CRUISE REPORT

ELENO

HABITAT TEMPLATE, MICROBIAL SIGNATURES AND ICONIC LIFE IN A CHANGING ARCTIC OCEAN

Le Commandant Charcot, Cruise No. CC260823,

August 26th 2023 – September 10th 2023

Longyearbyen (Svalbard Islands - Norway) – Reykjavík (Iceland)

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Summary

The ELENO project was an opportunity to expand the Arctic study area (Greenland Sea; 75°N transect) of the ongoing CASSANDRA project, funded by the Italian Arctic Research Program (PRA) in 2021 as part of Synoptic Arctic Survey (SAS) and contribute by studying a transect towards the North Pole in summer 2023, providing further integrative data of hydrography, as well as biological and ecosystem functioning.
The scientists of the ELENO project embarked on an extraordinary polar expedition aboard the specialized vessel “Le Commandant Charcot” (August 26 2023 – September 10 2023) which was designed to navigate the challenging route to the Geographical North Pole. The oceanographic expedition involved traversing dynamic ice floe landscapes, providing an opportunity for in-depth study of polar environments of the High Arctic and allowing the collection of unique samples of water, air and sea ice, as well as the sighting of marine mammals. The itinerary included also the possibility of sampling near the Svalbard Islands and the Greenland's fjords, increasing the possibility of sightings of marine mammals.

1. Research Programme/Objectives

The ELENO project mainly aimed at expanding the marine Arctic study area of the ongoing project CASSANDRA (who is the twin sister of ELENO in Greek mythology; PI Maurizio Azzaro), operating in the Greenland Sea in the historical transect at 75° N and funded by the Italian Arctic Research Program (PRA) and part of the Synoptic Arctic Survey (SAS; https://synopticarcticsurvey.w.uib.no/). The project included chemical-physical, biogeochemical and biological sampling at selected stations on the outward journey from the Svalbard Islands to the North Pole and on the return journey from the latter to Iceland (Figure 1). ELENO pursued 3 key foci: A) Physical and biogeochemical state; B) Ecosystem response; C) Carbon cycle. The aims of the focal areas are: A1) Characterize the thermohaline properties and the biogeochemistry of water masses; B1) Evaluate microbial abundances and diversity; B2) Quantify how remineralization processes varies in relation to the nutrient availability; B3) Estimate the biomass flux in the analyzed ecosystems; B4) monitor Arctic mammal populations to assess their conservation status; C1) Quantify the input and fate of organic carbon.

To these initial project objectives, the quantification of microplastics in the sea and the standing stock, functionality and biodiversity of the microbial component of the bioaerosol were added, given the importance of being able to access this unique and exclusive habitat up to the North Pole.

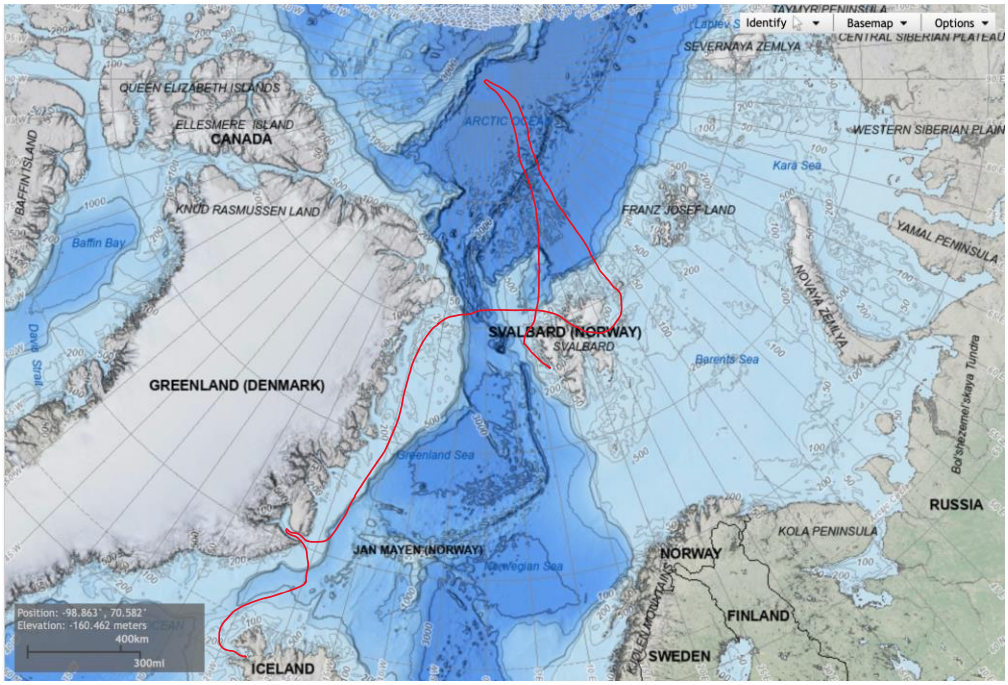

Fig. 1 Working area and track chart (red line) of R/V LE COMMANDANT CHARCOT during the oceanographic cruise ELENO.

2. Narrative of the Cruise

In total, the following activities were carried out during the entire cruise: 9 hydrological stations at different depths along the water column (Tab. 1), 10 bioaerosol samplings from the bow of the ship (Tab, 2), 15 hydrological samplings from peristaltic pump at a depth of 9 m (Table 3), a sea ice core at the North Pole and sightings of marine mammals lasted over 90 hours, providing a detailed picture of the presence and behavior of these species during navigation up to the North Pole.

August 26th 2023

The ELENO team boarded the vessel on August 26 at 12:00 AM (CEST) from the harbour of Longyearbyen. In the afternoon, safety protocols and briefings on emergency procedures took place. Personal protective equipment were distributed and the presence on board the ship was confirmed. A comprehensive safety training session followed, covering evacuation procedures and guidelines for polar conditions. An illustrative tour of the vessel was also carried out from the laboratories, to the work areas on the decks, and in general to all the common areas of the vessel.

After the safety checks, the team prepared scientific equipment and sampling equipment. Calibration and inspection were meticulously performed to ensure optimal functionality under polar conditions.

The setup of the laboratories (Dry Lab and Wet Lab) began on the afternoon of August 26th, after the delivery of the boxes of materials sent for the oceanographic expedition. Scientific sampling protocols included seawater collection of water from hydrological stations, seawater sampling for micro- and nano-plastics at discrete locations from the vessel's peristaltic pump (9 m depth; Ferrybox, on-board equipment), the sampling of bioaerosols from atmospheric samples, sea ice sampling, and the continuous observation of Arctic mammals.

On August 26, 2023, the ship set sail from the harbour of Longyearbyen at 16:00 PM (UTC) and the first sampling of seawater was carried out from the Ferrybox (16:26 PM, UTC) for the determination of the micro- and nanoplastics present, including the continuous acquisition of hydrographic parameters (Temperature, Salinity, Oxygen, CO_2) at a depth of 9 meters (P1; Table 3). The seawater was immediately filtered after sampling under the laminar flow hood and three replicates of the samples were stored together with blanks until the analysis which will be carried out in the laboratories of the Institute of Polar Sciences of Venice. For each sampling carried out during the oceanographic cruise the same procedure was repeated.

Each day of the cruise, the team convened for an evening briefing, engaging in discussions about the day's activities and collaborative planning for the next. Challenges and adjustments were addressed collaboratively, with seamless coordination between the ELENO team and the onboard scientific personnel. This cooperative approach ensured a harmonious integration of scientific efforts, maximizing the effectiveness of data collection and research activities throughout the expedition.

August 27th, 2023

During the morning, a briefing was held with the Captain of the Vessel and the Science staff dedicated by Ponant to plan and agree on the scientific activities that the Eleno project had to implement during the oceanographic cruise. The first Biaerosol sampling was done in the morning (EB1; Table 2) and the setup of the laboratories was completed on the 27th at midday. The bioaerosol samples were pre-treated immediately after sampling and stored (+4°C and -20°C) until the analyses which will be carried out in the laboratory of the Institute of Polar Sciences of Messina. In the afternoon the first hydrological station was made by Niskin bottles and the CTD up to 200 m depth (E1; Table 1). Water samples were collected, and depending on the oceanographic measurement processed, filtered, pre-treated, fixed and cataloged for subsequent analysis which will be performed in the laboratories of the Institute of Polar Sciences of Venice and Messina. On the same day in the evening the second sample was also taken from the Ferrybox. During this day a sighting of a group (3-5 specimens) of Harbour Porpoise (*Phocoena phocoena*) was made.

August 28th, 2023

On this day, as on the previous one, a Biaerosol sampling, a hydrological station and a sampling from the Ferrybox were carried out (Table 1, 2 and 3), as well as the related sample treatments which also continued during the night of the following day. No cetacean sightings occurred.

Furthermore, Dr. Maurizio Azzaro presented to passengers the activities planned for the scientific data collection campaign as part of the ELENO project in the vessel's theater.

August 29th, 2023

During this day, a Biaerosol sampling and a sampling from the Ferrybox were carried out (Table 2 and 3).The hydrological station was carried out in the early hours of the following day (Table 1). No cetacean were observed.

August 30, 2023

During the first hours of August 30th a hydrological station was made (up to 200 m deep), then during the day the vessel arrived at the north pole and the ELENO Team carried out a sampling of bioaerosol, a sampling of seawater at the Ferrybox and another hydrological station (up to 1000 m depth) (Table, 1, 2 and 3). This was certainly the most complex day during the oceanographic cruise in terms of activities and hours spent processing the samples and two sightings of Bearded Seal (*Erignathus barbatus*) and Polar Bear (*Ursus maritimus*) were made.

August 31st, 2023

On this day a sampling of bioaerosol, a sampling of seawater at the Ferrybox and a sighting of Bearded Seal (*Erignathus barbatus*) were made. Additionally, an ice core (Latitude 89°55'10.34" N; Longitude 87°52'8.45" E) was sampled for bromine studies and DNA analysis.

September 1st, 2023

On this day a Biaerosol sampling and a hydrological station were carried out (Table 1 and 2). No cetacean sightings occurred on this day. Moreover, a scientific lecture entitled "Navigating the Anthropocene: the Climate Crisis and Human Responsibility", aimed at tourists, was given by Prof. Carlo Barbante.

September 2nd, 2023

During this day, a Biaerosol sampling and a sampling from the Ferrybox were carried out (Table 2 and 3). No cetacean sightings were made on this day.

September 3rd, 2023

On this day, a Biaerosol sampling, a hydrological station and a sampling from the Ferrybox were carried out (Table 1, 2 and 3). During this day a sighting of a Polar Bear (*Ursus maritimus*) was recorded.

September 4th, 2023

On this day, as on the previous one, a Biaerosol sampling, a hydrological station and a sampling from the Ferrybox were carried out (Table 1, 2 and 3). During this day the sighting of 2 specimens of Killer Whale (*Orcinus orca*) and a Minke Whale (*Balenoptera spp.*) was made.

September 5th, 2023

On this day, as on the previous one, a Biaerosol sampling a hydrological station and a sampling from the Ferrybox were carried out (Table 1, 2 and 3). No cetacean sightings were recorded on this day.

Once we arrived in Ny-Ålesund (Svalbard Islands), we delivered most of the samples taken during the oceanographic cruise and the Bioaerosol sampler to the Italian logistics of the Institute of Polar Sciences for sending to the laboratories of Messina and Venice in Italy.

September 6th, 2023

During this day, two seawater samples were taken from the Ferrybox (Table 3) and no cetacean sightings were recorded. Moreover, a scientific lecture entitled "Written in Ice: Revealing Secret Messages from the Past", aimed at tourists, was given by Prof. Carlo Barbante.

September 7th, 2023

On this day, two seawater samples were taken from the Ferrybox (Table 3) and no cetacean sightings occurred.

September 8th, 2023

On this day, a hydrological station and a sampling from the Ferrybox were carried out (Table 1 and 3). No cetacean sightings were recorded.

September 9th, 2023

During this day, the last two seawater samples were taken from the Ferrybox (Table 3) and no cetacean sightings were recorded. On this day, having finished processing the samples, all the scientific equipment was placed in the boxes and the samples, which had to return on September 10th with the scientific staff in Italy, were arranged. The boxes of materials were left on the Vessel for the "TEMPLE LIFE" oceanographic cruise which will be conducted in January 2024 in Antarctica.

To provide insights to the passengers and crew of the vessel on life and scientific activities in Antarctica, on 9 September a live video call from the Vessel Theater with the Italian-French Concordia Station in Antarctica was organized by Prof. Carlo Barbante. During this session, the scientific staff of the Concordia Station shared with the "Le Commandant Charcot" staff and passengers insights into their daily routine, sampling techniques and the main scientific activities conducted by the Italian and French team at the Antarctic Station.

Furthermore, on the same day Dr. Maurizio Azzaro illustrated in the Vessel theater the activities carried out during the cruise period as part of the ELENO project.

September 10th, 2023

The cruise concluded in Reykjavík on September 10, 2023, marked by the disembarkation of both scientific personnel and tourists from the vessel “Le Commandant Charcot”. The oceanographic cruise ended on this day in Reykjavík. The ELENO scientific team landed at 12pm with the samples to return to Italy.

3. Station List

The hydrological stations (Niskin bottles, CTD) conducted during the ARICE program are listed below in Table 1:

TABLE 1	Date	Time	Latitude	Longitude	Water Depth	Gear	Remarks/Recovery
Station No.	**2023**	**[UTC]**	**[°N]**	**[°E]**	**[m]**		**Depth (m)**
E1	27.08	16:54	81°2'53.80"	18°15'15.96"	-279	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E2	28.08	19:28	84°8'28.66"	38°26'30.31"	-3935	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E3	30.08	01:21	87°59'3.36"	55°6'27.81"	-4339	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E4	30.08	14:32	89°59'14.51"	74°27'25.36"	-4207	Niskin/CTD	-1; -5; -20; -40; -100; -200; -500; -750; -1000 m
E5	01.09	23:23	86°1'20.25"	34°55'23.40"	-3508	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E6	03.09	05:36	80°55'44.54"	29°41'54.24"	-246	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E7	04.09	07:07	79°10'41.24''	19°29'16.90''	-135	Niskin/CTD	-1; -5; -20; -40 m
E8	05.09	18:16	79°00'12.14''	11°26'30.95''	-226	Niskin/CTD	-1; -5; -20; -40; -100; -200 m
E9	08.09	13:05	70°28'29.47''	21°58'42.27''W	-59	Niskin/CTD	-1; -5; -20; -40 m

The bioareosol samples were collected daily using a commercially available cyclonic collector (Coriolis®μ cyclonic system air sampler), which was placed in front of the vessel's bridge, only when the sampler was not downwind of the ship's exhaust to avoid contamination.

The bioaerosol sampling was conducted with the vessel in motion to filter the air over a wide geographic area of reference. Therefore, the table containing information on the sampling will include both the start and end coordinates, reflecting the dynamic nature of the sampling process.

The bioaerosol samplings carried out during the ELENO cruise are listed below in Table 2:

TABLE 2	Date	Time Start	Time End	Latitude – Start	Longitude - Start	Latitude - End	Longitude – End
Station No.	**2023**	**[UTC]**	**[UTC]**	**[°N]**	**[°E]**	**[°N]**	**[°E]**
EB1	27.08	08:39	11:16	79°19'40.72''	10°03'16.65''	79°57'31.46''	11°00'58.97''
EB2	28.08	12:46	15:16	83°29'20.12''	33°24'47.34''	83°57'11.25''	35°01'42.73''
EB3	29.08	13:34	16:17	86°21'26.41''	45°03'30.01''	86°49'17.38''	46°38'44.24''
EB4	30.08	12:43	15:00	89°33'53.67''	57°17'59.87''	89°50'49.53''	62°38'55.78''
EB5	31.08	12:42	14:55	89°58'36.57''	86°41'15.52''	89°58'36.57''	86°41'15.52''
EB6	01.09	12:10	14:03	87°17'24.05''	32°05'51.92''	87°05'27.99''	32°19'39.18''
EB7	02.09	13:33	15:47	84°05'30.68''	36°52'18.21''	83°40'49.57''	35°23'13.10''
EB8	03.09	13:07	15:23	80°05'53.70''	31°20'09.26''	80°05'53.70''	31°20'09.26''
EB9	04.09	13:05	15:16	79°20'02.23''	19°36'16.65''	79°20'02.23''	19°36'16.65''
EB10	05.09	06:25	08:38	79°20'17.01''	11°41'40.40''	79°20'17.01''	11°41'40.40''

The stations of surface samples (9 m depth) taken with pertistalc pump (Ferrybox) for the analysis of microplastics are listed below in Table 3:

TABLE 3 Station No.	Date	Time Start [UTC]	Latitude	Longitude
P0	26.08.2023	20:26	78° 13' 49,02" N	15° 37' 45,24" E
P1	27.08.2023	18:55	81° 2' 53,80" N	18° 15' 15,96" E
P2	28.08.2023	11:18	83° 14' 9,71" N	32° 32' 32,65" E
P3	29.08.2023	12:55	86° 17' 56,57" N	45° 23' 47.67" E
P4	30.08.2023	23:00	89° 59' 14,51" N	74° 27' 25,36" E
P5	02.09.2023	16:18	83° 34' 14,80" N	32° 26' 23,02" E
P6	03.09.2023	14:09	80° 5' 52,49" N	31° 20' 6.61" E
P7	04.09.2023	07:07	79° 10' 41,24" N	19° 29' 16.90" E
P8	05.09.2023	06:06	79° 20' 15,82" N	11° 41' 36.03" E
P9	06.09.2023	06:08	78°15' 51,41" N	15° 20' 35.92" E
P10	06.09.2023	18:58	77°19' 8,72" N	6° 16' 58.37" E
P11	07.09.2023	07:23	75°34' 35,72" N	4° 41' 50.24" W
P12	07.09.2023	18:05	73° 37’ 23,73” N	12° 58’ 40,83” W
P13	08.09.2023	07:28	70° 40’ 18,25” N	20° 40’ 33,13” W
P14	09.09.2023	10:44	66° 29’ 20,39” N	24° 50’ 51,89” W
P15	09.09.2023	19:07	64° 33’ 6,13” N	23° 27’ 10,22” W

Mammal sightings protocol

The monitoring of marine mammal sightings spanned over 90 hours, providing a detailed overview of the presence and behavior of these species throughout the polar exploration. A Dedicated Observer (DO) was stationed on one sides of the Observatory Lounge on Deck 9th of the “Le Commandant Charcot” vessels and continuously collected data on mammal presence from both sides. DO switched sides every 1-2 hours to prevent fatigue. DO collected data on mammals presence in "passing mode," which entailed continuous search efforts without approaching schools or animals, following the distance sampling protocol. DO primarily focused on a 130° arc in front of the ship and continuously scanned the area with the naked eye, occasionally using binoculars for scanning. The rear of the route was scanned only occasionally to avoid the risk of double-counting sightings. All data pertaining to the vessel's track (position, speed, and heading) were recorded form the onboard monitors present over all the Decks of the vessel. During sightings, data were collected using the "sight data collection sheet" (Weather; Sightings; Other Species). For each sighting, information on species, number of individuals, possible presence of juveniles, behaviour, and vessel presence were recorded. Sightings reported by crew members, trainee observers, or other non-DO individuals were recorded only if confirmed by the DO.

Binoculars and photographs were used to confirm sightings and determine species and group size. Unidentified species were registered as "US" followed by the designation "large whale species" (L), "medium cetacean species" (M), or "small dolphin species" (S).

Effort data were analyzed in terms of time (hours) spent and/or distance traveled (nautical miles or kilometers) during observations in good weather conditions (Beaufort ≤ 3). Beaufort scale assessments were based on visual observations and wind speed ranges. Distance traveled was generally preferred for coherence with spatial analysis.

Below is the weather data acquisition (ANNEX I) and sighting form for marine mammals and other species (ANNEX II) used in this observation campaign on board of "Le Commandant Charcot":

ANNEX I

Fixed Line Transect using ferries as platform of observation - Monitoring protocol I

Annex I Data collection sheet: meteo condition

Data collection sheet: Meteo

COD_Transect N. **Date** **Ship name** **Observers**

COD GPS	Time	Effort	Sea state	Wind direction	Rain	Visibility	Cloud cover	Lat	Long	Route	Speed	Other
		BEG=beginning off effort STOP=start of off effort START=start effort; END=end effort;			Mist, Fine, Drizzle	(optimus, good, mean, scarce)	%	Y	X			(es. predator fishing, fishing ship, naval traffic..)

Sea state	Wind (KN)	Description
0	0	Calm (glassy)
1	1-3	Calm (rippled)
2	4-6	Smooth (wavelets)
3	7-10	Slight
4	11-16	Moderate
5	17-21	Rough
6	22-27	Very rough
7	28-33	High
8	34-40	Very high
9	41-47	Phenomenal
10	48-55	Storm

Other:

! Remember the Naval traffic sheet!

ANNEX II

Fixed Line Transect using ferries as platform of observation - Monitoring protocol I

Annex II Data collection sheet: sightings

Data collection sheet: Sightings

COD Transect N. **Date** **Ship name** **Observers**

N/COD GPS	Time	Ship position		Side	Obs	Species	N° Tot			N° Juv	Distance	Angle	Direction of swim	Responce to ship			Behaviour	Ph	Collision or Near collision	Ships (see sheet)
		Lat (Y)	Long (X)				Min	Max	Best			(0-180°)	(0-360°)	Apr	Esc	Indif				

Behaviour

Behaviour: Travel, Rest, Play, Feeding Wild, Feeding Net, Mating, Unknown

Superficial: Half Leap, Full Leap, Dorsal Fin, Surfing, Blow, Breach, Spyhopp

Speed: Slow, Normal, Fast, Porpoising, Floating

Progress: Straigth, Irregular, None, Zig-zag

Direction: Same, Different, Circle

Group association (i.e. 2+2; 2+2+1)

Type: Males, Fem/juv, Mother/calves

Unidentified Species (US):

L LARGE "whale" species

M MEDIUM "cetacean" species
- **DD** Distinct Dorsa
- **ID** Indistint Dorsal
- **LF** Large Fin
- **SF** Small Fin

S SMALL patterned "dolphin" species

4. Preliminary Results

4.1 Water and Plankton Sampling

4.1.1 CTD Measurements, Seawater Sampling and Plankton Sampling

During the cruise seawater samples were taken at 9 stations (Tab.1, Tab. 4 and Tab. 5) at fixed depths. Sampling was carried out using Niskin bottles (8L capacity) and a CTD connected to a nautical rope and by the use of a winch. The water samples, once taken were pretreated on board for the analysis concerning the project, namely nutrients (Ammonium, NH_4; Nitrite, NO_2; Nitrate, NO_3; Phosphate, PO_4; dissolved inorganic nitrogen (DIN) and phosphorus (DIP); Total Phosphorous/Nitrogen, TN-TP), viral abundance (Virus; Cytometry), prokaryotic abundance and biomass (DAPI; Cytometry, Epifluorescence Microscopy), Bacterial Viability (Live/Dead), size-fractionated Chlorophyll *a* (CHL *a*; 0.2-2µm; 2-10µm; 10-200µm; spectrofluorimetric analysis), Phytoplankton diversity and abundance (Phytoplankton; Phytoplankton net), molecular detection of the prokaryotic phylogenetic composition and metabolic potential (DNA/RNA; next generation sequencing), Cultivable Bacteria, respiring cells (CTC), Metabolic profiles (Biolog), Particulate and dissolved organic matter remineralization (ETS). In addition, CTD probe recorded physical-chemical parameters (temperature, conductivity, pressure, oxygen, turbidity).

Water samples for determining the possible presence of Microplastics, Microfibers and Plastic additives were also sampled both by sampling from Niskin bottles (Tab. 1, Tab. 4) and by peristalitic pump (Ferrybox, Tab. 3). In both cases, the samples were filtered in triplicate and stored at temperature + 4 ºC.

4.1.2 First results

The water samples collected are yet to reach the workplace in Italy and will be analyzed upon delivery. The only preliminary results presented here pertain to enzymatic activity, as these samples were processed directly onboard the ship. The preliminary results obtained by the measurements of the enzymatic activities (leucine aminopeptidase, beta-glucosidase and alkaline phosphatase) provide information on the metabolic patterns of the microbial community. A significant spatial variability characterized the patterns of all the three enzymes, suggesting a patchy distribution of organic substrates used by microbes as a trophic resource. Leucine aminopeptidase activity, as a marker of proteolytic activity, was the predominant activity, suggesting that labile compounds referable to proteins were bioavailable and actively decomposed, especially at stations E1 and E6, with peaks at a 40 m depth. Alkaline phosphatase activity, detected at rates about two orders of magnitude lower than aminopeptidase, indicated that the mineralization of organic phosphates mediated by microbes was also an active process. Phosphorus regeneration was particularly fast at depths at station E2 and E6. Comparatively low levels of beta-glucosidase (with values generally lower than 1 nmol/l/h) were recorded, showing that polysaccharides were decomposed slowly. High glycolytic activity was recorded at stations E4-E5 and E6.

4.2 Bioaerosol Sampling

4.2.1 Airborne microbes sampling

The airbone microbes sampling were collected daily for the first 10 days of the cruise, using a commercially available cyclonic collector (Coriolis®µ cyclonic system air sampler), which was placed vessel's bow, only when the sampler was not downwind of the ship's exhaust to avoid contamination. The air sampling flow rate and duration were 300 L/min and 10 min, respectively. Air was aspirated twelve times into a sterile cone filled with 15 ml of sterile phosphate buffer saline (PBS 1x). The samples collected were

pretreated for the parameters regarding viral component, prokaryotic abundance and biomass, phylogenetic composition, Cultivable Bacteria, respiring cells (CTC), Metabolic profiles (Biolog), Bacterial Viability (Live/Dead).

Similar to the water samples, the bioaerosol samples have not yet arrived at the workplace and will be analyzed upon delivery. Results for the bioaerosol samples are pending, as they are still in transit.

Table 4

		Nutrients						Plastic		
Station	Depth	NH4	NO2	NO3	PO4	DIN-DIP	TN-TP	Microplastics	Microfibers	Plastic additives
E1	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E2	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E3	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E4	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
	500	x	x	x	x	x	x			
	750	x	x	x	x	x	x			
	1000	x	x	x	x	x	x			
E5	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E6	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E7	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x	x	x	x
E8	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			
	100	x	x	x	x	x	x			
	200	x	x	x	x	x	x	x	x	x
E9	1	x	x	x	x	x	x	x	x	x
	5							x	x	x
	20	x	x	x	x	x	x	x	x	x
	40	x	x	x	x	x	x			

Table 5

		Standing Stock					Microbial Activities						
Station	Depth	Virus	DAPI	CHLa	Phytoplankton	Phytoplankton net	DNA RNA	Cultivable Bacteria	CTC	Live Dead	ETS	Biolog	EEA
E1	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E2	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E3	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E4	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
	500	x	x				x	x	x	x	x	x	x
	750	x	x							x	x	x	x
	1000	x	x				x	x	x	x	x	x	x
E5	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E6	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E7	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
E8	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x
	100	x	x	x	x					x	x	x	x
	200	x	x				x	x	x	x	x	x	x
E9	1	x	x	x	x		x	x	x	x	x	x	x
	5												
	20	x	x	x	x	0-20 m				x	x	x	x
	40	x	x	x	x		x	x	x	x	x	x	x

4.3 Mammal Observation

4.3.1 Observation Effort and Sightings

The sampling effort for this study spanned 14 days and was primarily concentrated in the morning, in accordance with the other on board activities and data collection requirements of the campaign. During this study, we conducted over 90 hours of observation effort.

In total, we recorded six sightings of the following species during the observation effort (Fig. 5):

1. Harbour Porpoise (*Phocoena phocoena*)
2. Bearded Seal (*Erignathus barbatus*)
3. Polar Bear (*Ursus maritimus*)
4. Bearded Seal (*Erignathus barbatus*)
5. Polar Bear (*Ursus maritimus*)
6. Killer Whale (*Orcinus orca*)
7. Minke Whale (*Balenoptera spp.*)

In the observed instances, all mammals exhibited travel behavior, consistently refraining from any alterations or adjustments in response to the presence of the ship. Furthermore, the duration of mammalian sightings was brief, precluding the delineation of additional stereotyped behaviors, except in the case of polar bear sightings at the geographical North Pole. In this particular scenario, the animal engaged in exploration around the stationary vessel for a few hours before ultimately departing.

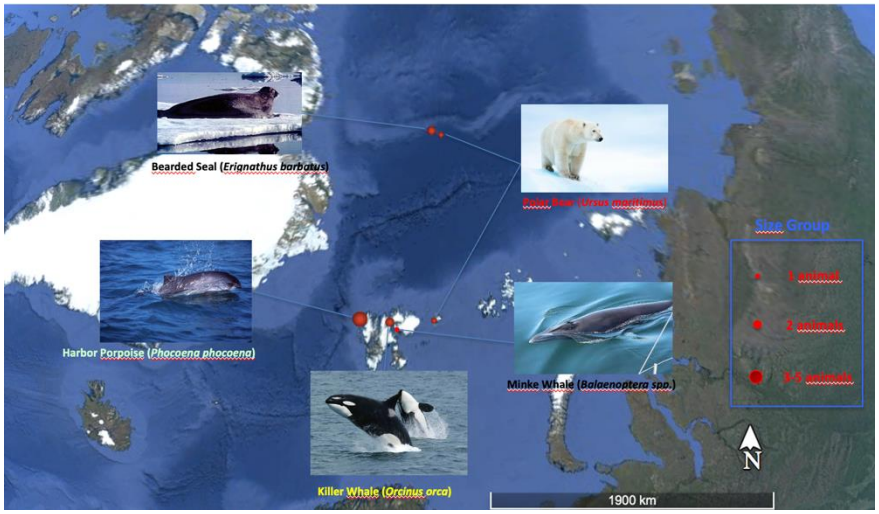

Fig. 5 The map above illustrates where sightings of marine and terrestrial mammals were made during data collection campaign.

Below, a summary table of all the sightings made during this period is provided, along with relevant information. Please note that the table above provides a summary of the sightings made during the observation effort, including species identification, the number of individuals, the presence of juveniles, behaviour exhibited, date and time of each sighting in Coordinated Universal Time (UTC), and the corresponding coordinates (latitude and longitude)(Tab. 6).

Tab. 6 Sightings recorded over the ELENO Cruise.

ARTIC REGION

Survey: ELENO Project on "Le Commandant Charcot" Route: Geographical North Pole

Operator/: Francesco Filiciotto

LEGEND:

Behaviour: Milling,Resting,Socializing,Travelling,Feeding.

	Wheater condition								MAMMAL SIGHTINGS								
				Wind								Behaviour					
Day	Time UTC	Lat. N	Long. E	Sea condition	C. nuvol.	Dir.	Speed (kts)	Angle sight	Distance sight	N. TRANSECT	Sighting n°	species	sex	N. min/ N. max	N° tot animals	M	R	S	T	F
27/08/23	08:42	79° 19'40,72"N	10° 3'16,65"E	calmo	coperto	196°		30°	150 m	1	1	Harbour Porpoise (Phocoena phocoena)		from 3 to 5	from 3 to 5					
30/08/23	22:05	89° 59'14,51"N	74° 27'25,36"E	calmo	coperto	127°		180°	50 m	4	2	Bearded Seal (Erignathus barbatus)			1					
30/08/23	23:00	89° 59'14,51"N	74° 27'25,36"E	calmo	coperto	127°		90°	150 m	4	3	Polar Bear (Ursus maritimus)	F		1					
31/08/23	22:00	89° 59'14,51"N	74° 27'25,36"E	calmo	coperto	265°		0°	200 m	5	4	Bearded Seal (Erignathus barbatus)			1					
03/09/23	10:59	80° 5'55,43"N	31° 20'35,28"E	poco mosso	coperto	124°	19	45°	1 km	8	5	Polar Bear (Ursus maritimus)	M		1					
04/09/23	17:35	79°40'57.14"N	18° 23'10,29"E	poco mosso	coperto	134°	10	90°	500 m	9	6	Minke Whale			1					
04/09/23	18:20	80° 9'30.98"N	17° 2'25.80"E	poco mosso	coperto	107°	8	90°	200 m	10	7	Killer whale(Orcinus orca)			2					

5. Data and Sample Storage / Availability

The hydrological stations and Arctic mammal sightings are accessible on the ArcGIS server at the following link https://www.arcgis.com/apps/mapviewer/index.html?webmap=2071003f78e74de89c5d2a4e57bab183. The website is currently undergoing updates, and information will be added gradually as samples are analyzed and results are graphed.

ArcGIS is a Geographic Information System (GIS) platform developed by Esri, offering a wide range of functions and capabilities for managing, analyzing, and visualizing geographic data. Here are some of its key features:

ArcGIS allows interactive visualization of maps and geographic data, providing zoom, pan, and query tools for exploring geospatial information. The platform provides various tools for conducting spatial analysis, such as layer overlay, buffer creation, pattern identification, and modeling complex geographic phenomena. Users can create customized maps with specific symbols, labels, and layouts to effectively communicate geospatial information. ArcGIS supports three-dimensional analysis and visualization, allowing users to explore geospatial data in more detail. In general, ArcGIS is a powerful platform that equips users with tools to fully leverage the potential of geographic data, facilitating location-based decision-making and enhancing understanding of spatial phenomena. In our case, through the portal, users can view the geographic locations of the water sampling stations, containing key information about the sampling. Regarding mammal sightings, the portal displays geographic points with the corresponding observed species.

All data collected during the cruise will be uploaded and stored on Italian Artic Data Center (IADC), link: https://metadata.iadc.cnr.it

6. Participants

No.	Name	Early career (Y/N)	Gender	Affiliation	On-board tasks
1	Maurizio Azzaro	N	M	ISP-CNR	Principal Investigator, Nutrients, Microbial respiration, Chlorophyll a, CTD, Phytoplankton net
2	Carlo Barbante	N	M	ISP-CNR	Sampling and initial treatment of Microplastics, CTD, Phytoplankton net
3	Francesco Filiciotto	N	M	ISP-CNR	Water samples collection, Mammal observations, CTD, Bioaerosol sampling
4	Alessandro Ciro Rappazzo	Y	M	ISP-CNR	Viral abundance, Microbial Abundance and Biomass, CTC, Live/Dead, Enzymatic activities, Microbial diversity, Cultivable bacteria, CTD, Bioaerosol sampling

ISP-CNR: Institute of Polar Sciences – Italian National Research Council

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