APPLICATION FOR CONSENT TO CONDUCT MARINE SCIENTIFIC RESEARCH IN WATERS (EEZ + territorial sea and internal waters) UNDER THE JURISDICTION OF GREECE

Date: December 18th, 2024

1 - GENERAL INFORMATION

1.1. Expedition's name: Expedition Tara Europa within the TRaversing European Coastline (TREC) PROJECT

1.2. Sponsoring institution

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1.3. Scientist in charge of the expedition:

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1.4. Local scientists involved in the planning of the expedition

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1.5. Submitting officer:

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2 - DESCRIPTION OF THE EXPEDITION

2.1. Nature and objectives of the expedition:

- Expedition Summary.

The new **Expedition ''Tara Europa'** of *Tara* will sample marine coastal waters along the European coastlines, in c.a. 120 sites from 21 countries from Finland to Crete, between April 2023 and July 2024 (see Fig. 1). This expedition is part of two larger exploration programs:

(*i*) the **TREC** - *Traversing European Coastlines* - expedition, led by EMBL (Heidelberg), EMBRC, and the Tara Foundation & Tara Oceans Consortium and, that will explore soil, aerosols, sediments, and water biodiversity in 'natural' and 'anthropized' contexts across c.a. 120 Land<>Sea Transects (LSTs) from Finland to Crete;

(*ii*) the **BIOcean5D** (B5D) European Horizon-2021 project, led by EMBL and CNRS, and uniting 29 European partners for a holistic assessment of marine biodiversity and its drivers, functions, and value, from viruses to mammals, from genomes to holobionts, across critical time scales and habitats.

In this context, the Expedition 'Tara Europa' is responsible for **water ecosystems sampling and assessment**. The main scientific objectives of TREC/B5D are to:

(*i*) holistically measure biodiversity at the Land-Sea interface across natural and anthropized ecosystems; (*ii*) unveil the evolutionary and ecological patterns shaping coastal micro- and macro-biodiversity in natural and anthropized contexts, at molecular, organismal, and community scales; (*iii)* develop new theories and models to predict biodiversity changes and preserve ecosystem services, including new methods for economic and legal valuations of biodiversity. For this, this expedition will measure physical, (bio)chemical, and biological parameters from the samples of water, eDNA, and plankton collected in every coastal ecosystem explored during TREC. A focus is given in biological data, which cover genomics, transcriptomics, proteomics, metabolomics, and phenomics. All data and samples will be barcoded and shipped to EMBL, before their redistribution into the international network of collaborators for data generation, analyses, and collective publication in open source journals and databases.

Last but not least, these data and sample collection efforts will largely contribute to the assessment of marine waters and human activities within the **Marine Strategy Framework Directive (MFSD)** and shall provide new insights towards future mechanisms for monitoring activities and public policies in Europe.

MAP

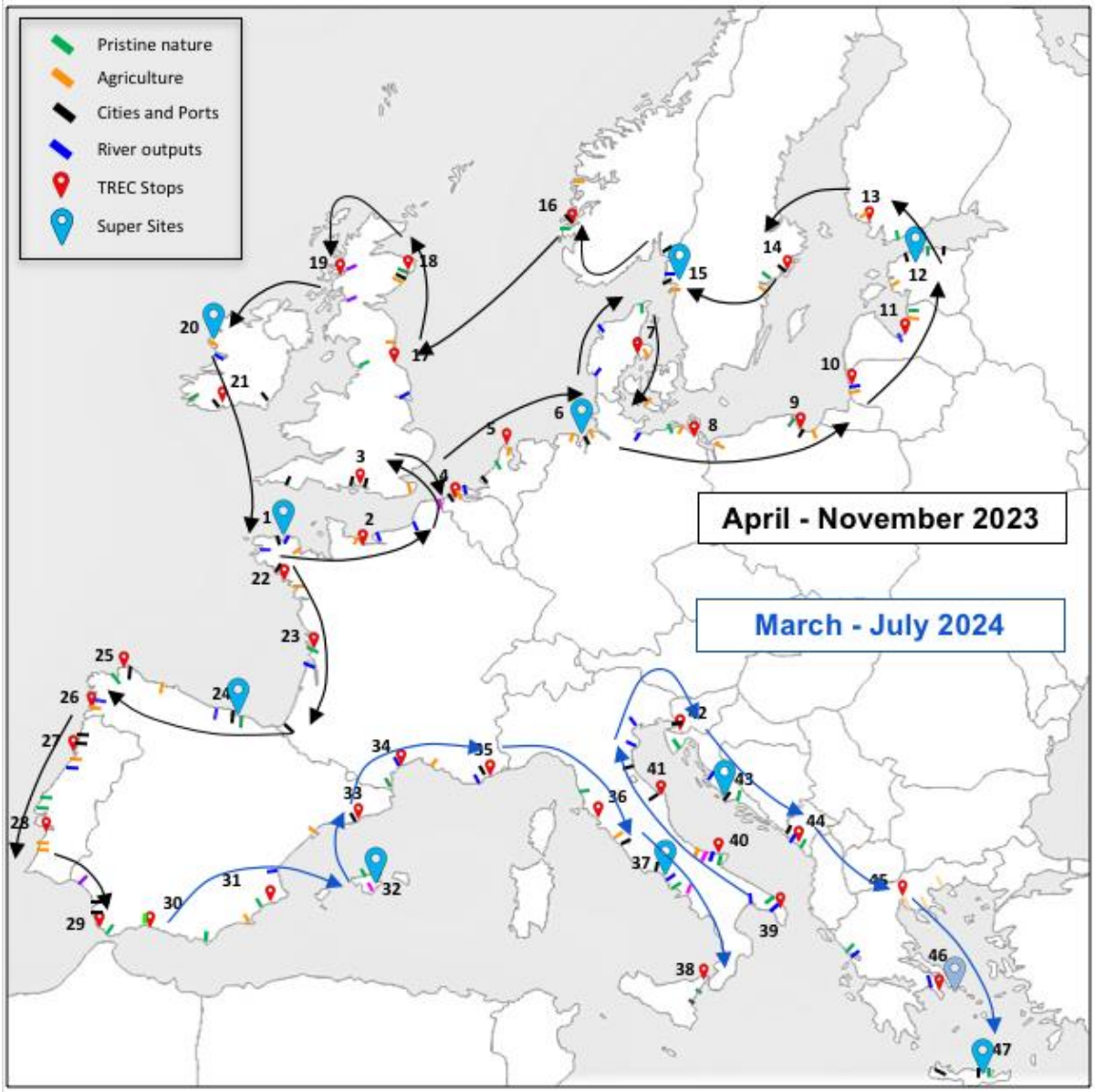


Figure 1: Location of the c.a. 120 Land-Sea Transects explored along the European coastline during the *expedition Tara Europa*, in the context of TREC and BIOcean5D. LSTs are colored according to broad ecological features; The 120 sampling sites correspond to 46 stops at marine stations, and the Super-Sites correspond to places where deeper explorations will be performed, thanks notably to a new EMBL mobile laboratory (on land) equipped with instrumentation to explore environmental biocomplexity at the single-cell and molecular levels.

- *Tara* Ocean Foundation

Figure 2. Picture of the *Tara* Schooner. ©Sacha Bollet / Fondation Tara Océan

The *Tara* Ocean Foundation is the first public interest group in France dedicated to the Ocean. We conduct high-level scientific research, in collaboration with international laboratories of excellence, to explore, understand and anticipate marine ecosystems' changes associated with climate and environmental risks. Our two key missions are exploring and sharing. To preserve the Ocean and make it a collective responsibility, the Tara Ocean Foundation raises awareness and educates young generations to protect this vital ecosystem.

The Expedition 'Tara Europa' will be conducted on board the vessel.
(*Tara* detailed particulars are in the Annexes)

2.2. Relevant previous or future research cruises:

Tara ARCTIC (2006-2008) – 2 year drift on the Arctic icepack
Tara OCEANS (2009-2013) – 4 year planetary study on plankton
Tara Mediterrannée (2014) – 7 months studying the impact of plastic debris on ecosystem in the Mediterranean sea
Tara PACIFIC (2016-2018) – 2,5 years pan-pacific study on coral reefs
Tara Microplastiques (2019) - 7 months studying the impact of plastic debris on ecosystem inside European rivers
Tara Mission Microbiomes (2020-2022): 2 years of studying the ocean microbiomes around the Atlantic.

2.3. Previously published research data relating to the expedition:

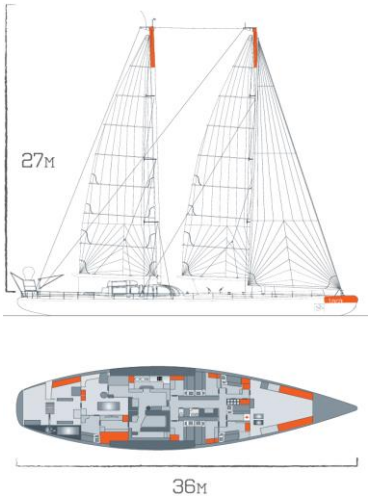
From Tara ARCTIC:
>http://oceans.taraexpeditions.org/en/m/science/results/
From Tara OCEANS:

>140 publications listed here:
https://sunagawalab.ethz.ch/web/taraoceans.php
From Tara PACIFIC:
>A pan-ecosystemic approach of the "-omics" complexity of coral reef holobionts across the Pacific Ocean. PLOS Biology 17:e3000483.
doi: 10.1371/journal.pbio.3000483
>Expanding Tara Oceans Protocols for Underway, Ecosystemic Sampling of the Ocean-Atmosphere Interface During Tara Pacific Expedition (2016–2018). Frontiers in Marine Science 6:750. doi: 10.3389/fmars.2019.00750
>Status of coral reefs of Upolu (Independent State of Samoa) in the South West Pacific and recommendations to promote resilience and recovery of coastal ecosystems. Marine Pollution Bulletin 129:392–398. doi: 10.1016/j.marpolbul.2018.02.044
>An improved primer set and amplification protocol with increased specificity and sensitivity targeting the Symbiodinium ITS2 region. PeerJ 6:e4816.
doi: 10.7717/peerj.4816
>Tara Pacific Expedition's Atmospheric Measurements of Marine Aerosols across the Atlantic and Pacific Oceans: Overview and Preliminary Results. Bulletin of the American Meteorological Society 101:E536–E554. doi: 10.1175/BAMS-D-18-0224.1

3 - METHODS AND MEANS TO BE USED

3.1. Particular of vessel

Name	TARA
Nationality	French
Owner	Tara Foundation
Operator	Tara Foundation
Overall length	35,98 meters
Maximum draught	3.5m
Net tonnage	50 UMS
Gross tonnage	169 UMS
Propulsion	Conventional fuel/sail; 2X 265Kw diesel engines
Cruising speed	7,5 knots
Maximum speed	10,5 knots
Call sign	FVNM



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N°immatriculation	RI 748443 A
N° MMSI	226070000

Method and capability of communication (including telex, frequencies)

Iridium	+8816 777 01732
E-mail	captaintara@fondationtaraocean.org
Mobile (GSM):	+ 33 (0) 68 91 81 87

Name of master	Martin Hertau / Samuel Audrain / Yohan Mucherie

Number of crew	6 persons
Number of scientists	6 persons
Number of supernumeraries	2 persons

3.2. Aircraft or other craft to be used in the expedition:
Two tenders : 1 rib boat outboard 50bhp and 1 rib boat outboard 70bhp

3.3. Particulars of methods and scientific instruments onboard:
The End-to-End approach to explore and assess marine aquatic ecosystems applied in Tara Europa Expedition is based on standards and best practices pioneered by the *Tara* Oceans consortium, and characterized by (1) "end-to-end" sampling of aquatic eco-systems, including in MLS its organic and inorganic pollutants; and (2) fractionating entities according to organismal size and taxonomy; in order to (3) select the best state-of-the-art chemical, imaging and genetic protocols to study entities in the different fractions. That approach provides a conceptual and practical framework that helps formulating scientific questions and making well-informed choices towards a coherent set of protocols and an adaptive sampling strategy for augmented observations.

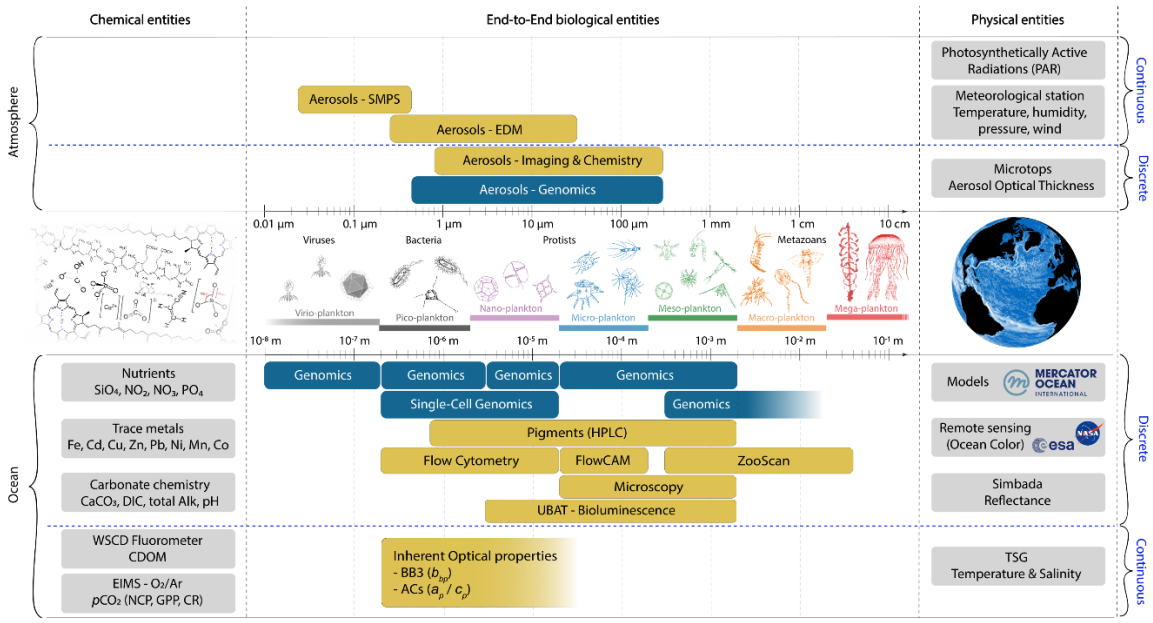


Figure 3: Schematic representation of the size and taxonomic spectrum of the marine aquatic microbiome (middle section). Yellow and blue boxes show the various imaging and genomics protocols that target different fractions of the ocean microbiome.

End-to-End biological and contextual measurements will last c.a. 6 hours for a single depth at a specific site. We will use automated sensors on board for measurements of greenhouse gases (with TSG for N2O, CH4 and CO2 partial pressure in the surface of the ocean).
We will assess biodiversity by *in situ* pumping and plankton net towing at different depth levels.
A suite of bio-optical measurements will be done with inline sensors. These include spectral absorption, attenuation and backscattering of the particulate fraction, fluorescence of the dissolved fractions and photophysiology of the photosynthetic machinery including chlorophyll fluorescence. On station (see paragraph 5 below) hyper-spectral remote sensing reflectance will be measured and samples will be taken to characterize the particulate (POC, POM, TSM, spectral absorption of particles and of phytoplankton) and dissolved fractions (absorption and excitation emission fluorescence).

Emergent organic and inorganic pollutants such as microplastics will be sampled using a manta net and Niskin bottles.
Other instruments considered on board will be:

- A Rosette (Seabird 911+) equipped with standard CTD, DO, fluorescence and optics sensors.
- ADCP
- Trace metals able to stimulate primary production will be measured through *in situ* pumping and a posteriori analysis.
- Wet lab on deck and sorting lab on board.
- Daily flow-cytometry sampling: monitoring of phytoplankton and bacterioplankton populations, using Accuri flow-cytometer. Fixation of samples with glutaraldehyde for viral quantification.
- Intra and extracellular metabolomics analysis: detection of small molecules (metabolites) present in the dissolved organic matter and microplankton associated metabolites through water filtration and concentration using SPE cartridges.
- Vesicle analysis: collection of extracellular vesicles for characterization in the lab through water filtration and concentration using 100kDa TFF.
- Microlayer sampling: better understand ocean-atmosphere interface by sampling water at the interface between ocean and atmosphere, using screen mesh water sampling.
- Glycan dynamics analysis: analyzing carbohydrate composition in POM and DOM.

3.4. Indicates whether harmful substances will be used:
Formaldehyde
Glutaraldehyde
Borax
Lugol
Ethanol
Methanol
37% HCL (1L total)

Chemical waste of Methanol and Paraformaldehyde will be stored in chemical waste containers.
Zinc chloride
Mercury Chloride
iron Chloride

For the storage of chemicals, a dedicated locked box has been placed outside the WetLab next to it (outside the ship). This locked box is meant to store all chemicals (e.g. formaldehyde, hydrochloric acid, ethanol, borax, glutaraldehyde, etc.). All the products in this box are stored in individual sealed bottles.

All chemicals are handled exclusively inside the WetLab. An *@Air Science* chemical hood, whose filter is replaced regularly, enables these products to be handled in complete safety. Strict handling protocols have also been put in place, so that chemicals are handled in a ventilated area, wearing protective gloves. Chemicals are never opened inside the ship, and when they need to be moved for sampling manoeuvres on the ship, they are moved using closed, hermetically sealed boxes.

For the removal of chemicals, a 25L drum is set up, stored outside the ship, to allow waste to be stored. On arrival at the local marine stations, these drums are handed over to the local services, which are responsible for removing the chemical waste. **No chemicals are discharged into the sea.**

3.5. Indicate whether drilling will be carried out: No

3.6. Indicate whether explosives will be used: No

4 – INSTALLATIONS AND EQUIPMENTS

4.1 Sampling of the Atmosphere

4.1.1 Continuous Atmospheric Measurements. A meteorological station (BATOS-II, Météo France) measures *continuously* air temperature, relative humidity, and atmospheric pressure at ~7 m above sea level. Wind speed and direction is measured at ~27 m above sea level. A Photosynthetically Active Radiation (PAR) sensor (Biospherical Instruments Inc. QCR-2150) is mounted at the stern of the boat (~7 m altitude). Data are recorded continuously and binned by minute.

4.1.2 Continuous Sampling of Aerosol Particles. One instrument is installed aboard *Tara* to *continuously* measure the size distribution and abundance of atmospheric aerosol particles. An optical particle counter (OPC; EDM180 GRIMM Aerosol Technik Ainring GmbH & Co. KG, Ainring, Germany) measuring particles in the size range 0.25 – 32 µm. Aerosols are pumped through an inlet mounted on the rear backstay of Tara near the mast (~27 m altitude). The OPC produces a particle size distribution every 60 sand is set to measure continuously throughout the expedition.

4.1.3 Interval Sampling of Aerosol Particles. A separate inlet is mounted on the rear backstay of Tara, next to the one used for *continuous* measurements (previous paragraph). Aerosols collected from this second tubing are vacuum-pumped (Diaphragm pump ME 16 NT, VACUUBRAND BmbH & Co KG, Wertheim, Germany) through three 47 mm filter holders mounted in parallel. One filter holders contains 0.45 µm pore size PVDF membrane filters, one filter holder contains a 0.8 µm pore size polycarbonate filter, and the third contains a cascade impactor (Dekati® Gravimetric Impactor) where the mass size distribution of particles < 2.5 µm is separated into five size fractions. Filters are generally changed twice a day, collecting aerosols for a period of at least 12 h at flow rates of >30 Lpm through each filter holder. PVDF filters are packaged in cryotubes and immediately stored into liquid nitrogen, whereas polycarbonate filters are packaged in sterile PetriSlide preloaded with absorbent pads and stored dry at room temperature, the size fractionated particles will be wrapped in aluminium foil and stored at -20C for chemical analysis. Particles from the PVDF filters will be extracted to assess microbial diversity using metagenomics and 16S and 18S rDNA amplicon sequencing. A scanning electron microscope Energy disperse X-ray analyzer (SEM-EDX) will be used to quantify the size, shape, quantity and elemental composition of all aerosols greater than 0.8 µm from the polycarbonate filters.
In addition, a high-flow air sampler (SASS® 3100) will be installed at the top of the mast, to sample for two hours during sailing and at 3 hour intervals when *Tara* is docked. The single electret filter media will be used for 16S amplicon sequencing. Also, time-lapse cameras will be installed to record the state of the ocean and clouds.

4.2 Underway, Continuous Sampling of Surface Water.
Surface seawater is pumped *continuously* on board through a hull inlet located 1.5 m under the waterline using a membrane pump (Shurflo), circulated through a vortex debubbler, and distributed to a number of flow-through instruments (Figure 4). The instruments comprise a thermosalinograph (TSG, SeaBird Electronics SBE45/SBE38), a spectrophotometer (WETLabs ACS), a CDOM fluorometer (WETLabs WSCD), a backscattering sensor (WETLabs ECO-BB3 and/or Sequoia Hyper-bb), and an Equilibrator Inlet Mass Spectrometer [EIMS] (Pfeiffer Vacuum Quadrupole 1–100 amu). The TSG is set to log time in coordinated universal time (UTC) synchronized with the ship's GPS. All instruments are then synchronized to the TSG. We hope to add two additional instruments, a Sequoia Horizon angular and polarized scattering meter and a Chelsea LabSTAF single turn over variable fluorometer (for phytoplankton physiology).

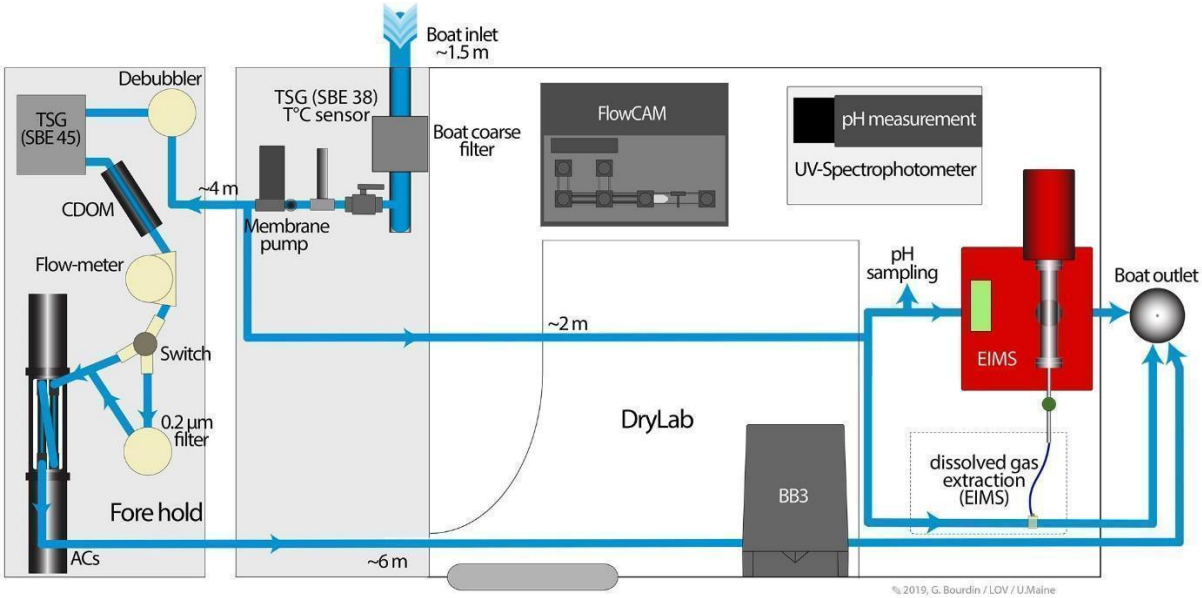


Figure 4. Schematic diagram of the underway, continuous sampling system, showing the setup of the various flow-through instruments and the distance between the water intake and each instrument. The water flow is shown in blue. The FlowCam and the UV-spectrophotometer were installed in the drylab for live imaging of microplankton and pH measurements.

4.2.1 Temperature and Salinity. The thermosalinograph (TSG, Seabird SBE45) is mounted 5.5 m downstream from the intake and measured sea surface temperature and conductivity at a sampling frequency of 0.1 Hz. A second temperature sensor (SBE38) is mounted 1.5 m downstream from the intake, upstream of the pump and the debubbler. Temperature and salinity measurements are quality checked throughout the expedition. The measurement errors of temperature are on the order of 0.01°C, whereas salinity records usually had uncertainties between 0.01 and 0.05. Salinity measurements are corrected after the analysis of unfiltered seawater samples and validated against Argo floats data in the vicinity of Tara when possible.

4.2.2 Net Community Production. The Equilibrator Inlet Mass Spectrometer (EIMS, Cassar et al., 2009) is mounted 3.5 m downstream from the intake and provides high-resolution measurements of the ratio of dissolved oxygen and argon (i.e., O2/Ar). While oxygen and argon have a similar solubility in seawater, argon is biologically inert, whereas oxygen is involved in biological processes such as photosynthesis and respiration. The O2/Ar ratio is therefore a measure of oxygen saturation associated with biological processes (i.e., biological O2 saturation), providing an estimate of net community production (NCP) (Cassar et al., 2009).

4.2.3 Inherent Optical Properties (IOPs). High frequency (1–4 Hz) measurements of IOPs are performed to derive numerous biogeochemical parameters. A WetLabs ACS (Figure 4) measures hyperspectral (~4 nm resolution) attenuation and absorption in the visible and near infrared; a WetLabs BB3 mounted in a ~4.5 L BB-box measured the volume scattering function (VSF) at 3 wavelengths

(470, 532, 650 nm) and a WetLabs WSCD measures fluorescent colored dissolved organic matter (CDOM). We use a calibration independent technique (Slade et al., 2010) to obtain particulate absorption (ap), attenuation (cp), and particulate backscattering (bbp) by subtracting measurements made without filter by measurements with a 0.2 µm filtered seawater, switching between those periods is done every 60 min (50 min total water, 10 min filtered).

4.2.4 pH. Seawater is collected once a week from the outlet of flow-through to determine pH. Surface seawater pH on total scale is determined using meta-Cresol Purple, a precise pH indicator dye (Clayton and Byrne, 1993; Dickson et al., 2007). Surface seawater samples are acclimated to 25°C for 2–3 h in closed, 5-ml polypropylene tubes. Absorbance at specific wavelengths is read, before and after of 40 µL meta-Cresol Purple dye addition using an Agilent Technologies Cary 60 UV-Vis Spectrophotometer equipped with an optical fibre.

4.2.5 Imaging FlowCytoBot (IFCB) and Cytosense*:* water will be analysed through these instruments, which take pictures of the microorganisms and a flow cytometer analyse the same sample to characterise shape, size and fluorescence of these organisms.

4.2.6 Discrete samples. Small volumes of seawater will be collected from the underway system, every day at noon. Samples will be prepared on board for chemical (mass spectrometry and HPLC), molecular (eDNA) and imaging (flow cytometry) analyses.

4.3 Processing discrete samples in the Wetlab.

Chemical preservation of samples is done outside the wetlab (Figure 5, left side, facing the stern). Inside the wetlab, two filtration systems are used, one for 142-mm-diameter filters, and the second for 25- and 47-mm-diameter filters. The first system is used for the serial filtrations of small plankton size fractions (<0.22, 0.22–3, and 3–20 µm) for genomic analyses. The second system is used to concentrate particulate matter in the large plankton size fractions (20–2000 and 300–2000 µm) for genomic analyses, and to collect material on glass fibre filters (0.7 µm) for pigment analyses (HPLC).

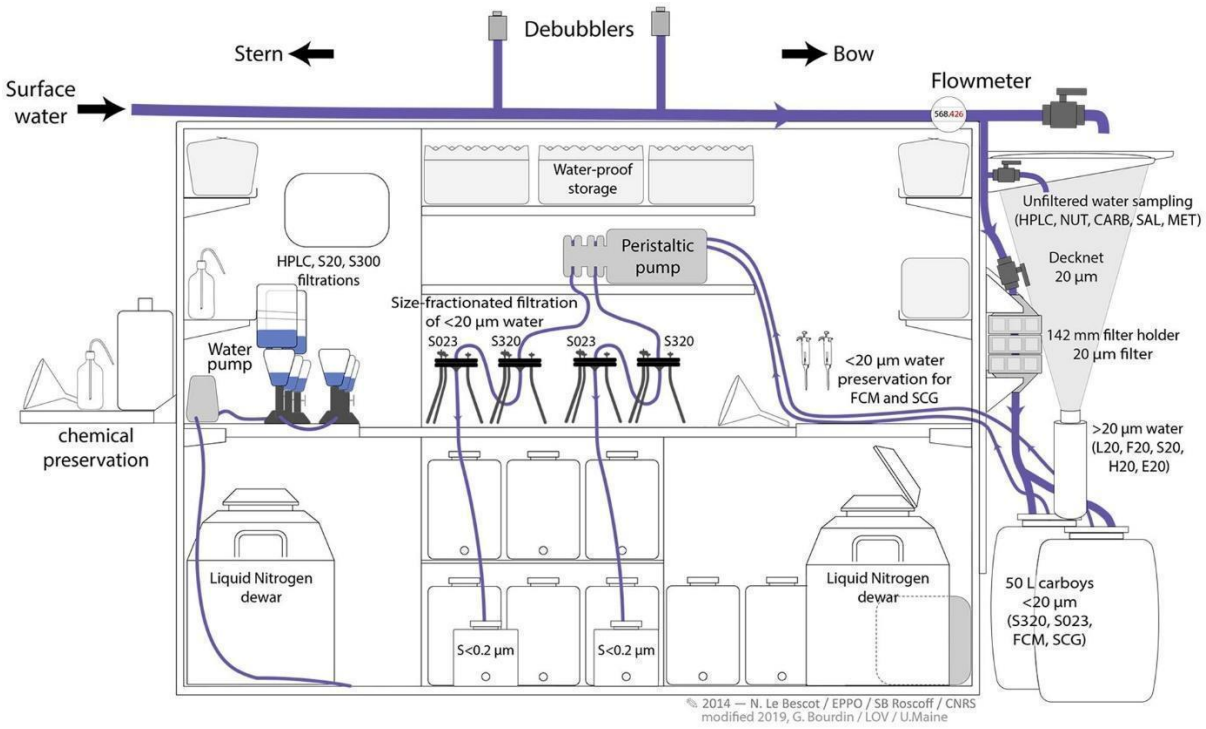


Figure 5. Schematic diagram of the wetlab on board Tara, modified from Pesant et al. (2015). Note the optimization of the inner space and outer surfaces of the wetlab. Two filtration systems, one for filters of 142 mm, the second for filters of 25 and 47 mm diameters are installed inside the wetlab. The outer wall facing the bow is adapted for the distribution of the surface pumped water into the circuitry.The wetlab wall oriented toward the stern is used for chemical preservation of samples.

4.4 Discrete sampling for water biogeochemistry.

Water samples are collected using Niskin bottles or, in the case of trace metals, using an extendable carbon-fiber pole equipped with a sturdy PVC bottle holder. The pole is hand-held off the bow of the ship. The uncapped bottle attached at the end of the pole is rinsed 4–5 times with surface seawater, then filled, and re-capped using polyethylene gloves.

4.4.1 Macronutrients. Seawater is collected from the unfiltered seawater circuitry (Figure 5) connected to the Dolphin at each sampling site to determine the nitrate (NO3), nitrite (NO2), phosphate (PO4) and silicate (Si(OH)4) concentrations. Seawater is immediately filtered through a 0.45 µm pore size cellulose acetate membrane with a syringe, dispensed into duplicate sterile 20 mL polyethylene vials, and stored at −20°C until analysis. The oxygen and nitrogen isotopic content of nitrate (DINO) will also be studied using the same sampling protocol, and stored in a 60 mL polycarbonate bottle in the freezer. Organic Nutrient such as Dissolved Organic Nitrogen and Dissolved Organic Phosphate will also be studied and sampled using the same protocols and stored in 60 mL plastics vials in the freezer. A bottle of 125 mL will be filled with seawater and acidified with 1 mL of 35% HCl to measure the mercury content of seawater (HG-M). The mercury content in particulates will be analysed on a 0.45 µm-pore-size glass fibre filter after filtration of 8L of seawater.

The filter are stored frozen (-20°C). The water passing through the filter will be collected in 2 glass vials of 40 mL to analyse dissolved mercury in seawater (total and methyl-mercury); they will be stored in the fridge (+4°C).

4.4.2 Carbonate Chemistry. Unfiltered seawater is collected once a week from the seawater circuitry (Figure Y). Glass bottles (500 mL) are filled with unfiltered seawater, poisoned with Hg2Cl2, and stored on board at room temperature until later analysis at the SNAPOCO2 facility at Sorbonne University in Paris, France. Total Alkalinity (TA) and Total Inorganic Carbon (TC) are measured, and all carbonate chemistry parameters are computed following the SNAPOCO2 protocol (Edmond, 1970; DOE, 1994). External reproducibility obtained for standard solutions for both parameters, TA and TC, is about 3 µmol/kg (0.15%). Deduced seawater pH values will be compared to the underway surface seawater pH measurements previously described.

4.4.3 Trace Metals. The task of quantifying extremely low levels of trace metals in seawater is complicated by sea vessels being a source of metal contamination, and therefore specialised oceanographic equipment and sampling procedures have been developed for collection in the water column (Measures et al., 2008). However, these procedures are typically specific to crane vessels, and sampling onboard the *Tara* schooner therefore required a basic surface-sampling protocol with similar metal-free handling precautions. Unfiltered seawater is collected using a custom-made hand-held bow-pole at each sampling site to determine total dissolvable iron (Fe), zinc (Zn), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb). Samples are collected off the bow of the boat while oriented toward the wind to minimize contamination, and polyethylene gloves are used during bottle handling. Low-density polyethylene (LDPE) bottles (125 mL) are cleaned on land by soaking overnight in 1% Citranox detergent, rinsed thoroughly, then soaked for at least 1 week in 10% HCl, followed by at least 8 rinses in ultrapure water, and individually enclosed in plastic bags to reduce contamination during transport and storage. Samples are stored in separate plastic Ziploc bags on-board at ambient temperature in the dark.

4.4.4 Pigments. Unfiltered seawater is collected with the Dolphin for the determination of pigment concentrations. Two liters of seawater are filtered on a 25-mm-diameter, 0.7-µm-pore-size glass fiber filter (Whatman GF/F). Filters are immediately stored in liquid nitrogen until later analysis by High Performance Liquid Chromatography for pigment analysis (Ras et al., 2008) at the Laboratoire Oceanographique in Villefranche-sur-Mer, France.

4.4.5 Dissolved Organic Matter and Dissolved Organics Carbon. 1 L of Filtered seawater is collected through a 0.45 um glass fiber filter and stored in glass vials and polycarbonate bottle, and acidified with HCl for the determination of dissolved organic carbon. 2L of filtered seawater is collected through a 0.45 um glass fiber filter, then acidified with HCl, and filter through a SPE cartridge

for the extraction of dissolved organic matter. The cartridge is then stored in the freezer.

4.4.6 Other chemical parameters of the seawater. Dissolved gases (DGAS) will be sampled in glass vials, and poisoned with zinc chloride and then stored in the fridge. The dissolved oxygen will also be sampled in a 20 mL glass vial and stored in the fridge.

4.4.7 Nanoplastics. seawater is collected and filter through a 5-µm-pore-size stainless steel filter and then through a 0.4-µm-pore-size glass fiber filter. Both filters are kept in the freezer at -20°C. Nanoplastics will be analysed on those filters.

4.4.8 *Other pollutants*. Chemical profiling of dissolved and particulate organic compounds: Particles will be samples using various filters (link to standard Tara protocol number). Concentrated particles will be stored frozen for further analysis, as well as cryopreserved for biobanking and subsequent cultivation efforts. Dissolved organic compounds will be concentrated using standard solid-phase extraction (SPE) procedures using absorption materials with complementary chemico-physical properties (e.g., PPL and C18). Organic compounds associated with particles and concentrated on SPE material will be extracted in the laboratory using organic solvents and analyzed by orthogonal analytical techniques, such as gas chromatography-coupled to mass spectrometry (GC-MS), liquid chromatography-coupled to mass spectrometry (LC-MS), and nuclear magnetic resonance spectroscopy (NMR). This will enable targeted and non-targeted profiling of organic compounds such as nutrients, endogenous metabolites, as well as man-made chemicals.

4.4.9 Extracellular vesicles and viruses' analyses. isolation of extracellular vesicles as well viruses for sequencing and characterization in the lab through water filtration and concentration using ultrafiltration (30kDa TFF) and ultracentrifugation on density gradient. Characterisation will include visualization via (transmission electron) microscopy, nucleic acids sequencing (DNA and RNA), metaproteomics, metabolomics, and lipidomics.

4.4.10 Marine invasive species. The expedition aims at identifying alien (introduced/invasive) species and associated native species (targeting invertebrates and vertebrates) present in ports, at their entrance and in their immediate vicinity, across different European Seas (from the North Sea to the Mediterranean Sea). Ports are targeted because they are points-of-entry of alien species and nodes for further alien spreading.
For that purpose, free DNA in seawater (environmental DNA) will be retrieved using in situ pumping and filtration. At each sampling site (3 sub-sites per site: in ports, port entrance, coastal water in the close vicinity), three small and autonomous pumping systems will be used for in situ seawater filtration using 0.45µm Waterra capsules. The filters contained in the capsules collected in each sub-site will be preserved in a non-hazardous Longmire buffer and kept

in a fridge until the DNA extraction in the laboratory.
The DNA will then be subjected to the construction of metabarcode libraries for the characterization of all the taxa composing the communities, targeting multi-cellular eucaryotes in particular marine invertebrates.
Genetic resources are used for biodiversity knowledge, and to assess the importance of one major driver of biodiversity change, namely alien species: Benefit sharing will consist of collaboration, cooperation or contribution to research, education, training, public and local professional awareness, skills transfer or technology transfer activities concerning the species mentioned in the declaration or related species. All the work will be published in scientific journals, and data provided in open public database.

4.5 Microbiomes in the <20 µm plankton Size Fraction.
Seawater is collected at each sampling site using Niskin bottles or a large-volume peristaltic pump, and is prefiltered through a 20 µm-pore-size nylon membrane. The filtrate is used to prepare the samples of the 3–20, 0.22–3, and <0.22 µm size fractions.

4.5.1 Flow cytometry. Two replicates of this filtrate (1.5 mL) are fixed with Glutaraldehyde (0.25% final volume) and Poloxamer (0.1% final volume) for 15 min at 4°C and then flash-frozen for later analyses using a FACS Canto II Flow Cytometer equipped with a 488 nm laser (Marie et al., 1999).

4.5.2 Single cell genomics. Two replicates of 4 mL filtrate are mixed with 600 µL of 48% glycine betaine aliquot and preserved in liquid nitrogen for later single cell genomics.

4.5.3 Prokaryotic and eukaryotic unicellular organism genomics. Two 50 L replicates of 20 µm prefiltered seawater are size fractionated in the wetlab into the 0.22–3 µm and 3–20 µm fractions using 142-mm-diameter, stainless-steel filter holder "tripods" (Millipore) and a peristaltic pump (Masterflex). Each replicate is filtered through a 142-mm-diameter, 0.22-µm-pore-size polyethersulfone Express Plus membrane filter and a 142-mm-diameter, 3-µm-pore-size polycarbonate membrane filter placed on top of a woven mesh spacer Dacron 124 mm (Millipore). The two filters are mounted in series to collect neuston and plankton in the 0.22–3 and 3–20 µm size fractions. To ensure that at least one of the two replicates yielded high quality RNA for transcriptomics analysis, the filtration of the first replicate is stopped after 15 min, whereas the second is allowed to go on for up to 60 min. Filters are packaged into 5 mL cryovials and preserved in liquid nitrogen immediately after filtration, until later sequencing at Genoscope, CEA, France. An additional sample will be taken by filtering 5L of seawater through a sterivex filter (eDNA). This filter will be stored in the freezer.

4.5.4 Virus genomics. Two 10 L replicates of the previously 0.22 µm filtered seawater are flocculated using iron chloride solution at room temperature for 1–12 h. The flocculated particulate matter containing viruses (John et al., 2011)

is concentrated onto a 142-mm-diameter, 1-µm-pore-size polycarbonate membrane filter placed on top of a woven mesh spacer Dacron 124 mm (Millipore). Filters are packaged in 5-mL cryotubes and stored at +4°C for later sequencing analysis at Ohio State University in Sullivan lab. Another sample of virus genomic will be taken with 2mL of seawater stored in a cryotube in liquid nitrogen (HI-C). 40 L of the previously 0.22 µm filtered seawater will be bring back to and and filter on a marine laboratory part of the EMBRC programme, using the ultrafiltration method. The sample is then aliquoted and centrifuged, and stored at room temperature.

4.5.5 Free metabolites, and unicellular organism endometabolites. Three 10 L replicates of 20 µm prefiltered seawater are size fractionated in the wetlab into the 0.22–3 µm and 3–20 µm fractions using 142-mm-diameter, stainless-steel filter holder "tripods" (Millipore) and a peristaltic pump (Masterflex). The cells are washed off into a buffer solution and preserved in the freezer. Free metabolites present in the filtrate are collected in a solid phase filtration cartridge and preserved in the freezer.

4.5.6 Prokaryotic and eukaryotic unicellular organism proteomics. Two 50 L replicates of 20 µm prefiltered seawater are size fractionated in the wetlab into the 0.22–3 µm and 3–20 µm fractions using 142-mm-diameter, stainless-steel filter holder "tripods" (Millipore) and a peristaltic pump (Masterflex). Each replicate is filtered through a 142-mm-diameter, 0.22-µm-pore-size polyethersulfone Express Plus membrane filter and a 142-mm-diameter, 3-µm-pore-size polycarbonate membrane filter and preserved in liquid nitrogen.

4.5.7: Scanning Electron Microscopy. 1L of seawater will be filtered using a 0.8-µm-pore-size polycarbonate filter and then dry at 60°C for 24h and store at room temperature. This sample will be analyse using a scanning electron microscopy to make pictures of the organisms at high definition.

4.6 Microbiomes and plastics in the 20–200 µm Size Fraction.
Microbiomes and plastics in that size-fraction are collected using a plankton net and manta nets with a 20 µm mesh size. Nets are lowered to the selected sampling depth and towed horizontally for 5–15 min at a speed of 0.3 m/s. Net samples from the two cod-ends are sieved through 200 µm and poured into an 8-L polyethylene bottle, The sample concentrated in the cod end is diluted to 2 L and divided into 6 subsamples.

4.6.1 Sequencing. 4 × 250 mL are filtered onto a 47 mm diameter, 10-µm-pore-size polycarbonate membrane filter, packaged in 5 mL cryotube and stored in liquid nitrogen for later sequencing analysis.

4.6.2 Inverted microscopy. 250 mL volume concentrated on a 20 µm mesh is preserved with acidic lugol in a 50 mL Falcon tube and stored at 4°C for enumeration and identification of microplankton using traditional inverted microscopy as detailed in Villar et al. (2015. Additionally, 45 mL is preserved

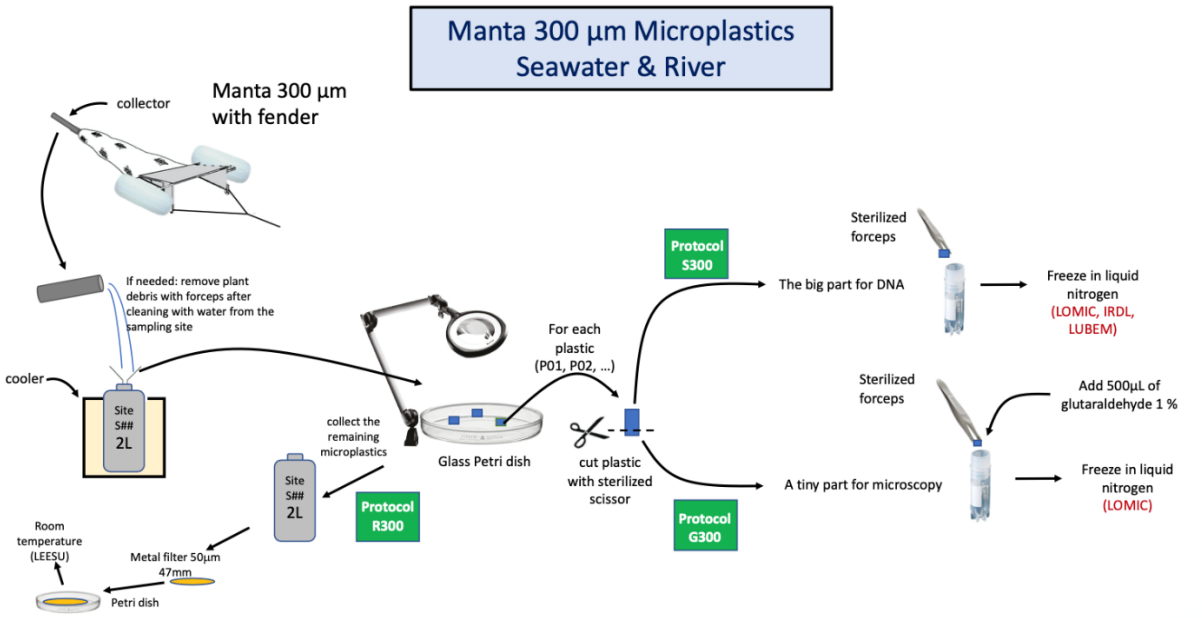
with 5 mL of buffered formaldehyde (4% v/v) in a 50 mL Falcon tube and stored at room temperature for enumeration and identification of microplankton using traditional inverted microscopy as detailed in Malviya et al. (2016).

4.6.3 Ethanol. 250 mL concentrated on a 20 µm mesh is resuspended in a 50 mL Falcon tube with pure ethanol. After 24 h the sample is concentrated again and preserved in a 15 mL Falcon tube with pure ethanol for genomic and morphological analyses

4.6.4 High-Content Fluorescent Microscopy. 45 mL from the 2 L cod-end content is transferred to a 50 mL Falcon tube and preserved with 5 mL aliquot of buffered 1% monomeric formaldehyde and 0.25% EM grade glutaraldehyde (Marie et al., 1999) for later environmental High-Content Fluorescent Microscopy analysis (eHCFM; Colin et al., 2017).

4.6.5 FlowCam. 250 mL is sieved through a 200 µm filter and the size fraction between 20 and 200 µm is analyzed live on-board using the FlowCam imaging system (Fluid Imaging Inc.; Sieracki et al., 1998).
4.6.6 Cryopreservation of microorganisms. The content of the cod-end will be divided in 2 fractions : the first fraction will be shipped back as fresh material to allow isolation of microorganisms and their culture *in vitro*. The second fraction will be cryopreserved in liquid nitrogen to be stored for long term duration, in order to be able to cultivate the organisms later in another laboratory.

4.7 Microbiomes and plastics in the >200 µm Size Fraction.
Microbiomes and plastics in that size-fraction are collected using a plankton net and manta nets with a 200/300/650 µm mesh size. The material collected in plankton nets (WPII-200, Bongo-300, Régent-650) is concentrated and preserved in a 250-mL plastic bottle with 25 mL tetraborax buffered formaldehyde (4%v/v) and stored at room temperature for later analysis with the ZooScan imaging system (Gorsky et al., 2010). A second sample is sieved through a 2000 µm metal sieve and the filtrate, 200–2000 µm size fraction, is concentrated on four 47 mm diameter, 10 µm pore size polycarbonate membrane filters, packaged in a 5-mL cryotube and stored in liquid nitrogen for later sequencing analysis. Additional tows of the Manta net are used to isolate sargassum samples & individual plastic particles in order to perform chemical (mass spectroscopy), molecular (DNA) and imaging (microscopy) analyses of their microbiomes, i.e. life on sargassum & plastics. The procedure is illustrated below.



4.9 The VMP.

The VMP-250 is a coastal-zone profiler for the measurement of ocean turbulence (micro-scale shears and thermal gradients) and hydrological parameters (temperature, conductivity, pressure, turbidity, fluorescence), deployable from surface and up to 500m deep. The profiler is designed for operation for small vessels with limited deck space (e.g., fish or sail boats, zodiacs), or where electrical power facilities are limited or missing (e.g., ice camps). The VMP-250 records data internally on a memory card, eliminating the requirement for a deck-side power supply and data recording system (i.e., laptop), and allowing to do repeated profiles without recovering the profiler between deployments.

4.10 On-board dimethylsulfoniopropionate (DMSP) sampling.

To understand sulphur cycling, a 200ml sample of water will be taken from the CTD in an amber bottle to which a Sodium hydroxide pellet will be added. These samples will be stored in a dark area and transported to Europe and South Africa for analyses.

Sampling the water for downstream analysis

Water biogeochemistry

- Nutrients: We will sample water to determine the nitrate (NO3), nitrite (NO2), phosphate (PO4) and silicate (Si(OH)4) concentrations. Seawater is immediately filtered through a 0.45 µm pore size cellulose acetate membrane with a syringe, dispensed into duplicate sterile 20 mL polyethylene vials, and stored at −20°C until analysis.
- Carbonate chemistry: Unfiltered seawater is collected using a pump. Glass bottles (500 mL) are filled with unfiltered seawater, complemented with Hg2Cl2, and stored at room temperature until later analysis at the SNAPOCO2 facility at Sorbonne University in Paris, France. Total Alkalinity (TA) and Total Inorganic Carbon (TC) are measured, and all carbonate chemistry parameters are computed following the SNAPOCO2 protocol (Edmond, 1970; DOE, 1994). External reproducibility obtained for standard solutions for both parameters, TA and TC, is about 3 µmol/kg (0.15%). Deduced seawater pH values will be compared to the underway surface seawater pH measurements previously described.
- Pigments: Unfiltered seawater is collected for the determination of pigment concentrations. Two liters of seawater are filtered on a 25-mm-diameter, 0.7-µm-pore-size glass fiber filter (Whatman GF/F). Filters are immediately stored in liquid nitrogen until later analysis by High Performance Liquid Chromatography for pigment analysis (Ras et al., 2008) at the Laboratoire Océanographique in Villefranche-sur-Mer, France.
- Trace metals: Unfiltered seawater is collected using a custom-made hand-held bow-pole at each sampling site to determine total dissolvable iron (Fe), zinc (Zn), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb). Samples are collected off the bow of the boat while oriented toward the wind to minimize contamination, and polyethylene gloves are used during bottle handling. Low-density polyethylene (LDPE) bottles (125 mL) are cleaned on land by soaking overnight in 1% Citranox detergent, rinsed thoroughly, then soaked for at least 1 week in 10% HCl, followed by at least 8 rinses in ultrapure water, and individually enclosed in plastic bags to reduce contamination during transport and storage. Samples are stored in separate plastic Ziploc bags on-board at ambient temperature in the dark.

Protist biology

- Bulk genomics: Water will be pumped using a peristaltic pump equipped with a 20um mesh to discard larger organisms. The water will be taken in 10L Nalgene bottles and then passed through a system of serial filtrations of small size fractions (<0.22, 0.22–3, and 3–20 µm) for genomic analyses on 142 mm polycarbonate membranes. Filters will be folded and stored in 5ml cryovials before flash freezing
- Flow cytometry: Two replicates of this 20um filtrate (1.5 mL) are fixed with Glutaraldehyde (0.25% final volume) and Poloxamer (0.1% final volume) for 15 min at 4°C and then flash-frozen for later analyses using a FACS Canto II Flow Cytometer equipped with a 488 nm laser (Marie et al., 1999).

- Single cell ecology: Water will be pumped using a peristaltic pump equipped with a 200um mesh to discard larger organisms and stored in a 10L tank. The water will be poured into a 20um sieve equipped with a pouring spout, placed on top of a 5um mesh net.
    - Biomass will be collected from the 20um sieve and stored for three different purposes: single cell barcoding (ethanol fixation, final volume 15mL), single cell transcriptomics (methanol fixation, final volume 2mL), single cell imaging (PFA fixation, final volume 50mL)
    - Biomass will be collected for the 5um cod end and stored for three different purposes: single cell barcoding (ethanol fixation, final volume 15mL), single cell transcriptomics (methanol fixation, final volume 2mL), single cell imaging (PFA fixation, final volume 50mL)
- Metabolomics: detection of small molecules (metabolites) present in the dissolved organic matter and microplankton associated metabolites through water filtration and concentration using SPE cartridges.

5 - GEOGRAPHICAL AREAS in GREEK WATERS (EEZ + territorial sea + internal waters)

Here is the list of the sampling sites with their coordinates. The sampled site might change from about 10 nm from the written GPS position to adapt to local conditions, including weather and marine traffic.

The objective is to sample close to the coast (a few nautical miles from the coast at maximum).
The research carried out at these stations involves surface sampling only. **No seabed survey is planned.**

Station's name	Latitude	Longitude
155	37.8893519	23.5123776
156	37.9381054	23.6126278
157	37.9240228	23.6730526
158	37.8860979	23.712878
159	37.9381042	23.561816
160	37.8955584	23.5935093
161	37.8438158	23.627734
162	38.0232393	23.5608544
163	40.0054698	22.6463704
164	40.301723	22.6497689
165	40.5450338	22.9372709

6 - DATES

6.1 Expected dates of first entry into and final departure from the research area of the research vessel:

The dates might evolve depending on any sanitary situation and weather conditions.

Entry date: May 15th, 2024, at the earliest, with a possible delay of 60 days at the maximum

Departure date: September 1st, 2024, at the latest, with a possible delay of 60 days at the maximum

6.2 Indicate if multiple entry is expected: NO

7 - PORTS CALLS

7.1. Dates and names of intended ports of call.

Athens: July 1st, 2024 – July 9th, 2024
Athens: July 14th, 2024 – July 15th, 2024

No other proper port calls, only moorings or nights at harbors, locations & dates to be defined, close to sampling sites.

7.2. Any special logistical requirements at ports of call.
TBC

7.3. Name/Address/Telephone of shipping agent (if available).
TBC

8 - PARTICIPATION

8.1. Extent of which the local scientists will be enabled to participate in the research expedition:

Local scientists are fully part of this European expedition through multiple local laboratories. As a part of this international and multidisciplinary scientific consortium, all institutions will fully be participating in the expedition, sampling and accessing raw and analysed data.

We will also participate to an **outreach stopover in Athens** in collaboration with local marine stations; and local NGOs or schools, the public and scientific and political institutions will be able to participate in:
- outreach activities and workshops
- public and scientific lectures
- exhibitions;
- exchanges with the crew and scientific team to visit the schooner and learn more about the mission and environmental challenges.

8.2.Proposed dates and ports or embarkation/disembarkation for local scientists as well as - if necessary a local observer.

Embark in Athens: July 9th, 2024.
Disembark in Athens: July 9th, 2024.

9 - SAMPLE SHIPPINGS

All data and samples taken aboard Tara will be stored & barcoded on board and shipped to EMBL (Germany), before their redistribution to GENOSCOPE (France) for sequencing and then into the international network of collaborators for data generation, analyses, and collective publication in open source journals and databases.

10 - ACCESS TO DATA, SAMPLES AND RESEARCH RESULTS

10.1. Expected dates of submission of preliminary reports which should include the expected dates of submission of the final results.

The Tara Ocean Foundation will follow guidelines and deadlines stated in the permit. Preliminary reports and final reports will be sent within these timelines.

10.2. Proposed means for access to data and samples

We wish to involve local scientists as much as possible as "Associated scientists and institutes". As a part of the European "BIOcean5D BIODIV-01-03" program, all visited marine institutions will fully be participating in the expedition, sampling and access to raw and analyzed data.
For example, the quality of samples collected during the Tara Oceans expedition enabled state-of-the-art imaging and sequencing, the vast potential of which was recently unveiled in *Science* and *Nature* (see references in Section 2.3 of this application). Data are progressively released in open access and already represent the largest coherent set of environmental, imaging and sequencing data collected in the global ocean. Yet only a fraction of available samples has been analysed, while replicates and aliquots are preserved in trusted collections and biobanks for the next generations.

The TREC program fosters an ambitious infrastructure of open access archives for sequences (ENA[2]), high throughput images (EuBI[3]) and environmental data (PANGAEA[4]), ensuring that its knowledge base remains FAIR[5] (Findable, Accessible, Interoperable and Reusable) to all. Sample provenance is key to connecting environmental, sequencing and imaging data, and Tara Oceans set the trend in marine science with the most comprehensive registries of sample provenance and context to date (see *Data Box 2*).

The sheer quantity of imaging and sequencing data, and the richness of their environmental context require innovative bioinformatics tools, next generation global ocean modelling, and cloud computing. A number of bioinformatics and modelling tools have started to spur in the framework of Tara Oceans (see *Data Box 3*) and several comprehensive data products are beginning to emerge (see *Data Box 4*). However, there is a pressing need to develop more comprehensive tools and interfaces that enable scientists to explore and exploit the full potential of Tara Oceans' knowledge base, in particular to further our understanding of interactions, adaptation and evolution of life.

[1]http://www.nature.com/articles/sdata201523
[2]http://www.ebi.ac.uk/ena
[3]http://www.eurobioimaging.eu/
[4]http://www.pangaea.de
[5]http://www.nature.com/articles/sdata201618

10.3. Proposed means of making research internationally available:

The database will be established in combination with existing databases and the scientific community. It will allow the scientific community to refine our knowledge about life evolution and will transform our capacity to model climate change. It would be a reference for future research on climate changes' impact. It would allow to precise data on marine organisms' evolution. These data will also be used to conceive a functional map of marine ecosystems, through models and interactive visualizations. The database will constitute a point of reference for future generations of researchers.
Idem, the international diffusion of scientific results will be freely accessible through the database and through scientific publications. The results will also be communicated to the competent UN agencies and other international institutions. The general information of the public and of children will be made through the usual media, the production of films and a specific pedagogic program.

ANNEXES

1 / Team

Coordinators	
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ssunagawa@ethz.ch	*Shinichi Sunagawa*
mbsulli@gmail.com	*Matt Sullivan*

Team on board during the mission: (14 pax maximum)

CREW MEMBERS (Tara Ocean Foundation) - TBC

	FIRST NAME	**LAST NAME**	**NATIONALITY**	**ROLE**	**PASSPORT NUMBER**
1	Martin	Hertau	French	Captain	
1	Samuel	Audrain	French	Captain	
1	Yohan	Mucherie	French	Captain	
2	Pierre	Landoeur	French	First Mate	
2/3	David	Monmarché	French	First Mate / Deck officer	
2/3	Mathieu	Oriot	French	First Mate / Deck officer	
4	Laurent	Rogniaux	French	Chief engineer	
4	Dave	Picaud	French	Chief engineer	
5	Elise	Le Baron	French	Deck officer	
5	Francois	Aurat	French	Deck officer	
6	Carole	Pire	French	Cook	
6	Sophie	Bin	French	Cook	
7			tbc	Journalist	
7			tbc	Journalist	
8			tbc	Artist	
8			tbc	Artist	

SCIENCE TEAM -

	FIRST NAME	**LAST NAME**	**NATIONALITY**	**ROLE**	**PASSPORT**	
1	Colomban	DE VARGAS	FRANCE	*Scientific Director for Tara Europa*	15DC85597	
2	Douglas	COUET	FRANCE	*Biological engineer*		
3	Thomas	LINKOWSKI	FRANCE	*Oceanography Engineer*		
4	Emmanuel	BOSS	USA	Underway inline sensing system		
5	Vittorio	BRANDO	ITALY	Remote sensing		
6	Lionel	GUIDI	FRANCE	Oceanography		
7	Patrick	WINCKER	FRANCE	DNA/RNA sequencing		
8	Hugo	BERTHELOT	FRANCE	Subcellular imaging		
9	Thorsten	DITTMAR	GERMANY	Biogeochemistry		
10	Sophie	ARNAUD	FRANCE	e-DNA		
11	Mak	SAITO	USA	Metaproteomics		
12	Georg	POHNERT	GERMANY	Metabolomics		
13	Michael	ZIMMERMANN	GERMANY	Chemistry & pollutants		
14	Ian	PROBERT	FRANCE	Culturing		
15	Michel	FLORES	ISRAEL	Aerosols		
16	Fabien	LOMBARD	FRANCE	Zooplankton and organimal imaging		
17	Tom	DELMONT	FRANCE	Protists		
18	Shinichi	SUNAGAWA	SWISS	Prokaryotes		
19	Matt	SULLIVAN	USA	VIruses & Vesicles		

2 / Map (*will most certainly evolve with time)*

3 / Tara particulars

Name: TARA

Call sign: FVNM
IMO number: 8817552
MMSI:226070000

Flag: French
Port of exploitation: Lorient
Port of registry: Marseille
Number of registry: RI748443A
Classification society: Bureau Veritas
Class: 1 *hull *mach special service-A unrestricted navigation
Buiding date: 1989 SFCN France

Length overall: 35.98m
Beam: 10m
Max draught: 3.5m
Air draught: 30m

Gross Register Tonnage:169 ums
Net Register Tonnage: 50 ums

Engines: 2 diesel engines Cummins 2x265 Kw
Diesel Generator: 1x 40 Kw and 2x 17 Kw
Sails: schooner type - 2x150 m2 and 1x300 m2
Tenders: 1 rib boat outboard 50bhp and 1 rib boat outboard 70bhp
Anchor: 1 anchor with 9 shackles