AtlantECO

Atlantic Ecosystems Assessment, Forecasting & Sustainability

# Deliverable number 4.5 - Report on augmented observations

Dissemination level: Public or Confidential

| | |
|---|---|
| Related Work Package | WP 4 |
| Related task(s) | Task 4.2 Generate new observations |
| Lead beneficiary | University of São Paulo (USP) |
| Author(s) | R.M. Lopes (USP), S. Pesant (EMBL) |
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***Declaration:*** *Any work or result described therein is a genuine output of the AtlantECO project. Any other source will be properly referenced where and when relevant.*

Table of Content

# 1 Version History

| Version | Authors | Summary of changes | Date |
|---|---|---|---|
| 0.1 | RM Lopes | Initial draft | 30 Aug 22 |
| 0.2 | RM Lopes | Initial draft with additions | 6 Sep 22 |
| 0.3 | RM Lopes | Interim draft | 29 Sep 22 |
| 0.4 | S Pesant | Interim draft with additions | 3 Oct 22 |
| 0.5 | RM Lopes | Second draft document | 10 Oct 22 |
| 0.6 | RM Lopes | Third draft amended after project monitoring | 28 Nov 22 |
| 0.7 | D Iudicone | Review before submission | 29 Nov 22 |
| 0.8 | S Pesant | Final corrections | 30 Nov 22 |
| 1.0 | E Trabut | Submission | 07 Dec 22 |

# 2 Executive summary

This Deliverable aims at describing the main activities carried out during years 1 and 2 within the scope of AtlantECO Work Package 4 relative to task 4.2. The objective of Task 4.2 is to generate new observations about microbiomes, plastics and the plastisphere. It was implemented during the first reporting period by partners EMBL, USP, EMBRC-ERIC, EMSO-ERIC, CEA, AWI, CNRS, CSIR, FTO, FURG, NIOZ, NOC, MBA, SU, SZN, UCT, UFSC, UFSCar, UniLIV and UP through six activities:

1. Flagship cruises (Subtask 4.2.1)
2. All Atlantic Ocean Sampling Day (Subtask 4.2.2)
3. Sail-4-Science (Subtask 4.2.3)
4. Continuous Plankton Recorder (Subtask 4.2.4)
5. Genomics, Carbon and Plastic analysis of Moored Sediment Trap samples (Subtask 4.2.5)
6. Third party cruises (Subtask 4.2.6)

Progress was achieved to various degrees by the six Subtasks, two of which (Subtasks 4.2.1 Flagships and 4.2.6 Third party cruises) saw several delays due to covid-19 restrictions, and two others (Subtasks 4.2.2 AA-OSD and 4.2.3 S4S) were rescheduled to ensure the success of our engagement with external partners and citizens.

Task 4.2 has benefited from activities covered by task 4.1, by means of two deliverables scheduled and completed during the first reporting period (M0-M20):

- D4.1 "AtlantECO Handbook of Standards and Best Practices (version 1)" was delivered with a few months delay to incorporate survey results and feedback from participants to AtlantECO's Webinar on standards and best practices (May 2021). It is available on Zenodo (https://doi.org/10.5281/zenodo.4897861).
- D4.2 "AtlantECO Handbook of Standards and Best Practices (version 2)" was delivered with a few months delay to incorporate the latest materials from AtlantECO's training course in South Africa (May 2022) and other activities. It is available on Zenodo (https://doi.org/10.5281/zenodo.4897860).

Handbook guidelines have been applied with success in flagship cruises belonging to Task 4.2, including the Tara Mission Microbiomes expedition, Eugene Seibold cruises and a RV Alpha Crucis expedition in the SW Atlantic off Brazil.

In addition, WP4 completed all milestones scheduled during the first reporting period (M0-M20), which also positively impacted activities belonging to Task 4.2:

- MS01 "Port calls schedule established for the first 2 years" was completed, and the schedule is publicly available on the AtlantECO website (https://www.atlanteco.eu/expeditions).
- MS02 "AtlantECO standard protocols workshop" was completed, and both the workshop proceedings (https://doi.org/10.5281/zenodo.4275504) and the resulting deliverable D4.1 are publicly available on Zenodo.
- MS09 "First South Atlantic CPR campaign" was completed, and the campaign summary report is publicly available on Zenodo (https://doi.org/10.5281/zenodo.6023403).
- MS10 "Workshop aimed at harmonising quality control and analysis methods for the South Atlantic CPR survey." was completed online and the resulting CPR control and analysis protocols are included in D4.2. A second workshop in person is scheduled for the second reporting period.

During the first reporting period, a single key exploitable result was produced by Task 4.1. The AtlantECO Handbook of Standards and Best Practices (D4.1 & D4.2) is a compendium of resources and will be consolidated throughout the second and third activity periods of the project (D4.3 & D4.4). A series of Webinars and training materials produced by the European Blue Biobank (EBB), a synergy initiative led by AtlantECO partner EMBRC-ERIC, are also exploited by AtlantECO together with the Handbook.

The Handbook is used to implement research activities in Task 4.2, notably the flagships and third-party cruises, the All-Atlantic Ocean Sampling Day, the citizen Sail-4-Science, and the Continuous Plankton Recorder (CPR) survey line between Santos and Durban. The Handbook is also exploited by WP4 to implement its Hub & Nodes approach to develop inter-comparable analysis pipelines across the project's imaging and sequencing centres in Europe (CEA & SU), South Africa (UP & UCT) and Brazil (UFSCar & USP). Finally, the Handbook was exploited by WP9's capacity building and training activities, such as the Port Calls of Mission Microbiomes (Sept 2021 - Sept 2022), and AtlantECO's 10-day training course in South Africa (May 2022). The exploitation of this key result will continue during the second and third periods of the project.

Exploitable results from Tasks 4.2 will become available during the second and third activity periods of the project, feeding into the activities of other data-driven Work Packages, and in the project Case Studies.

*This document is based on the terms and conditions established in the Grant Agreement (GA) and its Annexes, as well as in the Consortium Agreement (CA).*

# 3 Introduction

The AtlantECO Project aims to study the Atlantic Ocean from pole to pole to determine the structure and function of the Atlantic microbiome in the context of ocean circulation and presence of pollutants to assess 1) its role in driving the dynamics of Atlantic ecosystems at basin and regional scales; 2) its potential of being used as a sensor of ecosystem state and 3) the mechanism by which it drives the provision of ecosystem services.

In order to deliver this, the project builds on four activity streams (AS)

- **AS1: Asses the status of the ecosystem structures, functions, health and services** at regional, basin and all Atlantic scales and provide high quality gridded data products and maps
- **AS2: Enhance knowledge and innovate** by adopting standard optical and genetic observations protocols, cutting-edge network analysis methods and better parametrisation of connectivity and biogeochemical models
- **AS3: Assess drivers and stressors of change and forecast** their impact on tipping points and recovery of ecosystem structures, functions and services and develop eco-socio-economic models to predict their future states
- **AS4: Share and use** capacity and knowledge across the four continents bordering the Atlantic Ocean ensuring a seamless engagement between science, industry, policy and society.

AtlantECO addresses three major challenges **microbiome**, **plastics and the plastisphere** and **seascape and connectivity** and defines these as the scientific concepts underpinning the Project which, together with their interactions, will help address the gap in current knowledge and understanding on how they affect ecology, biodiversity, sensitivity to climate change and the potential for a sustainable exploitation of Atlantic natural resources. Building on the knowledge acquired about the status and dynamics of the Atlantic ecosystem, an additional challenge will be to optimize a set of tools and metrics to tightly couple ecosystem functioning and socio-economic activities, integrating them in a unified framework with Eco-Socio-Economic analyses and projections, which we consider a prerequisite for the sustainable management of the Atlantic Ocean. A final challenge to be addressed by AtlantECO is the design of a strategy and of procedures to guarantee that the new unified framework will be implemented in a systemic way so that all the components of the implicated societies in countries bordering the basin will contribute to the implementation and will fully benefit from it.

AtlantECO focuses on five ecosystem services:

- **ES1**: Climate support system: carbon drawdown and storage
- **ES2**: Deep ocean life support system: transfer of matter & energy to mesopelagic and seabed ecosystems.
- **ES3**: Food security and the trophic fluxes: fisheries and aquaculture.
- **ES4**: Healthy planet, healthy people: biohazards, cycling of plastics and pollutants
- **ES5**: Biodiversity: resilience, adaptation and contribution to the Bioeconomy, including new chemicals

One objective of WP4 is to generate new observations about microbiomes, plastics and the plastisphere. It is implemented by Task 4.2 through dedicated sampling activities in the North and South Atlantic (see details below), and its results are exploited in first instance by WPs 2, 5 and 6, and shared with the wider research community following the project's Data Management Plan (WP10). Progress was achieved to various degrees by the six Subtasks implementing this objective, two of which (Subtask 4.2.1 Flagships and 4.2.6 Third party cruises) saw several delays due to covid-19 restrictions, and two others (Subtasks 4.2.2 AA-OSD and 4.2.3 S4S) were rescheduled and are still in the planning phase to ensure the success of our engagement with external partners and citizens. These delays justified in part the extension of the project's calendar to 60 months to ensure the delivery of new observations during the second and third periods of the project with minimum disruption to their exploitation by other Work Packages. This deliverable is the first version of a series of reports on AtlantECO's augmented observations resulting from Task 4.3.

# 4 Activities

## 4.1 General objective of Task 4.2

The objective of Task 4.2 is to generate new observations about microbiomes, plastics and the plastisphere. It was implemented during the first reporting period by partners EMBL, USP, EMBRC-ERIC, EMSO-ERIC, CEA, AWI, CNRS, CSIR, FTO, FURG, NIOZ, NOC, MBA, SU, SZN, UCT, UFSC, UFSCar, UniLIV and UP through six activities:

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### ○ Sub-task 4.2.1 All-Atlantic flagship cruises (lead: USP)

***This sub-task has successfully launched four of its six flagships*** despite important delays due to covid-19 restrictions. While the three research sailboats (Eugen Seibold, Tara and Veleiro ECO) were able to navigate the restrictions and conduct their research with minimal delays, larger oceanographic vessels operating as national research facilities were largely impacted by the restrictions and had to cancel and/or postpone their cruises. A short (2-week) cruise off South Brazil was accomplished on board RV Alpha Crucis during July-August 2022. The RV Agulhas II (South Africa) carried out a polar cruise for a period of 3 weeks during austral winter. The RSS Discovery is also now operational and the proposed work on marine microbiomes is scheduled to start during the second period of the project. Partners EMBL, CNRS, UFSCar, UFSC, UniLIV and MPIC (external partner) presented the sampling accomplished during the flagships at the 2022 General Assembly meeting in Cape Town. Their presentations are publicly available on Zenodo (https://doi.org/10.5281/zenodo.6748938).

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Task 4.2.1 Generate new observations

***The all Atlantic Flagship cruises***

| Activity type | Activity name | Spatial coverage | Status |
|---|---|---|---|
| Flagship | SV Eugen Seibold | 65°N - 15°N North Atlantic | completed 2020 |
| Flagship | SV Eugen Seibold | 65°N - 15°N North Atlantic | on going |
| Flagship | SV Tara - Mission Microbiomes | 15°N - 60°S West Atlantic | completed 2021 |
| Flagship | SV Tara - Mission Microbiomes | 40°S - 15°N East Atlantic | on going |
| Flagship | SV Veleiro ECO | 0°- 30°S South Atlantic | on going |
| Flagship | SV Veleiro ECO | 0°- 30°S South Atlantic | on schedule 1st/4 2023 |
| Flagship | RSS Discovery - AMT30 | 45°N - 45°S Atlantic | postponed to 1st/4 2023 |
| Flagship | RSS Discovery - AMT31 | 45°N - 45°S Atlantic | postponed to 4th/4 2023 |
| Flagship | RV Alpha Crucis | 21°S - 29°S South West Atlantic | completed 2022 |
| Flagship | RV Alpha Crucis | 21°S - 29°S South West Atlantic | postponed to 4th/4 2023 |
| Flagship | RV Agulhas II | 35°S - 45°S Southern Ocean | completed 2022 |
| Flagship | RV Agulhas II | 35°S - 65°S Southern Ocean | postponed to 1st/4 2023 |

*Figure 1. Schedule of AtlantECO flagship cruises*

The flagship cruises are implementing the base protocols described in the AtlantECO Handbook on Standard Protocols & Best Practices (D4.1 & D4.2), using common sequencing and imaging analysis methods targeting consistent size-fractions of the marine microbiome. Each flagship nevertheless has the freedom to select

subsets or adapt the proposed base protocols (i.e., to use derived protocols), and to add additional protocols depending on the capacity of each research platform, and according to the scientific objectives and questions addressed. For example, microbiomes of the surface microlayer and of aerosols were studied on board SV Tara and SY Eugen Seibold using different sampling devices but using similar analysis methods. Microbiomes of the epi- and mesopelagic water layers are however sampled, size-fractionated and analysed using the same methods across all flagships.

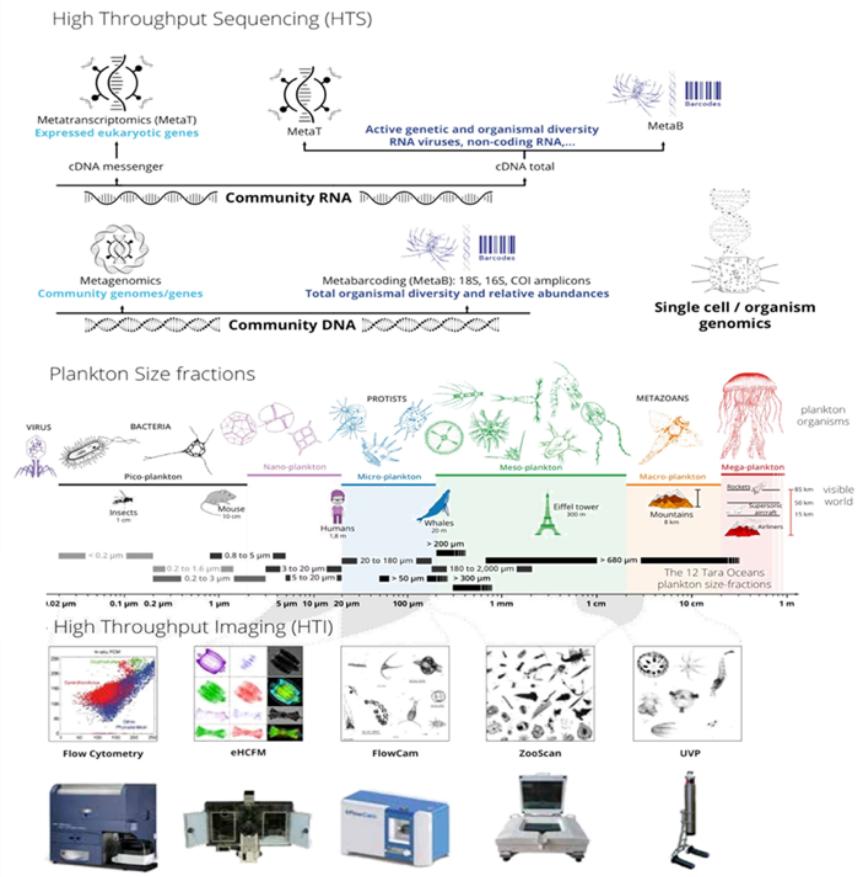


*Figure 2. The six base entities (organisms & size-fractions in the middle part), and a range of sequencing analysis methods (top) and imaging analysis methods (bottom) targeted by the sampling strategies on board the AtlantECO flagships (reproduced from Suanagawa 2020)*

***Flagship cruises contributed to capacity building***, especially with Early-Stage Scientists (ESRs) who participated actively in the different campaigns, gaining field work experience at sea with AtlantECO base protocols, and developing their international network. Over 25 ESRs from Brazil, South Africa, Europe, and North America participated in AltanECO's flagships so far:

- ***Brazil*** - Paula Huber (UFSCar), Pedro Junger (UFSCar), Erica Becker (UFSC), Andrea Green (UFSC), Gleice de Souza Santos (USP), Alessandra Gomes (USP)
- ***South Africa*** - Nicole Dames (UCT) Ndamona Mateus (UCT) Mancha Mabaso (UP), Suzana Nicolau (Angola), Damaria Boussiengue (Congo), Roland Ngomo (Congo), Ange Diedhiou (Sénégal)
- ***Europe*** - Couet, Douglas (CNRS), Léa Olivier (SU), Morgane Ratin (SU), Flora Vincent (EMBL), Clara Trellu (SU), Mathilde Bourreau (CNRS), Louis Caray (CNRS), Isabella Hrabe de Angelis (MPIC, Germany), Giancarlo Bachi (CNR, Italy), Antonella Ruggiero (SZN), Rémi Laxenaire (CNRS)
- ***North America*** - Guillaume Bourdin (UMaine, USA), Charlotte Begouen Demeaux (UMaine, USA), Nastassia Patin (NOAA, USA), Cora Hörstmann (OFI, Canada)

*Figure 3. Early-Stage Scientists participating in flagships cruises related to Task 4.2*

***The following Campaign Summary Reports (CSR)*** of the AtlantECO Flagships are available on Zenodo:

- SY Eugen Seibold - Iceland to Cabo Verde (2020.06.02-2021.05.02)
  CSRs coming soon on Zenodo
- SV Tara - Amazon plume - Fort-de-France to Macapa (2021.08.16-09.08)
  https://doi.org/10.5281/zenodo.6641687
- SV Tara - Amazon plume - Macapa to Belem (2021.09.11-18)
  https://doi.org/10.5281/zenodo.6641701
- SV Tara - Amazon plume - Belem to Salvador de Bahia (2021.09.23-10.09)
  https://doi.org/10.5281/zenodo.6641707
- SV Tara - Trinidad Seamounts - Salvador de Bahia to Rio (2021.10.15-11.03)
  https://doi.org/10.5281/zenodo.6641709
- SV Tara - Santa Marta Cape coast - Itajaí to Buenos Aires (2021.11.19-27)
  https://doi.org/10.5281/zenodo.6641715
- SV Tara - Coccolithophre bloom - Buenos Aires to Ushuaia (2021.12.04-27)
  https://doi.org/10.5281/zenodo.6641726
- SV Tara - Weddell Sea and iceberg experiment - KG Island to Punta Arenas (2022.01.25-02.23)
  https://doi.org/10.5281/zenodo.6641758
- SV Tara - Sub-mesoscale filaments - Punta Arenas to Cape Town (2022.03.05-04.23)
  CSR coming soon on Zenodo
- SV Tara - Benguela upwelling ecosystem - Cape Town to Walvis Bay (2022.05.01-06.01)
  CSR coming soon on Zenodo

## ■ Flagship SV Tara - Mission Microbiomes

The Mission Microbiomes initiative, as a major set of flagship cruises belonging to the AtlantECO project, took place between June 2021 and October 2022 on board the scientific schooner Tara. Partners SZN, SU, CNRS, CEA, EMBL and FTO coordinated this flagship, and partners UFSCar, UFSC, USP, CNRS, EMBL, UniLIV, UCT and UP have contributed significantly to the planning of the different campaigns by organising scientific meetings and leading/joining the fieldwork on board SV Tara. Partners EMBL, CEA and FTO have also contributed significantly to the logistics of the mission. Mission Microbiomes consists of topical studies that cover fundamental ecological patterns and processes of the Atlantic Ocean (see details below). A total of 121 stations were sampled so far, collecting ca. 9,500 samples for biogeochemical, imaging, and sequencing analysis (Table 1). Samples from the first 55 stations were transported from Rio to partner CEA (Paris) in November 2021 for a complete inventory, and then distributed to 14 labs in Europe and the USA to undertake the analyses. Samples from stations 56 to 109 were sent from Cape Town to partner CEA in April 2022 and are being inventoried. All remaining samples will be sent to partner CEA at the end of the mission when SV Tara is back in Europe in October 2022. A subset of samples collected in Brazilian and African waters remains in Brazil (partners UFSCar and USP) and Africa (partner UP) where they will be analysed using common imaging and sequencing methods. This extensive collection of genetic resources will allow us to study the surface and deep microbiome, and the plastisphere, combining cutting-edge molecular and imaging techniques.


*Figure 4. Mission Microbiomes – overview of the expedition route and snapshots taken in different locations*

The following major topics and geographic locations have been covered to date by the Mission Microbiomes expeditions:

- *Sargassum* proliferation in the Caribbean and northern Brazil;
- The Amazon River plume and its contribution to ecosystem dynamics and plastic input to the Atlantic;
- The role of the Vitoria-Trindade seamounts in providing habitat for different components of the microbiome, with a focus on molecular bioprospection;
- Coccolithophore blooms off Argentina and their importance to the carbon cycle;
- Sample across well-established physical and biogeochemical gradients in the Southern Ocean, and conduct a multi-day process study around an iceberg, topics related to the response of the microbiome to variability in the seascape in relation to climate change;
- Topical studies conducted along the coast of Africa will be presented in the next version of this deliverable (D4.6).

The Amazon plume and its interaction with the North Brazilian Current was studied from August to October 2021, including port calls in Fort-de-France, Macapa, Belem and Salvador. Chief scientists were Stéphane Pesant (EMBL) and Paula Huber (UFSCar). While rivers are an important source of pollutants from land into the Ocean, their plumes also transport large amounts of nutrients that fuel primary production in the coastal waters and open ocean, especially in oligotrophic basins. On the western side of the South Atlantic the two main river inputs are from the Amazon River and the Rio de la Plata, whose plumes are regulated by the local western boundary currents. One objective of this campaign was to characterise the river input to the ocean and subsequent dilution processes, and to study changes in the diversity of marine microbiomes and plastic pollution across those gradients. An important objective of this first campaign in the Atlantic was also to test new sampling strategies, equipment, and protocols.

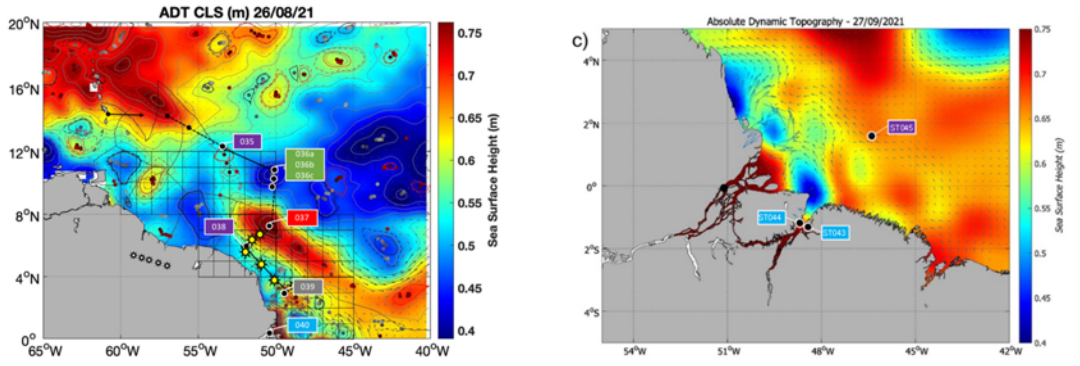


*Figure 5. Location of the sampling stations during the Amazon plume campaigns*

Biodiversity hotspots around Trinidad Seamounts were studied in October 2021 followed by an important port call in Rio de Janeiro. The chief scientist was Samuel Chaffron (CNRS). The main objective of this campaign was

to enable the potential discovery of new bioproducts & chemicals for industrial applications with high socio-economic value, such as new ways to produce pharmaceutical products or natural enzymes able to digest complex molecules such as pollutants or plastics. Given the bioprospecting focus of this leg, sampling stations were all located within international waters, along the Vitória-Trindade Chain (VTC) consisting of 11 heterogeneous seamounts. The sampling area will also be studied by the H2020 Atlantic project iAtlantic (Integrated Assessment of Atlantic Marine Ecosystems in Space and Time), so this campaign will hopefully lead to synergies and collaborations between AtlantECO and iAtlantic.

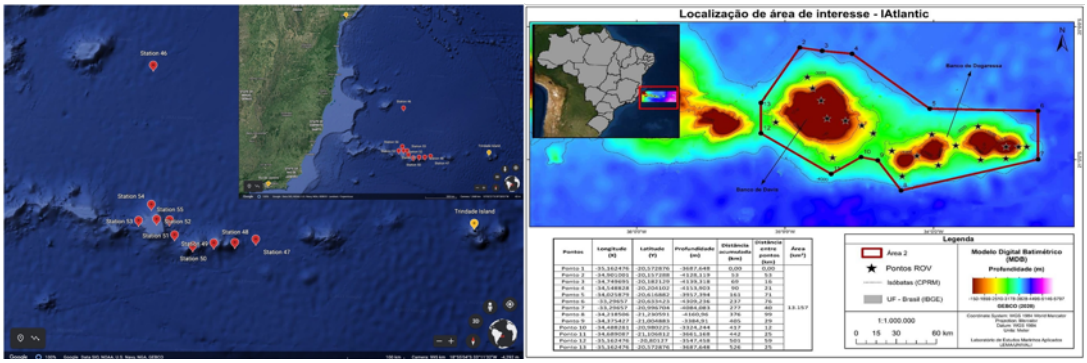


*Figure 6. Location of the sampling stations during the Tara campaign (left) and the sampling plan of iAtlantic (right)*

Dynamics of a coccolithophore bloom on/off the Patagonian shelf were studied in December 2021, including port calls in Buenos Aires and Ushuaia. The chief scientist was Flora Vincent (then Weizmann, now EMBL). Every spring, the Patagonian shelf and shelf-break experience massive phytoplankton blooms. While diatoms and dinoflagellates dominate at the beginning, gigantic patches of high reflectance waters associated with coccolithophores are detected towards December. These develop in long (100-1000 km) south-to-north streaks along the shelf-break for several weeks, on the track of the strong, northward-flowing Malvinas Current. Whether the bloom is only advected or also benefits from upwellings and therefore from fertilisation processes triggered by the current along the shelf break is currently not known. The main objective of this study was to study the ecosystem dynamics of a coccolithophore bloom and demise.

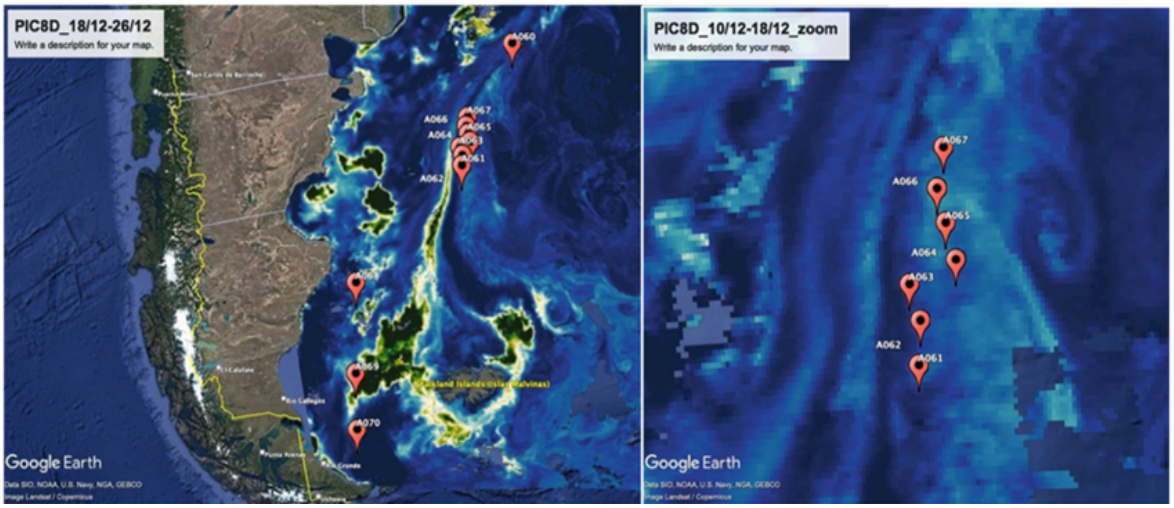


*Figure 7. Location of the sampling stations during the coccolithophore bloom campaign*

The Weddell Sea and the sub-mesoscale dynamics surrounding icebergs were studied in January and February 2022. The chief scientists were Alessandro Tagliabue (UniLIV) and Chris Bowler (CNRS). The main objective of the Tara Antarctic leg was two-fold: (1) to sample across well-established physical and biogeochemical gradients; and (2) to conduct a multi-day process study around an iceberg. Both are concerned with capturing the

response of the microbiome to variability in the seascape introduced by topography, frontal systems and freshwater (both melting sea ice, glaciers and icebergs). These scientific objectives are motivated by the urgent need to understand how the microbiome of the Antarctic seas will respond to climate change, as well as to generate baseline understanding in this under-sampled region.

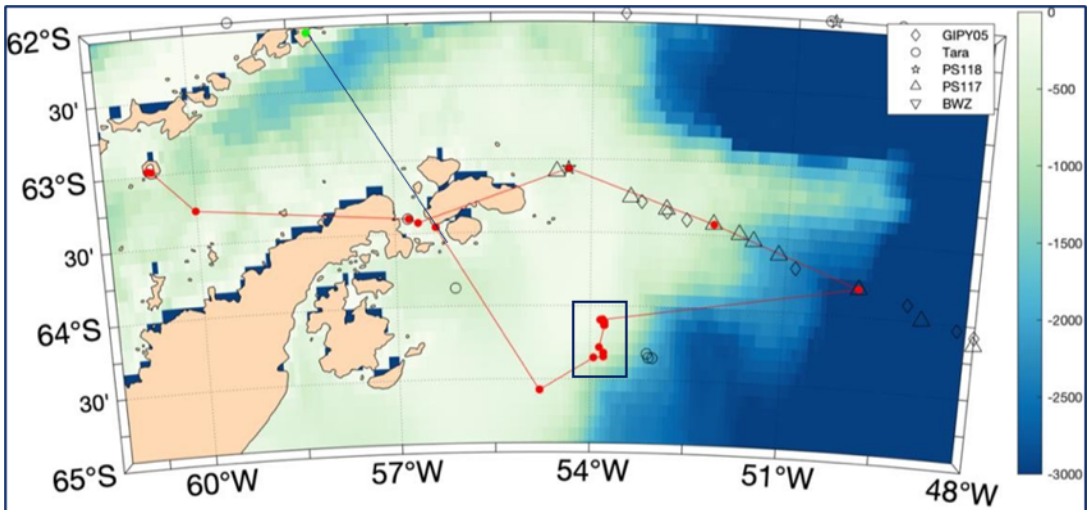


*Figure 8. Cruise track and location of sampling stations (red dots) during the Weddell Sea campaign. The black box shows the iceberg study area, and open symbols represent stations sampled during previous cruises*

Mesoscale eddies and sub-mesoscale filaments were studied in March-April 2022 from Punta Arenas to Cape Town. The chief scientist was Rémi Laxemaire (CNRS). The two objectives of this leg were (1) to study two mesoscale eddies and their meanders along the frontal zone between the South Atlantic and the Southern Ocean, and (2) to sample the Saint Helena Bay Monitoring Line (SHBML) near Cape Town. The two eddies located at approximately 48.5°S, 25°W and 38°S, 6°E were dissected using a combination of high-resolution underway sampling and in-depth sampling of the epi- and mesopelagic layers. Four stations along the SHBML were sampled using the suite of protocols used throughout Mission Microbiomes, thus extending the range of measurements routinely done by UCT along that monitoring line.

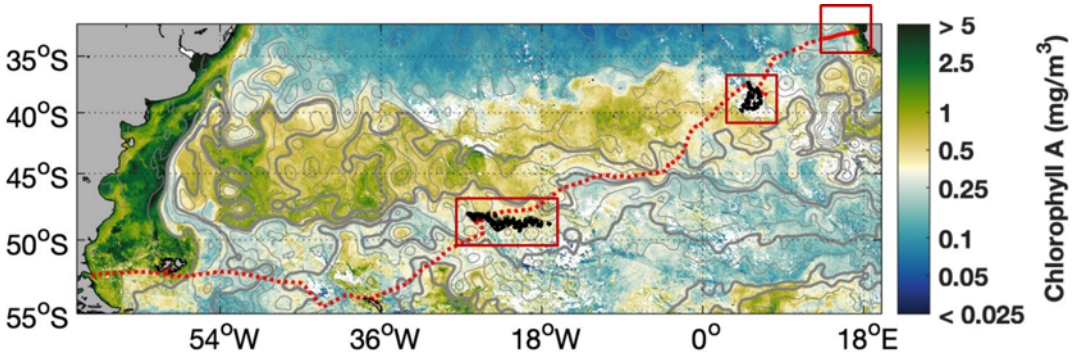


*Figure 9. Cruise track and three main sampling areas of the sub-mesoscale study*

***Table 1.*** *Summary of the sampling effort during the first seven topical studies of Mission Microbiomes. Effort is expressed here as the number of samples collected across the different types of protocols that target seawater biogeochemistry, the ocean microbiome, the plastic microbiome, and the aerosol microbiome.*

| metadata | | | | | water | ocean microbiome | | | | | | | plastic microbiome | | | aerosol microbiome | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Topical Study** | **Stations** | Number of stations | Number of targeted features, e.g. depths | Number of samples | biogeochemistry* | imaging (5 size-fractions) | short-read sequencing (5 size-fractions) | other sequencing** (3 size-fractions) | cell chemistry (3 size-fractions) | metabolomics (5 size-fractions) | long-read sequencing (5 size-fractions) | proteomics (2 size-fractions) | biogeochemistry (3 size-fractions) | imaging (1 size-fraction) | short-read sequencing (1 size-fraction) | biogeochemistry (4 size-fractions) | short-read sequencing (1 size-fraction) | imaging (1 size-fraction) |
| underway | MMB-000 | 0 | 1 | 1079 | 321 | 224 | 0 | 56 | 116 | 0 | 0 | 0 | 0 | 0 | 0 | 216 | 154 | 154 |
| Amazon plume | MMB-035-045 | 13 | 53 | 2473 | 472 | 311 | 281 | 239 | 219 | 251 | 147 | 104 | 195 | 93 | 77 | 112 | 28 | 28 |
| Trinidad seamounts | MMB-046-055 | 10 | 50 | 1183 | 284 | 150 | 226 | 98 | 90 | 96 | 61 | 40 | 58 | 10 | 10 | 80 | 20 | 20 |
| Itajai | MMB-056-058 | 3 | 10 | 346 | 70 | 35 | 46 | 33 | 21 | 54 | 21 | 14 | 22 | 6 | 6 | 24 | 6 | 6 |
| Coccolitophore bloom*** | MMB-059-070 | 12 | 14 | 749 | 189 | 81 | 119 | 59 | 94 | 49 | 49 | 18 | 19 | 0 | 0 | 96 | 24 | 24 |
| Weddell sea & Iceberg | MMB-071-090 | 19 | 44 | 1391 | 347 | 153 | 171 | 134 | 88 | 143 | 89 | 64 | 40 | 24 | 24 | 144 | 38 | 38 |
| Mesoscale eddies, meanders and filaments | MMB-091-105 | 15 | 42 | 1641 | 422 | 187 | 198 | 198 | 134 | 168 | 100 | 62 | 55 | 14 | 13 | 120 | 30 | 30 |
| St Helena Bay | MMB-106-110 | 5 | 13 | 679 | 203 | 58 | 91 | 60 | 64 | 64 | 42 | 32 | 35 | 0 | 0 | 40 | 10 | 10 |
| | **Total:** | 77 | 227 | 9541 | 2308 | 1199 | 1132 | 877 | 826 | 825 | 509 | 334 | 424 | 147 | 130 | 832 | 310 | 310 |

* water biogeochemistry protocols: salinity, nutrients, carbonate, isotopes (13C, 15N & 18O) & trace elements

** other sequencing protocols: EtOH, Single-cell Genomics, HiC, eDNA

*** this topical study is complemented by samples from incubations and additional protocols

## Flagship Veleiro Eco (UFSC)

UFSC carried out a pilot expedition on board the Veleiro ECO in April 2022 at the coast of Santa Catarina State (27.4° S) to test equipment and protocols that will be carried out during Veleiro ECO´s expedition along the Brazilian coast. It will carry on about 40 AtlantECO protocols for imaging and genetic analysis. The 2022-23 mission will start between October-November this year at the Itajaí-Açu River (26.9° S). At the beginning of 2023, Veleiro ECO expedition will comprise sampling from Espírito Santo to Natal during which the epipelagic layer will be sampled for marine microbiomes and microplastics along the coast and three estuaries: Mucuri or Doce (TBD), São Francisco (10.5° S) and Potengi (5.7° S). The La Plata River will be sampled on a separate expedition late 2023. Brazilian rivers are impacted to various degrees by human activity, including urbanisation, tourism, deforestation, shrimp farming, pulp & paper industry, and damming, and are characterised by environmental impacts such as flooding, eutrophication, and the release of heavy metals.

*Figure 10. Veleiro ECO team sampling plankton off Santa Catarina, Brazil*

## Flagship RV Alpha Crucis (USP)

A first oceanographic cruise onboard the USP research vessel Alpha Crucis within the scope of AtlantECO was organised in a partnership with the SAMBAR project (Interannual variability of the meridional transports across the SAMOC basin-wide Array) funded by FAPESP (https://bv.fapesp.br/en/auxilios/98525/interannual-variability-of-the-meridional-transports-across-the-samoc-basin-wide-array-sambar). The main goal of the mission was to recover and redeploy 4 PIES (Pressure Inverted Echo Sounders) in the Southwest Atlantic and collect water samples for the assessment of the microbiome diversity. The survey took place between 28th July and 13th August, 2022 (Figure 11).

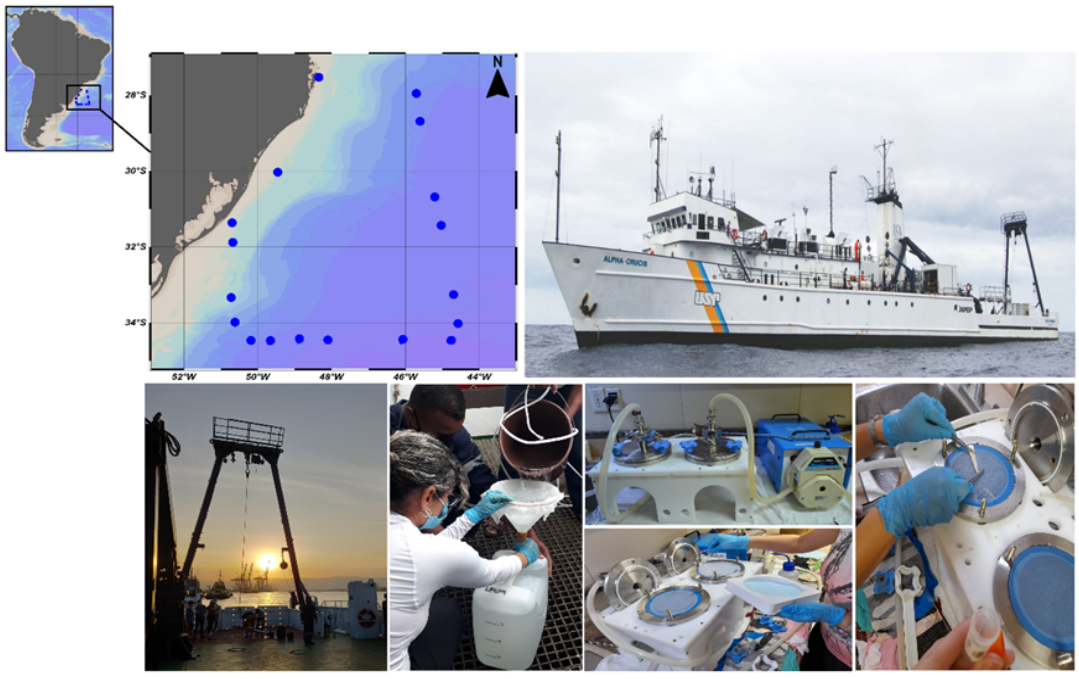


*Figure 11. RV Alpha Crucis sampling stations off Southern Brazil, July-August 2022, and on-board activities*

Sampling of microbial plankton on board RV Alpha Crucis was performed in 18 stations in coastal and oceanic waters following the AtlantECO protocols and using the AtlantECO kit. Water samples were obtained from the sea surface and temperature, salinity and chlorophyll were measured with a Rinko profiler (model ASTD102). On each station, from 10 to 20 L of seawater were sieved using a 20 µm mesh (to remove larger organisms) and filtered using a peristaltic pump with 142 mm filters of 3 µm and 0.2 µm to retain unicellular eukaryotes and prokaryotes, respectively. Subsequently, 1 mL of a stock solution of iron chloride (1 g FeCl3 diluted in 50 mL ultrapure water) was added to the filtrate and incubated for up to 24 hours, then filtered through a 0.8 µm 142 mm filter, targeting viruses. In the end of the filtration, 142 mm filters were recovered using two pairs of EtOH-cleaned tweezers. Each filter was stored in a 5-mL cryotube. The cryotubes containing the 3 µm and 0.2 µm filters were stored at -80°C in liquid nitrogen. The cryotubes containing the 0.8 µm filter were stored at 4°C. All samples were stored at USP and will be soon shipped to UFSCar for DNA extraction and sequencing.

## ■ Flagship SY Eugen Seibold – Northeast Atlantic Mission

Partners ETHZ and EMBL liaised with Ralf Schiebel from the Max Planck Institute for Chemistry (MPIC) in Germany, which operates this flagship and coordinates the missions. The SY Eugen Seibold is a 22 m long, 6 m wide sailing boat, with a 350 $m^2$ sail area, and place for 3 crew and 3 scientists. The research platform is equipped with state-of-the-art instruments for contamination-free, underway sampling of environmental parameters and microbiomes in surface water and aerosols, as well as in the epipelagic layer. Continuous water measurements include, among other parameters, photosynthetic activity, chlorophyll a, $pCO_2$, $O_2$, particle size distribution, $CO_2$, isotope ratio, dissolved Ar, $O_2$, $N_2$, and bacterioplankton. In air samples, data is continuously collected on total particle concentration and size distribution, black carbon, and bioaerosols.

The flagship completed its first mission in 2020-21. The Northeast Atlantic from Iceland to the Cabo Verde was studied from June 2020 to May 2021 (Figure 12), including several port calls along the route.

*Figure 12. Eugen Seibold cruise track and sampling activities in 2020-2021 and 2022. Far-right image depicts HYSPLIT 5 day back trajectories of airborne particles*

The mission consisted of eight campaigns that studied the biogeochemistry and ecology of marine ecosystems along a meridional transect. The campaign summary reports will soon be deposited in Zenodo, and a journal article is in preparation to summarise the mission and provide all metadata. A regular underway sampling of surface waters and aerosols, and a total of 13 stations that sampled the epipelagic layer yielded 280 genetic samples covering three size fractions. The extraction of samples has started, and their sequencing will proceed at the Max Planck Institute for Marine Microbiology (Bremen) and at the Genoscope (AtlantECO partner CEA). Additional discrete samples were collected at selected stations targeting a wide range of water-column measurements and the collection of biological samples (Figure 13).

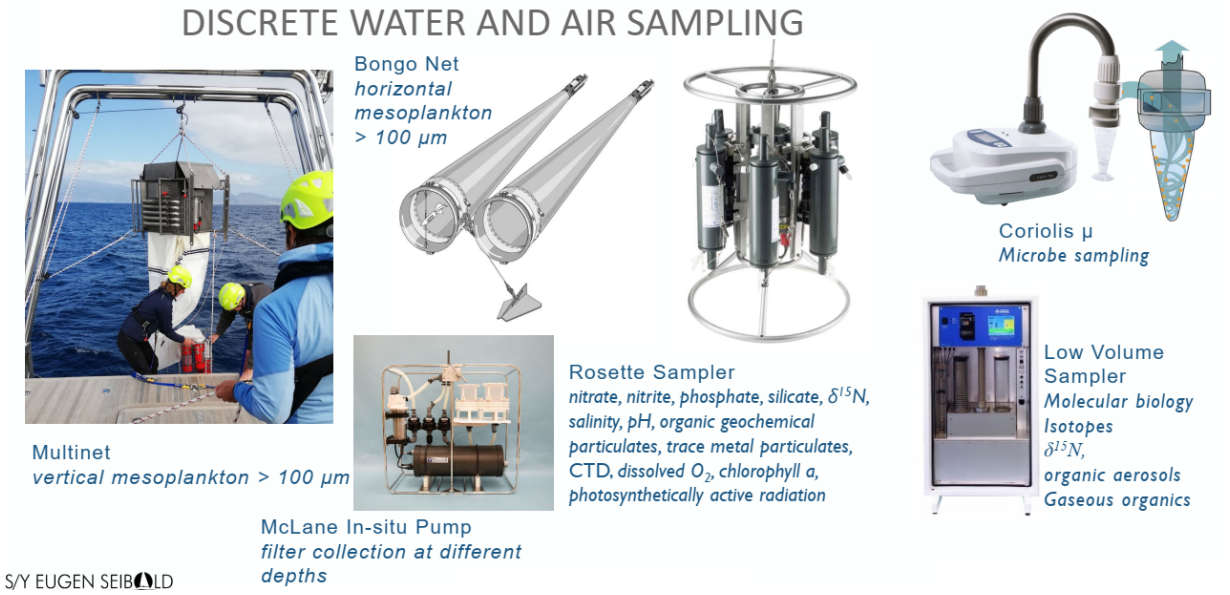


*Figure 13. Eugen Seibold instrumentation for discrete water air sampling at selected stations*

The SY Seibold started its second mission in March 2022, followed by a longitudinal transect across the Atlantic from Cabo Verde to Barbados. Information about this mission will be presented in the next version of this deliverable (D4.6).

## ■ Flagship SA Agulhas II

The Southern oCean Seasonal Experiment (SCALE) winter cruise 2022 started its mission from Cape Town on board SA Agulhas II for a period of 3 weeks (11 July 22 – 31 August 2022). The cruise was organised by the South African Polar Research Infrastructure (SAPRI) with the purpose to serve the scientific community involved in Southern Ocean research projects funded through the NRF (SANAP and other programmes). Our microbiomics team from the University of Pretoria was among the research groups that went on this mission to study the functional capacity and community structure dynamics of the Southern Ocean (SO) microbiota on a temporal and spatial scale using meta-omic approaches (Figure 14).


*Figure 14. S.A. Agulhas II winter 2022 cruise track and sampling*

The on-board activities involved coordinated sampling of water and ice along with ship-based incubation experiments:

- Water sample collection using Niskin-CTD from surface and deep (50m, 1000m and 2000m).
- Water sample collection using Trace metal free GoFlow-CTD from surface and deep (50m and 500m).
- Water filtration using 142mm filters (3µm, 0.2µm) and flash freezing in liquid N2 and storage at – 80°C for further meta-omic analysis according to AtlantECO protocols- Unravelling the bacterial diversity and function of sub-Antarctic and Southern Ocean ecosphere.
- Fe2Cl3 treatment for viral particle enrichment in water samples followed by filtration in 142mm filters (0.8 µm) and storage at 4°C until further meta-omic analysis according to AtlantECO protocols – Understanding the taxonomy and role of Viruses in Ocean.
- Nutrient co-limitation (Fe and Mn) studies by setting up on-board mesocosms in trace-metal free containers and sub-sampling in clean-room for studying different parameters along filtration and meta-omic analysis- Trace metals like Fe, Mn and others are important micronutrients that form an integral part of metalloenzymes, which take part in various cellular processes essential for the survival of microorganisms. The effect of nutrient-colimitation (Fe and Mn) was studied on the bacterial and phytoplankton community of sub-Antarctic waters through a mesocosm experiment.
- Collection of water samples to understand metabolic pathway for Dimethylsulfoniopropionate synthesis and catabolism in the Southern Ocean – Dimethylsulfoniopropionate (DMSP) is an organic sulphur compound synthesized from its precursor methionine, principally by marine eukaryotic microorganisms including (algae, phytoplankton and diatoms) and some bacteria that aid as osmoprotectant, antioxidant and cryoprotectant. Degradation pathways (Demethylation) of DMSPs are significant for ecological sustainability by providing microorganisms with their sulphur and carbon requirements.
- Collection of water samples to explore the chemical ecology of marine fungi and discovery of important biosynthetic pathways involved in the biological carbon pump- Investigating the role of marine fungi in Southern Ocean for carbon transfer between microbial organisms through metagenome assembled genomes (MAGs) and high-resolution mass spectrometry (LC-MS).
- Ice core sampling from ice- pancakes – 2 cores per pancake (split into top and bottom each) were procured through coring, melted at room temperature and processed for meta-omic analysis.
- Underway water sampling from different stations for the meta-omic studies- The collected water was again filtered using 142 mm filters (3µm, 0.2µm) and flash freezing in liquid N2 and storage at – 80°C for further meta-omic analysis according to AtlantECO protocols.

Sampling of microorganisms including iron chloride flocculation of viruses was done using the Tara protocol as illustrated in Figures 15A and 15B.

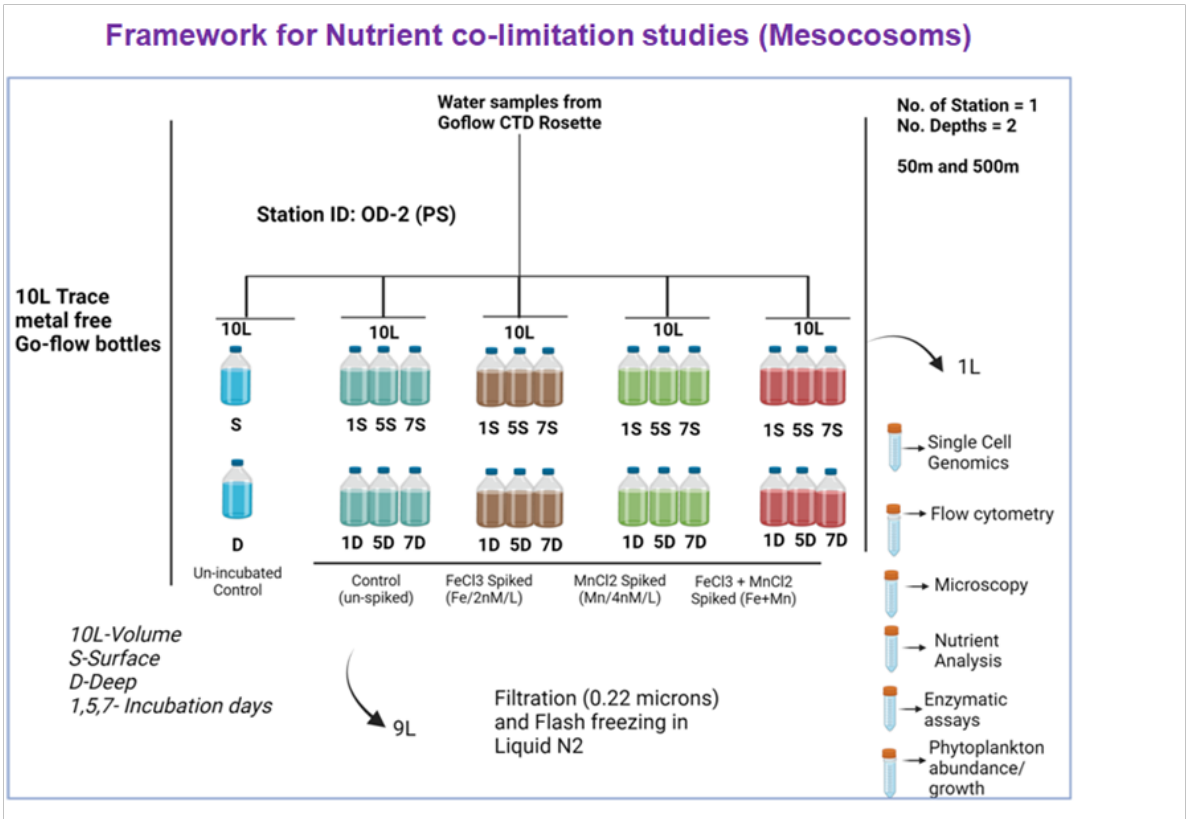


*Figure 15A. SA Agulhas II sampling protocol in mesocosms studies during winter 2022*

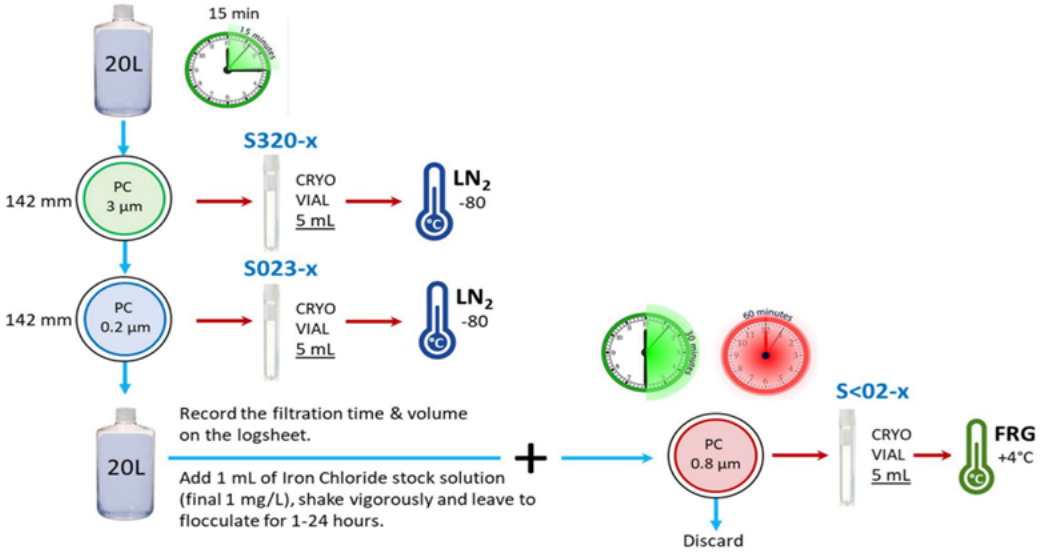


*Figure 15B. SA Agulhas II sampling protocol in mesocosms studies during winter 2022*

## ■ Flagship RSS Discovery

One scientist from AtlantECO will join the AMT-30 cruise on board RSS Discovery in January 2023 to carry out extended genomics and imaging sampling.

### ○ Sub-task 4.2.2 The All Atlantic Ocean Sampling Day (lead: EMBTC-ERIC)

***All Atlantic Ocean Sampling Day (AA-OSD)*** – This task considered the scientific interests and the logistic/practical issues related to the timing and synchronicity of sampling in the North and South hemispheres. It was decided that:

- The main objective of the Ocean Sampling Day (OSD) is to share capacity and best practices
- The All Atlantic OSD will happen on the same date in both hemispheres
- The timing of the AA-OSD does not need to be consistent with the one selected in the previous editions of the OSD, i.e., the summer solstice in the northern hemisphere (21st June)
- AtlantECO wishes to prioritise the preferences of new participants in the southern hemisphere
- The AA-OSD will be held on the 21st of September 2022 and 2023.

A pilot AA-Ocean Microbiome Sampling will be held during the three-month period from 21st of September to 21st December 2022 at sites shown in Figure 16.

Candidate sites in South America and Africa attended the Webinar on Standards and Best Practices (May 2021) and participated in a survey about their best practices and their capacity to adopt the standard protocols proposed by AtlantECO. Partner EMBRC (Uvigo) has reviewed the policy documents of the regular OSD, which will be updated for the AA-OSD. Training of candidate sites in Africa was achieved in part via the AtlantECO Training Course in Cape Town and via the participation of local scientists to Mission Microbiomes. Training of candidate sites in South America was achieved via the participation of local scientists to Mission Microbiomes and by online meetings. Two students from the IMBRSea international master's programme did a professional practice (internship) hosted by partner EMBRC (HCMR) in Crete during the months of April-June 2022. They contributed to the curation of sample log sheets and metadata from the OSD 2021 campaign and worked on the communication with candidate sites from South America and Africa.

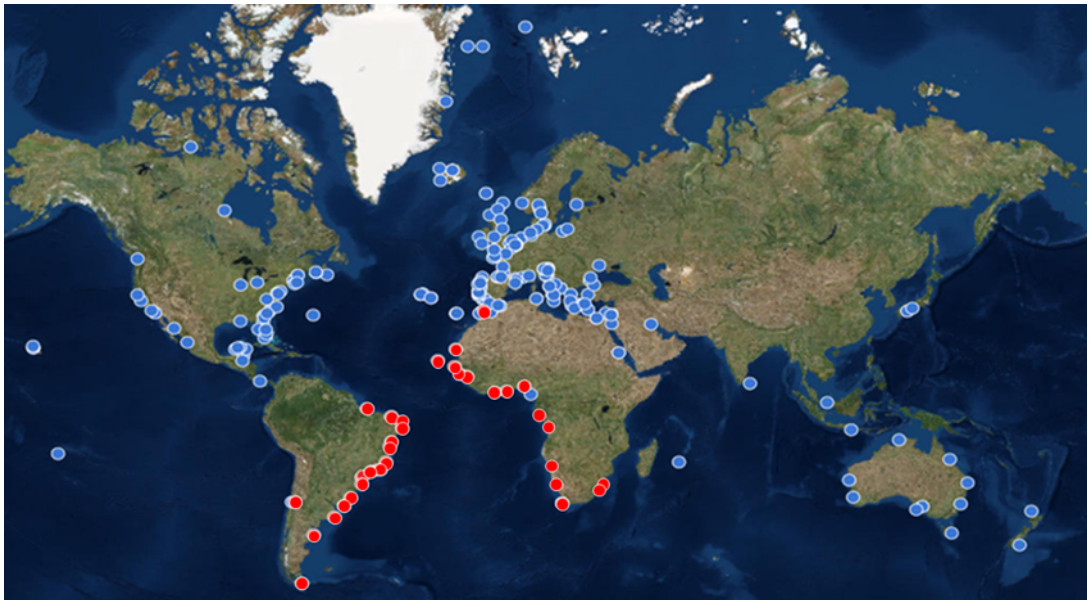

*Figure 16. Location of the regular OSD sites (blue) and candidate sites for the All Atlantic OSD (red)*

***Regular Ocean Sampling Day*** – Partner EMBRC (HCMR & VLIZ) in collaboration with GFBio worked towards the FAIRification of the OSD data from years 2018 & 2019. This is an extremely time-consuming process when working with such a large number of datasets that need to be put together uniformly, while adapting to ENA, OBIS and PANGAEA individual standards. All metadata, as well as metabarcoding and metagenomics data have been uploaded to the ENA database (partner EMBL). In 2021, sampling took place on 21st June, and all samples

were sent to HCMR for DNA extraction and sequencing. DNA extractions are completed, and sequencing will begin in the second half of 2022.

***Review of preservation methods*** – In synergy with the EMO BON network, partners EMBRC (HCMR) and CEA conducted a pilot study to review the preservation methods of sequencing samples. Samples were either immediately frozen at -80 0C or preserved using a DNA/RNA Shield (Zymo Research) and stored at different temperatures. Data analysis revealed that the extraction yield, the quality of sequences, and the taxonomic annotation of microbial communities from the two treatments were not significantly different. Since the preservation of samples with a DNA/RNA shield facilitates the transport of samples at room temperature, the EMO BON network decided to favour this preservation method. The decision regarding the preservation of samples from the AA-OSD is pending further discussions.

### ○ Sub-task 4.2.3 The All Atlantic Sail-4-Science (lead: SU)

The goal of subtask 4.2.3 is to develop and implement a lighter version of the AtlantECO's protocols to be carried out by citizen scientists. This subtask has progressed with the development and testing of an affordable plankton sampling and imaging kit designed for the study of microplankton by citizen scientists (Figure 17). The kit is composed of a high-speed surface plankton net, the Coryphaena, and a portable in-flux automated imaging device, the Planktoscope. Partner SU tested the kit in January 2021 along a latitudinal transect across the Atlantic Ocean on board AtlantECO flagship SV Tara at the beginning of Mission Microbiomes. The Coryphaena was able to collect microplankton samples at speeds up to 11 knots, and the Planktoscope was benchmarked and compared against the FlowCam, the equivalent state-of-the-art instrument available commercially at a much higher cost. All samples were analysed on board and images were classified and annotated for taxonomy using EcoTaxa:

- Flowcam (66243 images) https://ecotaxa.obs-vlfr.fr/prj/3892
- FlowCam (88465 images) https://ecotaxa.obs-vlfr.fr/prj/3891
- Planktoscope (98260 images) https://ecotaxa.obs-vlfr.fr/prj/4356
- Planktoscope (13176 images) https://ecotaxa.obs-vlfr.fr/prj/4362
- Planktoscope (115119 images) https://ecotaxa.obs-vlfr.fr/prj/4363
- Planktoscope (17203 images) https://ecotaxa.obs-vlfr.fr/prj/4343

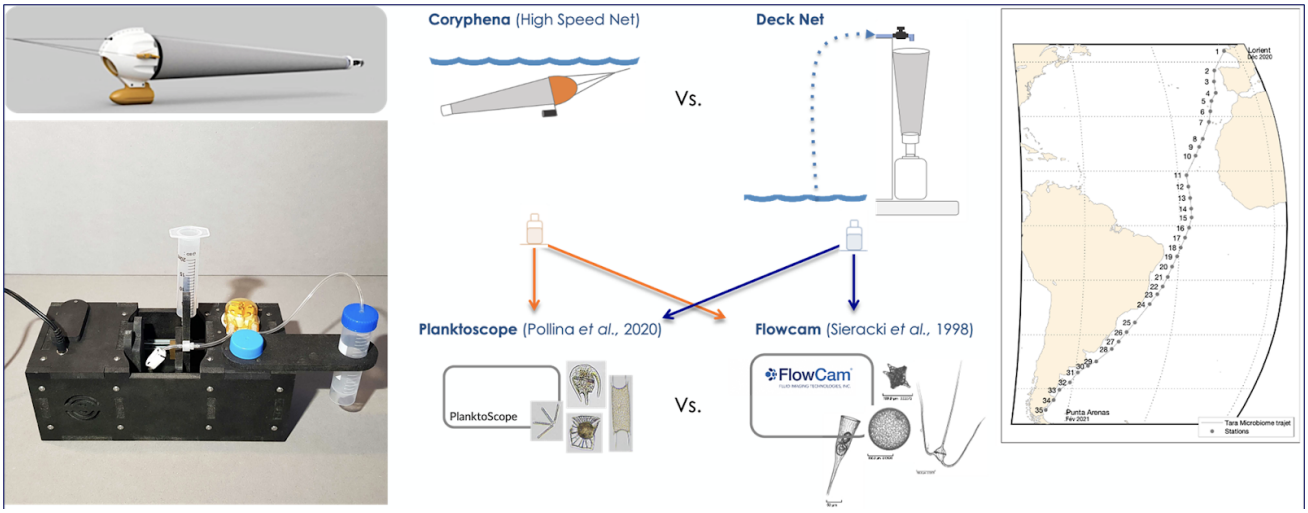


*Figure 17. Coryphaena net and PlanktoScope v2.5 (left) and summary of tests performed on board SV Tara (right)*

The results are published (https://www.frontiersin.org/articles/10.3389/fmars.2022.916025) and show that the PlanktoScope and the FlowCam provide comparable analyses of surface microplankton composition and biogeography. Overall, the prototype plankton collection and imaging kit opens the way to a cooperative, citizen-based plankton observatory network at planetary scale.

Several improvements were implemented after the tests at sea and led SU to develop v.2.5 of the Planktoscope. Five new units are now deployed in different AtlantECO laboratories in France, Brazil, South Africa and Italy for a six-month testing before the final version can be finalized and deployed on sailing boats. A user manual is now available online on the protocols.io platform (https://www.protocols.io/view/planktoscope-protocol-for-plankton-imaging-bp2l6bq3zgqe/v1) and will be updated based on both AtlantECO laboratories' and citizen groups' feedback.

Partners SU, ETHZ, UFSCar, EMBL and SZN have selected a first set of sampling routes in the South Atlantic (Figure 18) that offer interest to both the sailing and scientific communities. A first route going from Cape Town to Europe will be sampled on board luxury sailboats built by Southern Wind Shipyard in Cape Town. A second route between St Helena and Ascension Islands would involve volunteer sailboats travelling that route and borrowing kits from marinas on either ends of the route, and a collaboration with the Ascension Island Marine Protected Area (MPA) could use the kit during volunteer surveys of the MPA. A third route would be across the North Atlantic and involve RéAlizés, a project initiated by engineering students of Centrale Supelec and INSA (France) who will cross the Atlantic in December 2022 from France to Antilles to test new low-tech technologies.


*Figure 18. Selected routes between Cape Town and Europe (left), St Helena and Ascension Islands (middle) and North Atlantic (right)*

Partner SU started to prototype kits for the filtration of plankton samples and their preservation for genetic analyses. The filtration kits will be tested at sea and deployed together with the Planktoscope and the Coryphaena on selected Sail-4-Science routes in 2023.

### ○ Sub-task 4.2.4 A Continuous Plankton Recorder (CPR) survey (lead: MBA)

The objective of this sub-task was to conduct the first tows of the Continuous Plankton Recorder (CPR) between Brazil and South Africa. Tow number 1 took place using the LODUR, IMO: 9219381 MMSI:3045040000 CALL SIGN: V2CY, between 23/10/21 and 31/10/21 (Figure 19). Whilst the CPR Survey has towed over 7 million nautical miles, and been running for over 90 years, this is the first tow in the area, collecting phyto- and zooplankton, using a standard device – making it comparable to all previously collected CPR data.

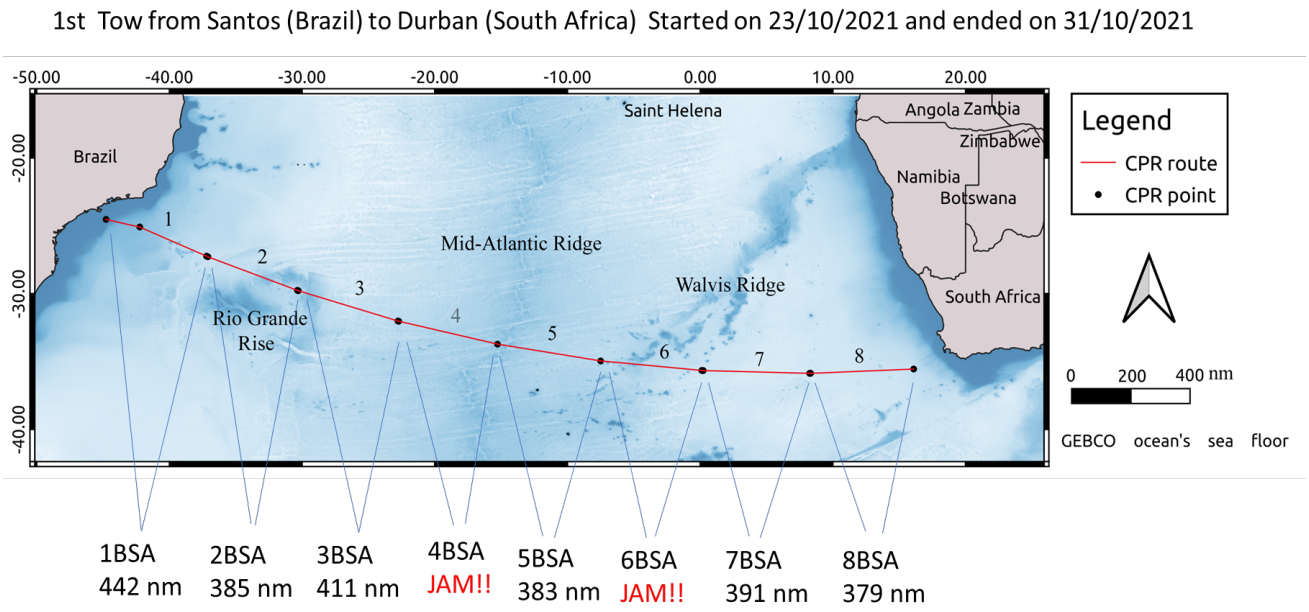


*Figure 19. Sampling deployments carried out along the Brazil to South Africa (BSA) survey line, each representing a tow of ca. 450 nautical miles.*

World Courier transported the CPR internals from the first AtlantECO CPR line containing the silk rolls from South Africa to Brazil where they were delivered to partner FURG. Out of 8 internals deployed during the first crossing, two did not work properly, leaving six silk rolls to be unrolled, measured and analysed. A script in R, based on a code provided by the Australian Antarctic Division (AAD), was used to determine the length and

number of each 5 nm segment. The Phytoplankton Colour Index (PCI), which determines the greenness of the silk was measured on each segment using Pantone Charts. One out of five segments are analysed, so that silks were cut sequentially into 10 nm, 5 nm and 10 nm segments, resulting in 240 5-nm samples to be analysed and 118 10-nm samples to be biobanked for future analyses. All 44 samples (5nm segments) of the first internal (CPR189/0) have now been analysed by FURG, resulting in a total count of 102 individuals identified to either species, family, order or class level belonging to the following taxonomic groups: Foraminifera, Ostracoda, Pteropoda, Euphausiaceae, Calanoida, Cyclopoida, Harpacticoida, Appendicularia, Salpidae and Chaetognatha.

The second crossing of the MV Lodur, towing the CPR along the Brazil - South Africa (BSA) route, occurred between 18$^{th}$ and 25$^{th}$ of January 2022, samples returned to Cape Town and dispatched to Brazil in early April 2022. The third and fourth crossings are due to take place in July and November 2022. As anticipated, the challenge for this task during the second period of the project is to plan the sustainability of CPR deployments along this South Atlantic line, and to ensure the analysis and exploitation of the resulting samples. A new DFFE facility dedicated to the analysis of CPR samples is now operational in Cape Town, which may help resolve our challenge by sharing capacity with Brazil to analyse samples. AtlantECO partner MBA will provide a one-week CPR training at that facility in July 2022.

### ○ Sub-task 4.2.5 Genomics, Carbon and Plastic analysis of Moored Sediment Trap samples (lead: NOC)

NOC (Alice Horton, Katsia Pabortsava, Christopher Feltham, Sue Hartman, Richard Lampitt) will generate new observations on downward fluxes of particulate organic carbon (POC) and microplastics from three Atlantic

locations: Porcupine Abyssal Plain (PAP), Northern Oligotrophic Gyre (NOG) and Southern Oligotrophic Gyre (SOG). Sediment traps at these locations are deployed at 3000 m depth.

Samples from the years 2009-2016 (Figure 20) have been selected for analysis based on the flux magnitude: 2x high-flux events and 2x low-flux events in each year, based on observations of dry weight flux (PAP samples) and estimated volume flux (NOG and SOG samples). A total of 96 samples will be analysed per variable.

AWI (Christina Bienhold, Katja Metfies) will generate new observations on molecular biodiversity of sediment trap samples from station HGIV, the central station of the LTER HAUSGARTEN, Fram Strait, from 200 m water depth. Acknowledging recent anomalies in melt-water stratification, samples cover the years 2016-2020 and include seasonal coverage.

PLOCAN (Eric Delory) will make data from the ESTOC observatory (1991-2009) publicly available for reuse. Additionally, through a collaboration with the University of Barcelona, samples from the years 2018/2019 will be analysed for organic carbon (OC), inorganic carbon (IC) and biogenic silica (BioSi).

*Figure 20. Left: Sediment trap being deployed; right: time-series of sediment trap samples collected at the SOG site.*

Microplastics analysis

Microplastics extraction from the complex sediment trap material involves two chemical digestion steps followed by density separation in hypersaline solution. For digestions and density separation steps, particles are filtered onto acid-washed hydrophilic 5 µm PTFE filters. All the reagents used for digestion and density separation are prepared and kept in acid-clean (25% HCl + 2% H2O2 (v/v%) solution) and pre-combusted glassware (500°C for 8 hours); all the reagents are also filtered through pre-combusted 0.8 µm GF/F filters. All PTFE filters are kept in glass Petri dishes with MilliQ until use.

The microplastics extraction procedure involves the following steps:

1. *Separation of particles from buffered formalin preservative*: filter the sample onto a PTFE filter; rinse with 50% (v/v%) ethanol solution; transfer particles into a glass beaker using small silicon spatula.

2. *Digestion step 1 – removal of organic material:* add 7.5% (v/v%) NaClO solution to the beaker with particles and heat overnight at 60°C; filter the particle-solution mixture onto the same PTFE filter and rinse with 50% ethanol; transfer particles into the same beaker using small silicon spatula.

*Digestion step 2 – removal of inorganic biogenic material:* add 10% (v/v%) HCl solution to the beaker with particles and heat at 60°C overnight; filter the particle-solution mixture onto the same PTFE filter and rinse with 50% ethanol; transfer particles into the glass vial using small silicon spatula.

3. *Density separation – removal of lithogenic material:* add supersaturated $ZnBr_2$ solution to the glass vial with particles in it, gently vortex and allow particles to settle overnight. Without disturbing settled particles,

pipette out the overlying solution and filter onto a respective PTFE filter; transfer the particles into another glass vial using the silicon spatula and 50% ethanol solution.

4. Return the filtrate ($ZnBr_2$ solution) back into the original glass vial and gently vortex the particle-solution mixture; let the particles settle overnight again. Repeat steps 4 and 5 twice.

5. Filter the particles in 50% ethanol mixture onto 0.8 µm Ag filter; rinse with 50% ethanol, dry overnight. The sample is ready for FTIR imaging. Note that the remaining dense/settled particles are filtered as in step 4 and stored at 4°C.

To date processed and IR-imaged 8 samples from the NOG site and 4 samples from the SOG site (years 2009 and 2010). Data analysis and interpretation are in progress (following Pabortsava and Lampitt 2020). Common polymers encountered in NOG samples include polypropylene, nylon, polyethylene, polyethylene and polypropylene copolymer, various plasticisers, adhesives and varnishes.

POC analysis

POC analysis for PAP samples is complete. The NOG and SOG samples are being prepared for POC (and microplastics) analyses, which involves estimated volume flux measurements (completed), sample splitting and removal of zooplankton swimmers (all in progress). The POC splits will then be prepared for POC analysis on CHN analyser following the established procedure (Hedges and Stern 1984).

Molecular analysis

Samples from the central HAUSGARTEN station, Fram Strait (79°0'25.92" N, 4°20'3.12" E) collected at 200 m water depth, are currently being prepared for sequencing of 16S/18S amplicons and metagenomes. There are 15-20 samples per year, covering all seasons and the years 2016-2020. The opening times of the cups vary between approximately 7 and 30 days, depending on season. Preparation of sediment trap samples for sequencing includes: 1. Visual inspection of cups, 2. Picking of zooplankton (swimmers) under a dissecting microscope, 3. Splitting into 1/32 volumetric splits, 4. Filtration on 0.22 µm membrane filters, 5. DNA extraction using Dneasy PowerWater Kit.

Samples from 2016/2017 have been extracted and have been sent in May 2022 to Genoscope for 16S and 18S metabarcoding as well as metagenomic sequencing of selected samples. Samples for the remaining years are currently being prepared. Contextual biogeochemical flux data (e.g. POC/PON) for the ecological analysis of these samples will be available through existing internal collaboration at AWI. In addition, molecular data from water samples already available as part of the FRAM (Frontiers in Arctic Marine Monitoring) Molecular Observatory (e.g. Wietz et al. 2021) will provide further context to link the seasonal development of microbial communities in surface waters with their export.

New availability of existing data

PLOCAN have now made all data from the ESTOC observatory between the years 1991-2009 publicly available. These data were previously used in the recent publication by Fischer et al (2020). The data can be accessed here:

http://data.plocan.eu/thredds/catalog/estoc/mooring-watercolumn/Sediment-Trap/catalog.html

## ○ Sub-task 4.2.6 Synergies with third party sampling programmes (lead: EMBL)

During the first two years of the project, five third party cruises in the Southern Hemisphere (Figure 21) participated in this Subtask, in synergy with AtlantECO's Mission Microbiomes on board SV Tara. The cruises implemented AtlantECO's base protocols for the sequencing of marine microbiomes, or complemented measurements made on board SV Tara when sampling occurred concurrently, i.e. during the Amazon plume study. Data sharing agreements are not yet signed, but in most cases, samples collected by third party cruises will be analysed by one of AtlantECO's partner sequencing centres. We do not expect any issue regarding early access to the results.

- RV Antea (France) – Amazon plume study (September 2021)
- RV Houssay (Argentina) – Coccolithophore bloom study (November 2021)
- RV Hesperides (Spain) – Southern Ocean study (February 2022)
- RV Polarstern (Germany) – Southern Ocean study (March 2022)
- RV Algoa (South Africa) – Benguela ecosystem study (May 2022)


*Figure 21. Synergies with third party cruises*

Additionally, sampling for the imaging and genomic analysis of marine microbiomes in the Eastern North Atlantic was carried out in April 2021 during the international EXPORTS programme on board three research vessels (R/V Sarmiento de Gamboa, RRS James Cook and RRS Discovery) and involving several AtlantECO partners.

# 5 Concluding remarks

The augmented observations carried out during the first 24 months of the AtlantECO project (September 2020-August 2022) provided significant contributions on microplastic characterization and microbiome diversity, distribution, and functional role in the marine ecosystem, with an emphasis in the South Atlantic. The Mission Microbiomes cruise series on board flagship Tara collected an impressive amount of over 9,500 samples aiming at gene sequencing, evaluation of biogeochemical proxies, assessment of plankton size spectra, biomass and taxonomic composition, and studies on the plastic and aerosol microbiome, among other measurements. Knowledge gaps in remote, under-sampled areas such as the Amazon River mouth, the tropical seamounts in the vicinities of Trindade island, the Patagonian shelf and polar and subpolar regions of the South Atlantic will be filled once samples are analysed at different laboratories belonging to the AtlantECO consortium in Europe, South America and Africa. Other flagship cruises carried out by the SA Agulhas II (South Africa) and RV Alpha Crucis (Brazil) represented important complements for the suit of observations performed on board Tara. The flagship SY Eugen Seibold covered additional sampling sites in the Northeast Atlantic.

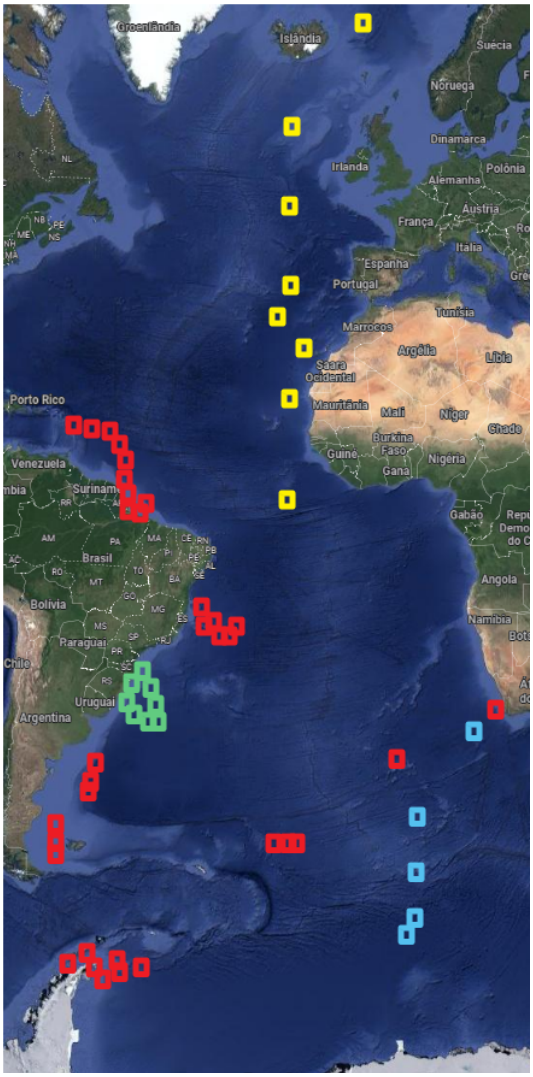


*Figure 22. Overview of sampling sites covered by AtlantECO flagship cruises up to August 2022. Red: Tara; Green: Alpha Crucis; Blue: Agulhas II; Yellow: Eugen Seibold. A single point may represent several nearby stations. The CPR line between South Africa and Brazil is not shown (refer to Figure 19).*

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