at AWI (image: J. Lemburg und F. Wenzhöfer, AWI)

In addition to samplers, technologies are emerging that allow for the identification of organisms based on gene-based sensors or even for *in situ* sequencing. An unique and ongoing in situ instrument for such observations is the ‘Environmental Sample Processor’ (ESP), developed at MBARI and currently modified in order to reduce size and to improve performance for eDNA-based

[5] Fontanez, K. M., Eppley, J. M., Samo, T. J., Karl, D. M., DeLong, E. F. (2015) Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre. Frontiers in microbiology, 6, 469

[6] https://www.atlantos-h2020.eu/download/deliverables/AtlantOS_D6.3.pdf

monitoring of organisms present (see AtlantOS D6.3). The pocket-size MinION Nanopore sequencer[7] represents another new and exciting technology that may soon be adopted for marine scientific applications. As with the ESP the development of modules for automated *in situ* DNA extraction from organisms or particles as well as DNA purification and preparation for subsequent analysis remains a major technological challenge.

Assessment of different preservation agents

At least until *in situ* sequencers are fully operational and affordable, molecular time-series will depend on the successful preservation of samples taken over the time-course of the autonomous observation platform deployment. Maintenance of OceanSITES fixed-point observatory platforms is typically carried out once a year, i.e. a flawed preservation may easily introduce biases between samples taken right after deployment that reside at in situ water temperature for many months and those obtained right before retrieval. In addition, there are also large repositories of archived samples that have collected at fixed-point observation platforms and preserved, namely sediment trap material. Any study of past biological communities with omics approaches would depend on a proper preservation of the DNA with the agents typically added (mainly formalin, the trap-preservative recommended by JGOFS[8] and mercury chloride).

To investigate potential biases in omics-based microbial community analyses associated with storage and the effect of different preservation reagents, a fixative experiment was carried out by AWI (Fig.4). Five common preservatives were added to 105 technical replicates (V=50ml) taken from a common environmental sample collected at the established time series station at Helgoland Roads in the southeastern North Sea (Wiltshire et al., 2010). Apart from agents common in omics studies (RNA-later, DNA-gard, Phosphate buffer), also typical trap sample preservatives (formalin and mercury chloride) have been included (Fig. 3). The preserved samples were kept in the dark at the *in situ* temperature typically found in Fram Strait (0°C). This was chosen as Fram Strait represents the focus area of AWI’s microbial observatory established in the framework of the infrastructure project ‘Frontiers in Arctic Marine Sciences’ (FRAM; Soltwedel et al., 2013). After periods of 10, 28, and 52 weeks, microbial cells were harvested from 5 replicate samples for each preservative by means of filtration. The DNA was extracted with the NucleoSpin Plant II kit (Macherey-Nagel), and sequenced on an Illumina MiSeq sequencer after PCR amplification of the V4 region of the 18S rRNA gene. Protist taxa identified based on the operational taxonomic units (OTUs) obtained from the sequences of the different samples were compared to the communities found in unpreserved samples at t=0.

The results indicate large differences in performance of the different preservatives that get more pronounced with time. Taxonomic composition of protist communities found for samples preserved with formalin- and mercury chloride show closest resemblance to those obtained from unpreserved samples. The impact of preservation with different fixatives on bacterial community composition will be addressed in analogy to the approach for protists. Sequencing of bacterial communities is currently underway and a joined manuscript on the study results is currently in preparation (Metfies et al., in prep.) and will be finalized soon. On the FOO readiness level scale this study helps to raise time-series observations of the GOOS EOVs ‘Phytoplankton biomass and diversity’ and ‘Microbe biomass and diversity’ with autonomous sampling devices attached to moored fixed-point observatories from the concept to the pilot readiness level - at least in cold high-latitude areas.

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[7] https://nanoporetech.com/products/minion

[8] http://ijgofs.whoi.edu/

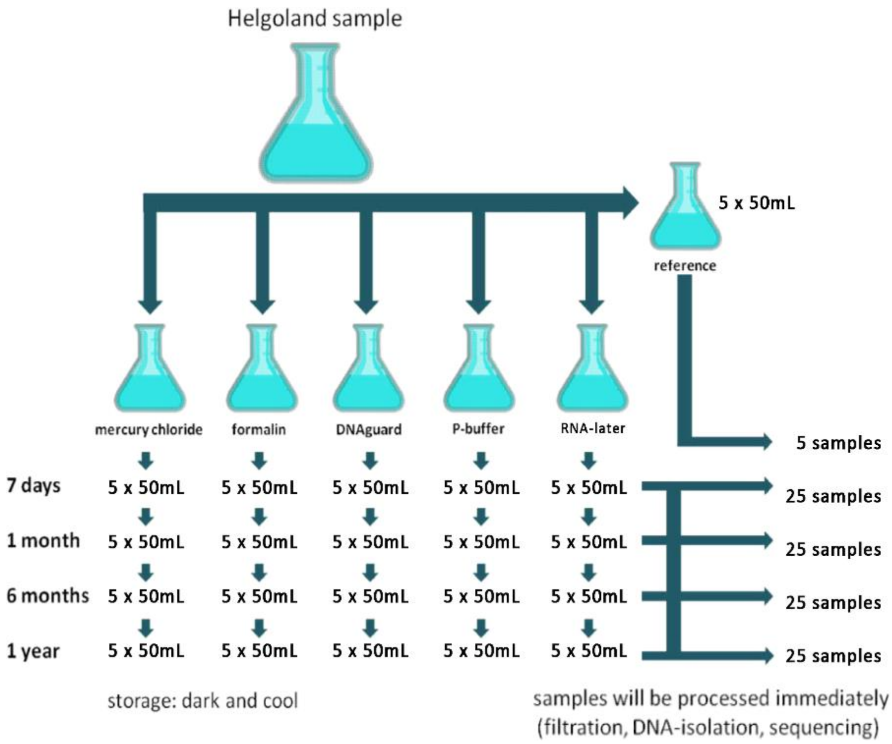

Fig. 5. Schematic of the experiment to compare the performance of different preservatives (scheme: C. Wolf, AWI)

Demonstrating feasibility of omics time series observations

Molecular analyses of the taxonomic composition of eukaryotic algae were carried out based on archived samples obtained at the LTER observatory HAUSGARTEN between 2001 and 2012. As omics approaches are just beginning to be used in the context of ocean observation, there is a strong need for a proper method validation. This relates to the ability of omics-based observations to resolve temporal patterns in community composition and their feasibility to address scientific and societal needs, e.g. global change effects on biological communities and connections of microbial community taxonomic composition to biogeochemical cycles. Focusing on eukaryotic algae, the study is particularly relevant for the GOOS ‘Phytoplankton biomass and diversity’ EOV, that recognizes genomics (in addition to pigment analysis and microscopy) as observation method. The study aims to demonstrate the capability of an omics approach to observations and to validate the proof of concept *in situ*. By this means, it directly contributes to establishing the ‘Pilot’ level at the FOO readiness level scale. Particle traps have been selected as the sampling device for this study because of the availability of archived samples that cover recent transitions in global climate. Legacy samples available from HAUSGARTEN, and several other times-series stations worldwide, hence have the potential to reconstruct baselines of the oceanic microbiome that can be compared to recent conditions observed by ongoing particle trap sampling programs at the same stations. Thanks to the fact that a large sample set was readily available the same analysis could be employed for all samples, thus avoiding any bias introduced by the ongoing evolution of omics methods. As particle traps quantitatively collect sinking particles, the taxonomic information can be combined right away with information on water column biogeochemistry (i.e., particulate organic matter export fluxes).

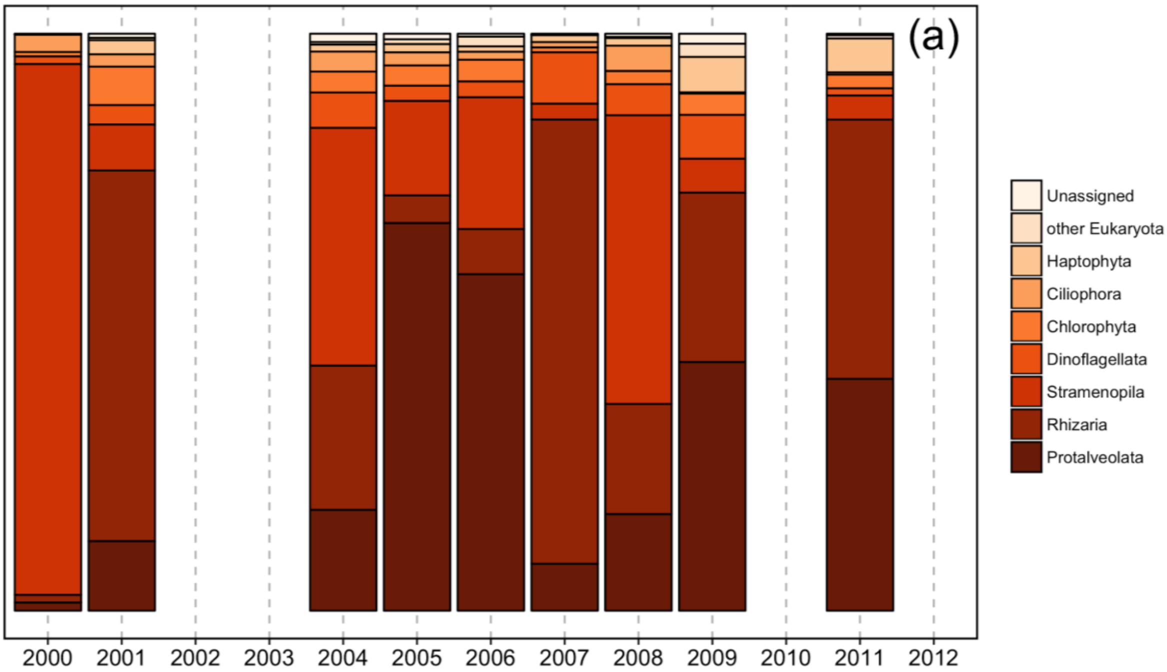

Fig. 6. Evolution of the relative contribution of different taxonomic groups to the composition of the protist communities in surface water of eastern Fram Strait at the time of the summer peak POC export flux in the period 2000 to 2011 (graph I. Salter et al., unpublished)

The study focused on the central HAUSGARTEN site in eastern Fram Strait (see above). To synchronize the protist community observations with the timing of the productivity cycles, samples selected for molecular analyses originated from the periods when export fluxes of particulate organic carbon (POC) peaked in spring and summer. DNA was successfully extracted from all samples that had been preserved with mercury chloride and stored for more than 10 years. After amplification of the V4 region of the 18S rRNA gene, sequencing was performed with an Illumina MiSeq sequencer. Protist communities identified based on the detected OTUs showed convincing temporal patterns that indicate multiannual variations in prevailing primary producers at the site (Fig. 6). Modeling-based data analysis revealed that characteristic communities were associated with specific environmental conditions and particularly with the respective levels of POC fluxes. The study integrated a variety of multidisciplinary observations, carried out in situ as part of the FRAM observational framework and by satellite remote sensing. This provided further evidence that these variabilities are not preservation or analysis artifacts but represent real environmental patterns in protist communities that are related to specific productivity regimes. In summary the study demonstrates the feasibility and relevance of time-series observations of microbial communities by means of simple sampling with well-established samplers (i.e., particle traps) in combination with standard omics techniques (i.e., amplicon sequencing). A manuscript on the study is currently under preparation and is expected to be published soon (Salter et al, in prep c).

Enhancing the FAIRness and comparability of omics data in observatory settings

Omics-based approaches produce large amounts of raw data (e.g. DNA and/or RNA sequences) which can be analyzed with a wide variety of bioinformatics tools and methods. The results derived from these tools can vary considerably, and a lack of global coordination of “in silico” methods across omics-observatories hinders inter-comparability, interoperability, and consolidated reporting. Mainstreaming omics in global assessment and monitoring requires greatly improved harmonization of omics data products in what is still largely an innovation- and research-driven field.

This is especially true as more marine observatories begin to deploy omics technologies in operational settings, often creating local and ad hoc data standards which make data integration across observatories difficult. Indeed, without a core set of common tools and reference resources (reference gene catalogues, taxonomies, etc.) operational usage of omics technologies loses practicality.

Three key outcomes of AtlantOS WP3 have addressed these needs and have contributed to a firm foundation for future improvements and network building:

1. Through GLOMICON (compare AtlantOS deliverable D6.4), the omics network seeded by AtlantOS, AWI and Ribocon, with support from external partners, have launched strategic efforts to harmonize the datascape, information resources, and knowledgebase of the global omics observatory community and link them with other key information resources and services:
    a. In cooperation with GFBio (www.gfbio.org; seeded by the FP7 project Micro B3: www.microb3.eu), these partners have established an online registry of omics observatories, harvesting key metadata about the observatories themselves (glomicon.org/registry). This is linked to a web page to improve visibility of GLOMICON’s mission, coordination resources (see below) and activities.
    b. A coordination space for key bioinformatics code and software tools has been launched on GitHub (github.com/GLOMICON) to support collective development and testing in the community
    c. A coordination space for established and developing omics sampling and field protocols (which are key metadata for downstream in silico analyses) has been established in protocols.io (protocols.io/groups/glomicon). This allows the network to avail of protocol.io’s advanced document management, versioning, and sharing capabilities. From this growing pool of protocols, we will submit a candidate “best practice” workflow to the UNESCO/IOC Ocean Best Practices System (WP6)
    d. An ontology – a human and machine-readable knowledge representation which addresses the “I” in FAIR – for the omics observing domain has been established using the best practices of the Open Biological and Biomedical Ontologies Foundry and Library (github.com/GLOMICON/omicon) and will be linked to the development of ontology resources for the GOOS EOVs (initiated in WP6, with endorsement from GOOS) as the community matures.
    e. AWI has initiated a GLOMICON-driven extension to the metadata standards of the Genomic Standards Consortium (GSC) to support omics in the cryosphere/marine realm. “MIxS-cryo” is now gathering input from experts and a manuscript is being refined for publication.
    f. A meeting (December, 2018) at the European Bioinformatics Institute between representatives of the Global Biodiversity Information Facility (GBIF), the European Nucleotide Archive (ENA, an INSDC node), SILVA, and GLOMICON secured an agreement on the implementation of data exchange between omics data stores and global biodiversity occurrence aggregators like GBIF. This major step in mainstreaming omics will be pursued in Q1 of 2019, and announced at two workshops pending acceptance of session proposals (submitted).
    g. A satellite meeting at the 21st meeting of the GSC (May, 2019) has been organized to discuss the merger GLOMICON with the US-based Genomic Observatory Network (genomicobservatories.org), with particular focus on data and metadata harmonization through resources such as GeOMe (geome-db.org), to further consolidate omics observatories on a global scale.

2. Building on the above efforts (particularly 1b and 1c), Ribocon has established a “baseline” bioinformatics analysis procedure for data exchanged by GLOMICON members. This will create an anchor for intercomparison and coordination between observatories using different methods as the community develops standard practices. This pipeline can be applied to raw sequences from all partners, and adopts the well-established SILVA NGS data analysis pipeline.
3. To systematically demonstrate the utility of 2 within the informatics framework established in 1, an international intercomparison study coordinated by AWI and MBARI was initiated at the AtlantOS and Genome Canada/Agriculture and Agri-Food Canada Workshop on enhancing interoperability & coordination of long-term omics observations (January 2018). Six omically enabled observatories and institutions (inside and outside AtlantOS) joined the experiment and allocated funds for lab and bioinformatics analyses. Sampling is near complete and distribution of samples among participants has been planned. The sequencing and first round of bioinformatics analyses are expected to take place within the time frame of AtlantOS. The method comparison focuses on analyses of protist communities by means of amplicon sequencing and includes the entire pipeline from DNA extraction to bioinformatics analyses. Environmental samples from different regions and DNA extracts from mock communities are being exchanged between participants for replicate analyses according to the procedures established at partner institutions. The results of this exchange will be needed to estimate the stability of data in our setting, and allow us to test the effectiveness of data exchange and FAIR compliance over the next year. The approach is introduced in AtlantOS D6.5. The activity represents a first step towards harmonization across observatory programs, which represents a key requirement by the FOO for any mature contribution to EOV observations as part of the Global Ocean Observation System.

In conclusion, through the coordination of data-focused activities in close association with global experimental activities, the AWI and Ribocon partners have leveraged and added significant momentum to existing efforts to enhance FAIR data exchange in omics observatory. There is still work to be done in order secure sustained and robust data coordination; however, an extensible foundation, supported by growing community endorsement, has been laid and linked to GOOS activities (e.g. see Section 2.9 in GOOS report #232[9]). As AtlantOS concludes, multi-lateral efforts to sustain this work are being pursued by both internal and external partners.

[9] GOOS Biology and Ecosystem Panel (GOOS BioEco) (2018). Report of the Third Panel Meeting, 7-9 November 2018. GOOS Report #232, UNESCO, St. Petersburg, Florida, USA. 63 pp. 3 annexes. http://www.goosocean.org/index.php?option=com_oe&task=viewDocumentRecord&docID=23380

3. Biogeochemical EOV Innovations

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Dissolved Oxygen

Sensor technology for dissolved oxygen has reached full maturity for operational observation on a wide range of (autonomous) platforms.

The classical Clark-style electrochemical technique had already matured prior to AtlantOS and, owing to its very short response times and corresponding applicability for fast profiling applications, has remained the method of choice for profiling CTD deployments. However, due to remaining long-term drift issues and resulting accuracy limitations, this method is considered suboptimal for long-term deployments such as on moorings, Argo floats etc.

The oxygen optode technology has now also reached full maturity. Its superiority with respect to long-term stability, together with other properties such as small size and power consumption, robustness, lends itself to long-term deployments. The major available optode types (Aanderaa 4330, Sea-Bird SBE63) have been extensively tested both in the laboratory and in the field, so their response (as a function of oxygen, temperature, salinity, pressure), response time and ageing characteristics are now well documented (Bittig and Körtzinger, 2015; Bittig et al., 2013; 2015; 2017; 2018). Furthermore, data processing and quality control routines (e.g., MATLAB functions, SCOR WG 142 recommendations) are available. The different operation modes of the two major optode types, i.e. unpumped (Aanderaa 4330) vs. pumped (Sea-Bird SBE63), provide optimization potential to specific platform requirements. Where a pumped seawater flow can be provided, the flow-through SBE63 sensor is applicable yielding slightly faster response times, easier biofouling protection and the possibility of near-synchronous measurements with other sensors on the same sample. The open Aanderaa 4330 sensor in contrast, can be used most favourably in situations where regular exposure to air is possible during the deployment thereby allowing for in-air calibration and hence highest data accuracy. Response times of these two optode types are ideal for stationary and generally good enough for slow profiling applications (e.g., float, glider, profiling mooring). Where necessary, deconvolution procedures have been applied successfully to correct for the smearing effect of the response time by back-folding the data (Fiedler et al., 2013).

In order to overcome the response time as a limiting factor for fast moving/profiling platforms, a novel oxygen optode sensor (HydroFlash O2, KM Contros GmbH, Kiel/Germany) was extensively tested in the laboratory and the field within AtlantOS (jointly with Task 6.1, see deliverable D6.3 and Fig. 7). The sensor shows a temperature-dependent response time (t_{63}) of about 4 s (weak-turbulent flow) and is thereby significantly faster than the other optical oxygen sensors Sea-Bird SBE63 and Aanderaa 4330. It has also been shown to achieve comparatively high accuracy by individual sensor multi-point calibration (RMSE < 1 µmol · L^{-1}). A remaining issue appears to be the sensor's sensitivity to direct sunlight, which comprises its suitability for profiling floats.

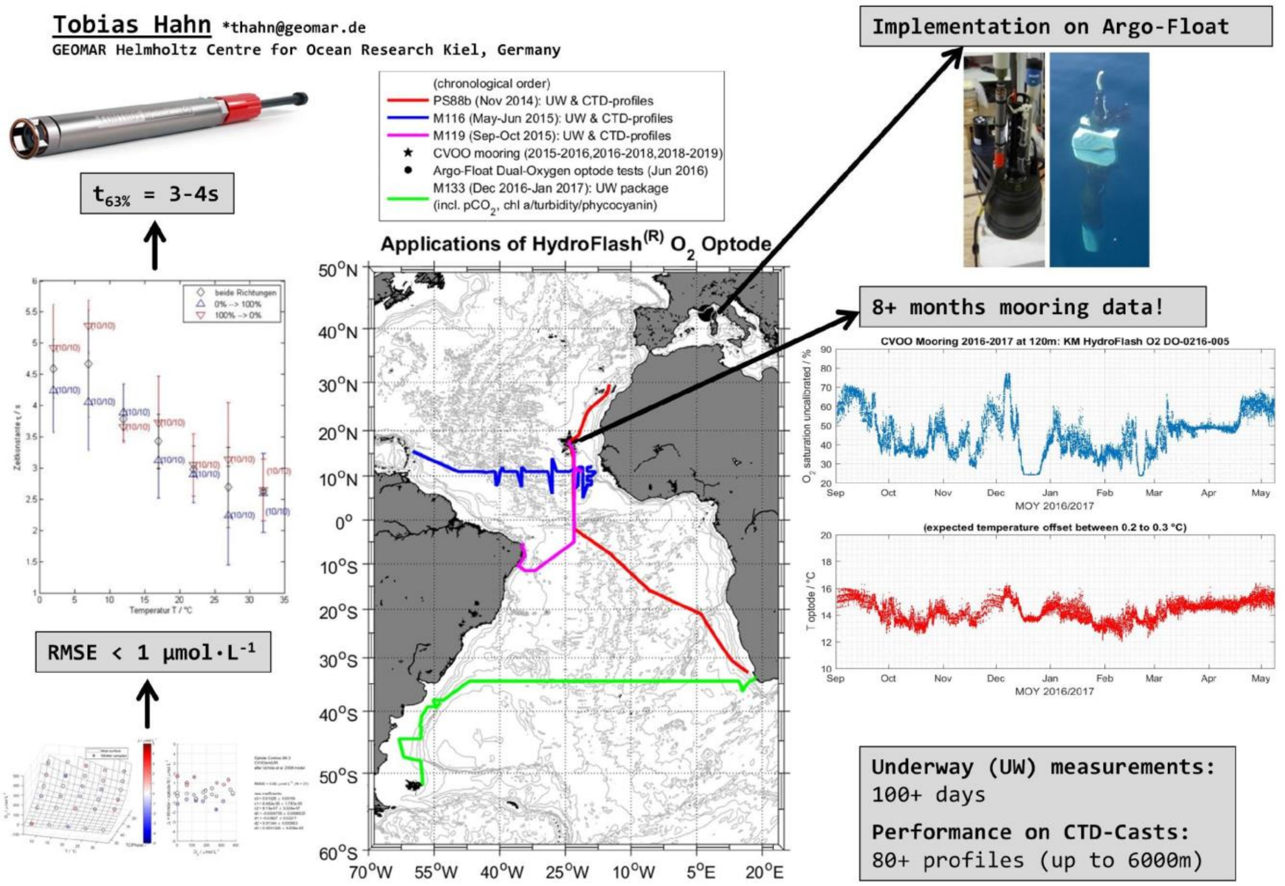

Fig. 7: Applications and major outcomes of HydroFlash O2 optodes in the framework of AtlantOS (Data and figure: T. Hahn, GEOMAR; work carried out in conjunction with Task 6.1).

Fig. 8: (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 (from AtlantOS D6.3).

Carbonate System Parameters

Work carried out in the AtlantOS project has focussed on measurement/sensing technology for 3 of the 4 measurable parameters of the marine carbonate system (**pH**, ***p*CO₂**, DIC, **TA**).

pH: Laboratory tests performed on a KM Contros prototype pH optodes using commercially available sensing foils have been conducted on buffer solutions of defined pH value for the determination of the optode’s precision (jointly with Task 6.1, see deliverable D6.3 and Fig. 8). For this, universal opto-electronics were realized and tested that can apply the time and frequency domain dual lifetime referencing measuring method (f-DLR and t-DLR, resp.) for direct comparison. The current precision achieved with this prototype sensor for pH does not meet the quality requirements by more than a factor of 20 (f-DLR). The time based DLR (t-DLR) was tested as well, yet provided even less reproducible results.

CO_2 partial pressure (*p*CO_2): Autonomous in-situ measurements of CO_2 partial pressure (*p*CO_2) until today rely mostly on well-established technologies which employ gas-phase equilibration via planar or tubular membranes and subsequent NDIR-based CO_2 detection or water-phase equilibration of a measurement solution and subsequent spectrophotometric pH detection. These submersible *p*CO_2 sensor technologies have gone through major development and improvements levels and can be considered mature. They have been shown to be capable to achieve long-term accuracies of as good as 1% under most stringent operation and deployment schemes (Fietzek et al., 2014).

An early type of optode-based *p*CO_2 sensors (Aanderaa) had already been available at the start of the AtlantOS project. Our own tests as well as those carried out elsewhere in the scientific community revealed serious issues with the *p*CO_2 optode technology which are associated with shortcomings in the foil technology. Very long response times, serious drift problems and salinity memory effects were the major limiting factors which severely limited utility of the sensor. Our hopes were for this technology to see rapid advancement and adoption by other manufacturers. It was thus planned to test and deploy novel versions of this sensor type in various types of field tests. Other than expected, however, since then none of the few manufacturers of the critical sensing foils for *p*CO_2 sensors (PreSens, Pyroscience) were able to deliver an improved foil. Our SME partner (KM Contros) therefore decided to dispensed the development of an own *p*CO_2 sensor, as the market perspective for this sensor technology deteriorated and until today has remained too small.

Tests carried out under Task 3.2 during the R/V Meteor Cruise 133 across the South Atlantic (Dec. 2016/Jan. 2017, Cape Town/South Africa – Stanley/Falklands) featured established membrane-based systems both for submersible use (HydroC CO_2, KM Contros) and bench-top flow-through (HydroC CO_2 FT, KM Contros), both of which were operated against the internationally established equilibrator-based reference *p*CO_2 system (GO model 8280, General Oceanics, Miami/USA, Pierrot et al., 2009). These confirmed earlier findings of the achievable accuracy of < 1 %.

Total Alkalinity (TA): Under Task 2.2, the first commercially available automated flow-through system for TA was extensively tested in the laboratory and in the field (R/V *Meteor* Cruise M133, R/V *Maria S. Merian* Cruise MSM 68/2, “Voluntary Observing Ship” M/V *Atlantic Sail*). A major issue observed during these tests were leakages in the degasser unit, which allows the CO_2 after sample acidification to be stripped from the sample. This and other observations led to various improvements of the commercial version. According to our findings, the revised and improved current version is suitable of performing autonomous high-quality TA measurements (Seelmann et al., 2019). Ideas exist for miniaturization of the instrument that would allow submerged operation on autonomous platforms (e.g., Wave Glider, float). However, this would involve serious development work and testing which is beyond the reach of the AtlantOS project.

4. Observation Platform Innovations

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Profiling Winch Mooring for Eulerian Time-Series

Eulerian time-series observations are typically carried out by autonomous moorings (both with buoy or sub-surface top floatation element). Such moorings have limited vertical resolution which can be increased either by the number of fixed point sensors (very expensive) or by integration of moored profilers which can crawl up and down to mooring wire with a sensor payload (frequent problems with fishing longlines entangled around moorings in tropical oceans). A further complication is the fact that upper ocean observations, which given the magnitude of the signals often are of highest scientific importance, are severely compromised by sensor biofouling issues.

As an alternative way to vertical profiling and prevention of major biofouling we developed a submersible winch system with a multi-sensor profiler. The system is designed to allow profiling measurements from depth of up to 150 m to near the sea surface from where data can be telemetered via a communication tether attached to the profiler. While successful deployments have been achieved (see Fig. 9) and demonstrated the functionality of the approach significant issues with the reliability of the rather complex system remain. The approach is therefore still in need of further development aiming at reduced complexity and increased robustness.

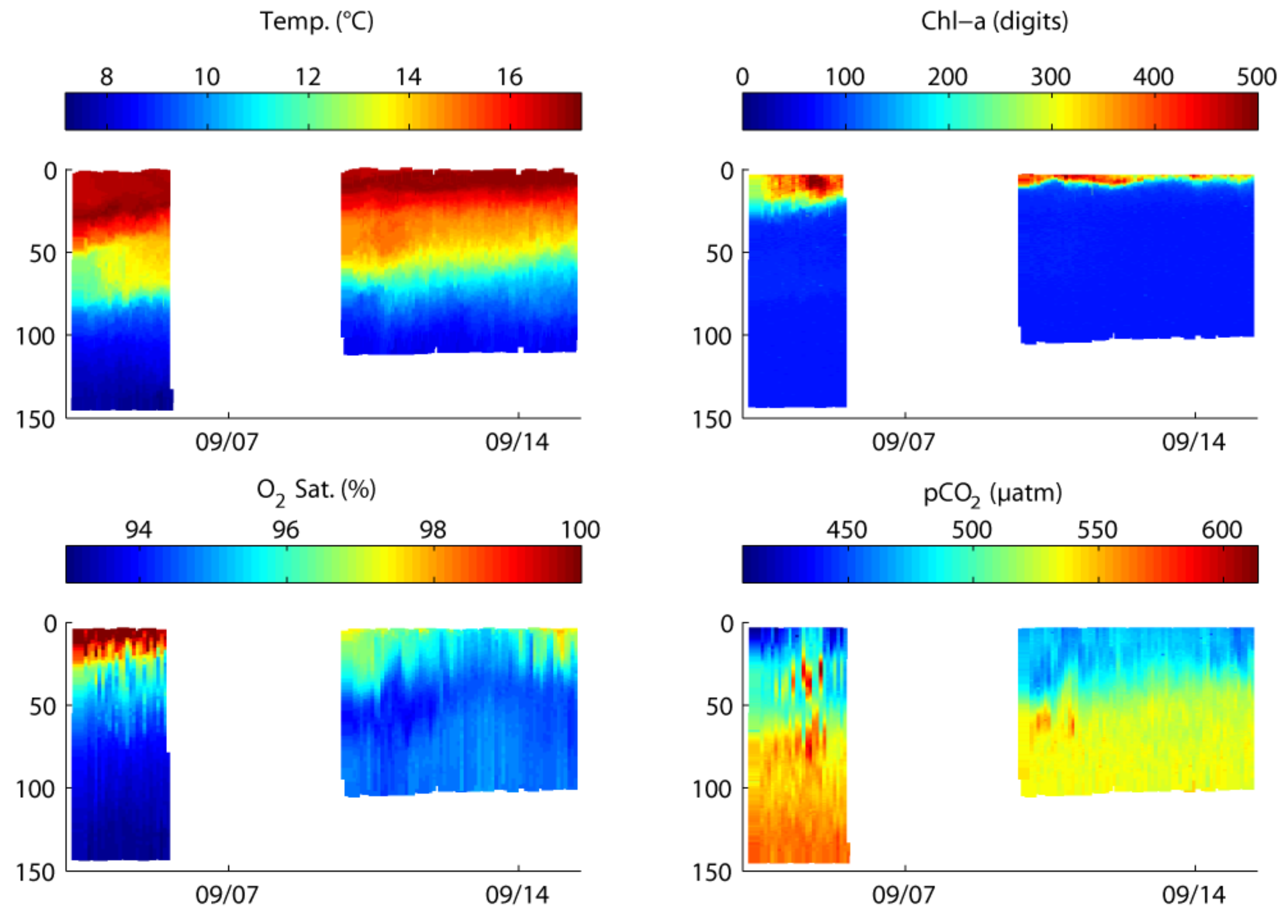

Fig. 9: Time-series of 94 vertical profiles of temperature, chlorophyll a, oxygen saturation and pCO_2 as acquired in September 2015 in 2-hour intervals during test deployments of the GEOMAR submersible winch system in the Koster Fjord, northeast Skagerrak, Sweden (Data and figure: B. Fiedler, GEOMAR).

Virtual Mooring for Surface Time-Series

As an alternative to truly Eulerian observation approaches (mooring, buoy), we have explored the possibilities of employing mobile autonomous platforms (float, wave glider).

For the float-based approach, we had already demonstrated prior to AltantOS (Fiedler et al., 2013; Fig. 10) the feasibility of time-series observations mimicking a Eulerian observatory using modified Argo floats (Nemo, Optimare) in combination with state-of-the-art oxygen optodes (Aanderaa 4330) and membrane-based pCO_2 sensors (HydroC pCO2, KM Contros). This approach, that in our early work that was still compromised by power and response time limitations, is now near-operational with the technologies and procedures developed as part of the Biogeochemical Argo Program (Biogeochemical-Argo Planning Group, 2016). Modern SeaFET pH sensor (see above) permit high-quality long-term carbon system observations from autonomous platforms.

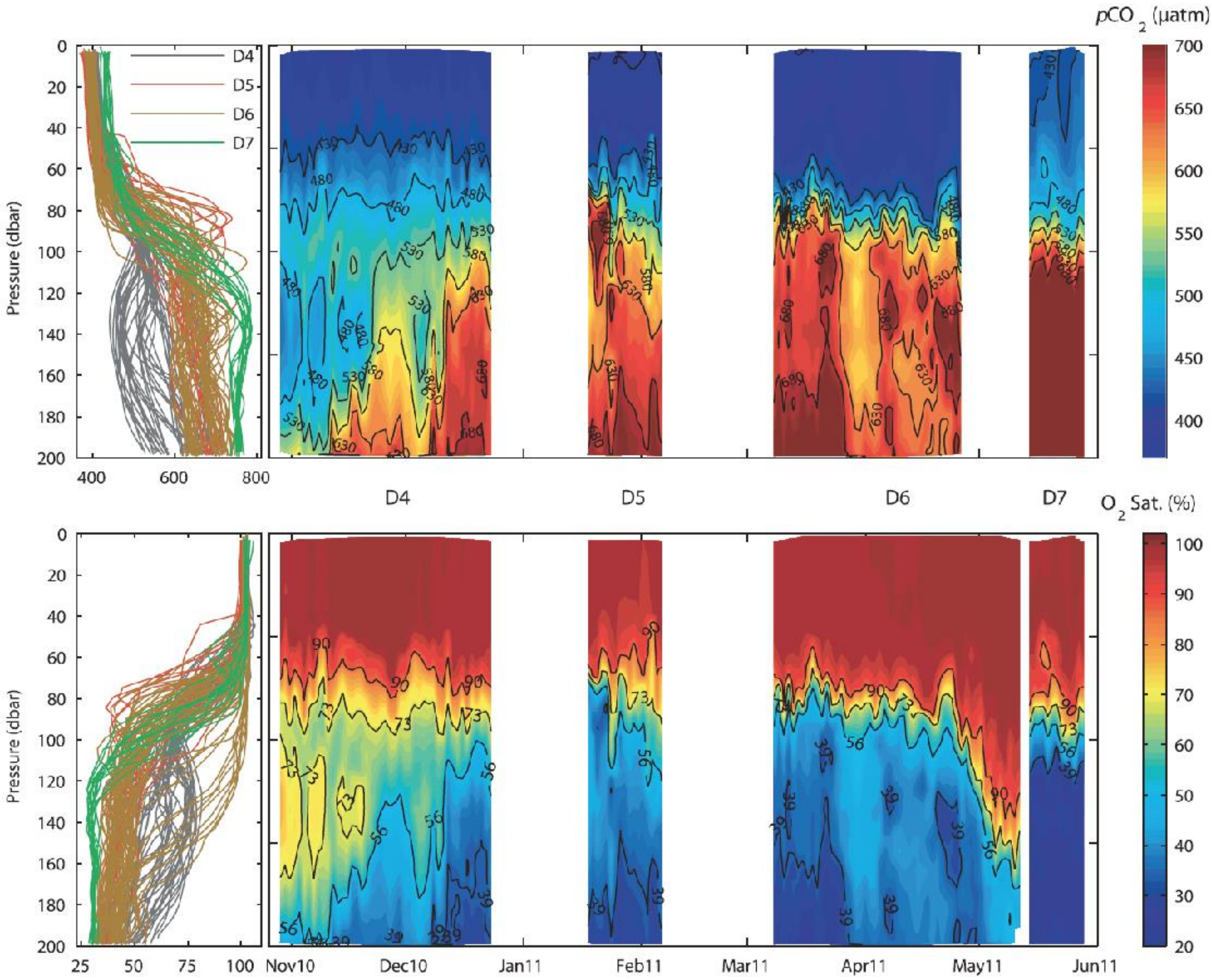

Fig. 10: Final pCO_2 (top) and O_2 data (bottom) collected in the vicinity of the Cape Verde Ocean Observatory. Time series for both parameters are strongly complementary to each other. Blank areas indicate times when the instrument was refurbished on land–ship for the next deployment (from Fiedler et al., 2013).

As a further alternative to surface ocean Eulerian observation, which otherwise can only be achieved from buoy-based systems and with dedicated anti-biofouling measures, the wave glider technology has been explored extensively. For this purpose, custom-made flow-through sensor packages featuring T, S, chlorophyll a, turbidity, O_2, pCO_2, and total gas tension sensors were successfully integrated into the payload bay of Liquid Robotics wave gliders SV2 and SV3. It could be shown that this mobile platforms allow to mimic Eulerian time-series observations for surface ocean properties (Fig. 11).

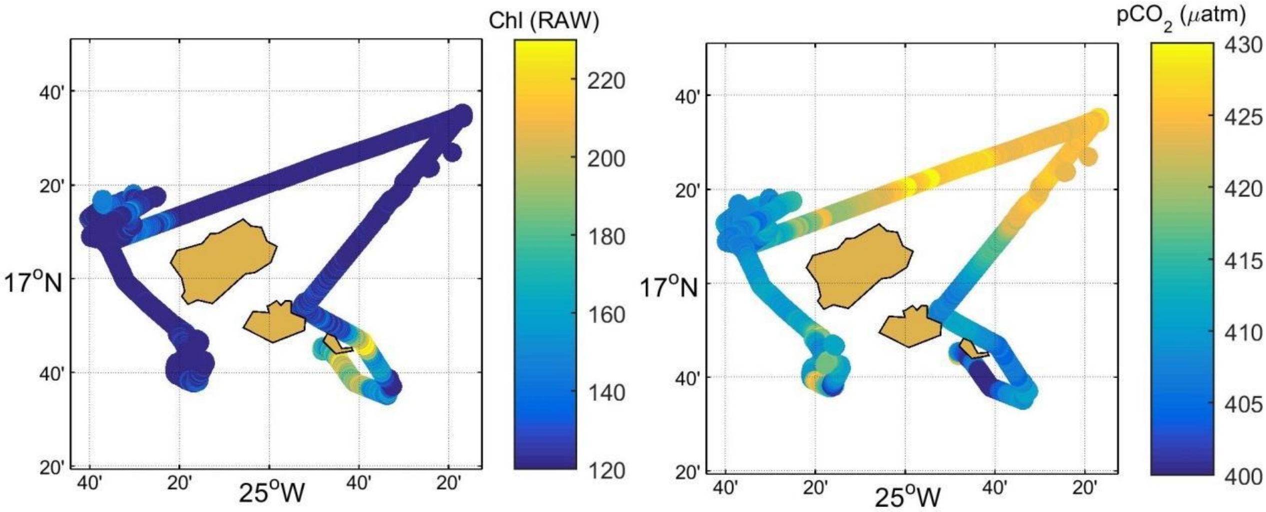

Fig. 11: Surface water pCO_2 and O_2 measurements performed from a Liquid Robotics SV2 water glider around the Cape Verde Ocean Observatory during a 3-week test deployment in May/June 2015 (Data and figure: B. Fiedler, GEOMAR).

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