

| Start | 2024 | 02 | 27 | Malaga, Spain |
|-------|------|----|----|------------------|
| End | 2024 | 03 | 17 | Barcelona, Spain |

Fondation

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SCIENTIFIC INTEREST & CAMPAING OBJECTIVES

The leg from Malaga (Spain) to Barcelona (Spain) in February/March 2024 was the first leg of the second phase of the Tara EUROPA expedition that focuses on the Mediterranean Sea in 2024 (April 2023 – July 2024; https://fondationtaraocean.org/en/expedition/tara-europa/). Tara EUROPA is the TREC ocean part of а larger program, _ Traversing European Coastlines (https://www.embl.org/about/info/trec/) -, whose main goals are to: (i) study the invisible biodiversity at the land-sea interface across 19 European countries from Finland to Greece, and understand the effect of environmental changes on the interactions and evolution within and between ecosystems (soil, sediments, air, water); (ii) understand the impact of human activities (pollution and global change) on marine coastal biodiversity and ecosystems; (iii) share systems bio/ecology knowledge and advanced technologies with scientists and the general public from all coastal European countries.

Within this framework, a team of 6 international researchers and engineers on board *Tara* realize the sampling of coastal waters and aerosols in synchronization with a team from EMBL that conducts sampling of shallow water, sediments, and soils. The sampling sites are pre-selected along the coastline or in estuaries and represent either pristine or human-impacted (agriculture, urban areas, ports, industry) environments. Usually, *Tara* samples a single station at regular coastline sites, and 2 to 3 onshore to offshore stations in estuarine settings to allow for the characterization of environmental gradients from brackish to marine waters, with a putatively decreasing impact from land biogeochemistry. The positions of the coastal stations are pre-adjusted based on historical remote-sensing (ocean color) and oceanographic (bathymetry, currentology) data, and can be slightly modified depending on real-time remote sensing data and weather/navigation conditions. Overall, the *Tara* Europa stations represent coastal water masses with varying degrees of land-sea connectivity and anthropogenic impacts.

At each *Tara* Europa station, the team onboard conduct a complex set of at least 50 protocols and collect ~ 100 samples that are stored in appropriate conditions for future analyses in land-based laboratories. The different methodological approaches focus on the characterization of the biological community composition and diversity present in the water (from viruses to animals, from genomes over expressed genes, metabolites, proteins, to cellular and organismal features), together with contextual physical, biophysical, chemical and biogeochemical properties.





PERSONNEL

Table 1: Crew and Scientific personnel onboard Tara from 27th February 2024 to 17th March 2024

| ROLE | | NAME, Surname, Affiliation |
|------|---|--------------------------------|
| 1 | CREW - Captain | Samuel Chaffron |
| 2 | CREW - 1st Officer | Morgann Andrieux |
| 3 | CREW - Mecano | Dave Picaud |
| 4 | CREW - Deck | François Aurat |
| 6 | CREW - Cook | Sophie Bin |
| 7 | CREW - Media | Louise Cognard |
| 8 | GUEST - Artist | Enrique Ramirez, Yoichi Ochiai |
| 9 | GUEST - Observer | |
| 10 | SCIENCE - A - Oceano. Engineer | Thomas Linkowski |
| 11 | SCIENCE - B – Bio. Engineer | Morgane Guillam |
| 12 | SCIENCE - C - W-Lab genomics | Odette Beluche |
| 13 | SCIENCE - D – Deck Chemical profiling/ Chief Scientist | Jessika Füssel |
| 14 | SCIENCE - E – Deck | Marta Furia |
| 15 | SCIENCE - F - S-Lab sorting/imaging | Erwan Legay |





REALIZED STATIONS



Figure 1: Satellite view of the geographical sampling locations of Tara EUROPA between Malaga, Spain

and Medes Islands, Spain.



CAMPAIGN SUMMARY REPORT (CSR)

Mission Microbionies

SUMMARY OF ACTIVITIES

Table 2: Dates, Station number, geographical Location and samples obtained during the Tara EUROPA leg of March 2024.

| Date | Station # | Name | Latitude | Longitude | Cas t | Pump A20 | Net 5 µm | Net 20 µm | Net 200 µm | Net 680 µm | Bow Pole | ASM | eDN A | HTSRB | SML | Mercury | Aliens in Port | # of samples |
|----------------|--------------|-------------------------------|-------------|-----------|----------|-------------|-------------|--------------|---------------|---------------|-------------|-----|----------|-------|-----|---------|-------------------|-----------------|
| 29/02/202 4 | 100 | Marbella | 36.462 | -4.815 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 124 |
| 01/03/202 4 | 101 | Marbella offshore | 36.345 | -4.715 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 126 |
| 02/03/202 4 | 102 | Motril | 36.713 9 | -3.5290 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 123 |
| 04/03/202 4 | 103 | Almeria | 36.798 9 | -2.4332 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 125 |
| 05/03/202 4 | 104 | Almeria offshore | 36.658 3 | -1.5159 | 3 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 154 |
| 07/03/202 4 | 105 | Offshore Mallorca | 38.713 4 | 2.51 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 124 |
| 08/03/202 4 | 106 | Bassal de s'Estanyol | 39.244 1 | 3.0636 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 126 |
| 09/03/202 4 | 107 | Sa Coma offshore | 39.515 2 | 3.6895 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 115 |
| 11/03/202 4 | 108 | Mallorca/Portocolom | 39.420 3 | 5.2828 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 123 |
| 12/03/202 4 | 109 | Sa Coma shore | 39.548 7 | 3.5545 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 125 |
| 13/03/202 4 | 110 | Mallorca / Badia d'alcudia | 39.804 5 | 3.1714 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | 1 | 1 | 0 | 123 |

Legend Table 2:

Pump A20: Deployment of a tubing system in sub-surface waters, connected to a peristaltic pump installed in the wetlab on *Tara*'s deck. Water is then filtered through large membranes to concentrate plankton biomass for genetic analyses.

Net: Deployment of various types of plankton nets with specific mesh-sizes (5 μm, 20 μm, 200 μm or 680 μm), either on Tara's deck (Decknets, 5 μm, 20 μm) or overboard (200 μm or 680 μm).

Cast: Deployment of the Rosette water sampler equipped with a CTD (holding 5x12L and 8x8L Niskin bottles and sensors) to collect a suite of biophysical data and water samples along the water column.

Bow pole: Manual handling of a long stick for clean, contamination-free collection of small volumes of surface water stored for laboratory analyses of trace element analysis.

ASM (Aerosol Sampling Mast): Pumping system installed on Tara's mast to collect and concentrate aerosols.

HTSRB (Hyperspectral Tethered Spectral Radiometer Buoy): Deployment of a floating device equipped with sensors to measure optical properties (hyper-spectral radiometry) of surface seawater.

SML (Surface Micro-Layer sampler): Deployment of a screen sampler to collect 1L of surface microlayer water.

Mercury: Specific filtration protocol to measure mercury from a Niskin bottle, performed at estuarine sites.

Aliens in port: Deployment of an in-situ pumping system (Watera capsules) to concentrate biomass from 30L of subsurface water for eDNA analyses.

| 16/03/202 4 | 111 | Medes offshore | 42.001 5 | 3.4431 | | | | | | | | | | | | | 154 |
|----------------|-----|----------------|-------------|--------|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| 15/03/202 4 | 112 | Medes middle | 42.043 2 | 3.2561 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 124 |
| 17/03/202 4 | 113 | Medes shore | 42.042 2 | 3.2066 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 124 |

CAMPAIGN SUMMARY REPORT (C

explore and share INVENTORY OF SAMPLES COLLECTED DURING THE CAMPAIGN

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Table 3: Total number of samples preserved and storage conditions for each of the 64 protocols performed during the scientific stations onboard Tara.

| protocol | Protocol category | Storage T° | TOTAL of | protocol | Protocol category | Storage T° | TOTAL of |
|-----------------------|---|------------|----------|----------------|----------------------------------|------------|----------|
| name | Flotocol category | Storage i | samples | name | Flotocol category | Storage 1 | samples |
| PM | Oceanography/Biogeochemistr v | - 20°C | 49 | DGAS | Oceanography/Biogeochemistr v | 4°C | 132 |
| FOI | Oceanography/Biogeochemistr y | - 20°C | 42 | DGAS-EXE | Oceanography/Biogeochemistr y | 4°C | 39 |
| PA | Oceanography/Biogeochemistr y | - 20°C | 14 | FC-P | imaging | LN2 | 32 |
| S023-L (long read) | Nucleic Acids/Sequencing | - 20°C | 16 | SML-FC | imaging | LN2 | 28 |
| S320-L (long read) | Nucleic Acids/Sequencing | - 20°C | 16 | FC-G | imaging | LN2 | 32 |
| E20 | Nucleic Acids/Sequencing and imaging | - 20°C | 14 | HPLC | Oceanography/Biogeochemistr y | LN2 | 18 |
| MB320 | Chemical profiling | - 20°C | 14 | SML-320 | Nucleic Acids/Sequencing | LN2 | 42 |
| MB033 | Chemical profiling | - 20°C | 14 | SML-023 | Nucleic Acids/Sequencing | LN2 | 42 |
| PPL | Chemical profiling | - 20°C | 56 | нс | Nucleic Acids/Sequencing | LN2 | 112 |
| HLB | Chemical profiling | - 20°C | 56 | HC-G | Nucleic Acids/Sequencing | LN2 | 112 |
| S20-L | Nucleic Acids/Sequencing | - 20°C | 14 | CP-G | Nucleic Acids/Sequencing | LN2 | 48 |
| S200-L | Nucleic Acids/Sequencing | - 20°C | 14 | SG | Nucleic Acids/Sequencing | LN2 | 32 |
| S680-L | Nucleic Acids/Sequencing | - 20°C | 14 | S023-S | Nucleic Acids/Sequencing | LN2 | 32 |
| MB20 | Chemical profiling | - 20°C | 14 | \$320-S | Nucleic Acids/Sequencing | LN2 | 32 |
| NUT | Oceanography/Biogeochemistr y | - 20°C | 42 | \$20-S | Nucleic Acids/Sequencing | LN2 | 28 |
| ASM | Aerosol | - 20°C | 14 | S200-S | Nucleic Acids/Sequencing | LN2 | 28 |
| рМеНg | Oceanography/Biogeochemistr y | - 20°C | 0 | P023 | Nucleic Acids/Sequencing | LN2 | 16 |
| pTHg | Oceanography/Biogeochemistr y | - 20°C | 16 | P320 | Nucleic Acids/Sequencing | LN2 | 16 |
| Hg-20 | Oceanography/Biogeochemistr y | - 20°C | 14 | SG5 | Nucleic Acids/Sequencing | LN2 | 32 |
| Hg-200 | Oceanography/Biogeochemistr Y | - 20°C | 14 | S02-2000 QN | Nucleic Acids/Sequencing | LN2 | 0 |
| Hg-680 | Oceanography/Biogeochemistr y | - 20°C | 14 | SML-CP | Nucleic Acids/Sequencing | LN2 | 42 |
| NAT | Oceanography/Biogeochemistr y | - 20°C | 52 | DICTA | Oceanography/Biogeochemistr y | RT | 16 |
| PPN2 | Oceanography/Biogeochemistr y | - 20°C | 39 | SAL | Oceanography/Biogeochemistr Y | RT | 9 |
| fMeHg | Chemical profiling | 4°C | 16 | MTE | Chemical profiling | RT | 14 |
| fTHg | Chemical profiling | 4°C | 0 | F200 | imaging | RT | 14 |
| ufTHg | Chemical profiling | 4°C | 2 | F680 | imaging | RT | 14 |

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|------------------------|----------------------------------|---------|------|---|---------|---------|------|----|
| THg (from bow pole) | Chemical profiling | 4°C | 14 | | F2000 | imaging | RT | 1 |
| CDOM | Oceanography/Biogeochemistr y | 4°C | 42 | | Flowcam | imaging | Data | 14 |
| DOC | Oceanography/Biogeochemistr Y | 4°C | 42 | | | | | |
| тос | Oceanography/Biogeochemistr Y | 4°C | 42 | | | | | |
| S<0.2 (Virus) | Nucleic Acids/Sequencing | 4°C | 32 | | | | | |
| FM5 | imaging | 4°C | 32 | | | | | |
| FM20 | imaging | 4°C | 28 | | | | | |
| eDNA | Nucleic Acids/Sequencing | 4°C | 14 | | | | | |

TOTAL SAMPLES Tara Europa Malaga-Medes

Table 4: Total number of samples preserved for each of the 4 protocols performed underway during the Tara

 Europa navigation. The details of the number of samples per protocol per stations can be found @here.

| protocol name | Protocol category | Storage T° | TOTAL of samples |
|---------------|------------------------------|------------|------------------|
| AF | Aerosols | - 20°C | 31 |
| AS | Aerosols | LN2 | 31 |
| HPLC underway | Oceanography/Biogeochemistry | LN2 | 1 |
| FC-P underway | Imaging | LN2 | 1 |
| FC-G underway | Imaging | LN2 | 1 |
| SAL-underway | Oceanography/Biogeochemistry | RT | 1 |
| AI | Aerosols | RT | 31 |

TOTAL SAMPLES UNDERWAY Tara Europa Malaga-Medes

97



STATION COMMENTS

| Tara Europ a Statio n # | Comments |
|-------------------------------------|--|
| 100 | First station of 2024! We started the station at sun rise and took about 6 h for all protocols. The station was long as we had to instruct the Operators E/F who had no experience with the work yet and we also included some new protocols. |
| 101 | Offshore station without the land team. The weather is grey and windy. We decided to use the 5- μ m decknet again instead of filtering water through a 5 μ m filter, which didn't work well as we had received the wrong filters. |
| 102 | Station started in the early morning (5:40 UTC) and we deployed the CTD rosette before sunrise. Wind and waves increased during the station. The 200µm net broke during the tow. Too much drift to deploy secchi disk. |
| 103 | Station is located very close to shore. There are a lot of greenhouses along the coast and we expect to detect some pollutants in the water samples. We sampled close to a small river output. A clear oxygen minimum between 8 and 10 m water depth, the oxygen saturation drops from 90 to72%. We see a slight DCM at 10 m and relatively stable salinity and T°C. |
| 104 | We are sampling a 2nd depth for the first time during this mission! We deploy the second CTD to >1000m to record a profile and then sample at the base of the mix layer (MLD), as there is no DCM. The only ChI a peak is within the mixed layer at about 10 m. At stations with two depths, we do vertical tows for the large nets from 200 m rather than horizontal tows. To obtain enough water for all protocols that are performed at both depths we require to CTD rosette casts. The sea is calm, there is no wind and the sun is shining. 3 measures of salinity (at surface, 60m, and 1500m). |
| 105 | A lot of mucilage in the 680 and 200µm nets. Thomas had to rinse them very thoroughly much longer than usual. There is little wind. |
| 106 | Strong wind today with waves. The MilliQ system failed, ~16M Ω instead of 18.2 M Ω . We are still using the water for rinsing but we will need to change the filter pack. |
| 107 | We started the station directly at sun rise. Everything went smoothly. |
| 108 | The weather is excellent, no wind and sunshine. The land team is sampling in a bay while we are located just behind the cliff that forms the barrier on one side of the bay. |
| 109 | For the first time we observe slight thermal stratification with a thermocline between 4-5 m, the salinity remains constant throughout the water column. There is an interesting oxygen peak at 45m that we can't explain but that we can observe |

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| | in both down- and up-cast. There is hardly any chl-a fluorescence at the surface and no DCM. Good weather, calm sea |
|-----|---|
| 110 | Station in a bay close to a small river. Again developing thermal stratification with the thermocline at 6-7 m and no change in salinity throughout the water column. |
| 111 | Offshore station. We are sampling a 2nd depth again at the base of the mixed layer at the depth of the oxygen minimum at 75 m. There is no thermal stratification in the upper water column. |
| 112 | Very calm sea. We are at Medes Islands and sample the site of the Medes Island time series that is classified as pristine. |
| 113 | We are sampling along the land-sea transect between the shore and the Medes Island benthic time series station The area is beautiful and we are very close to the shore and the land team |