SWEDISH POLAR RESEARCH SECRETARIAT

POLARFORSKNINGSSEKRETARIATET

EXPEDITION REPORT

SWEDARCTIC
Synoptic Arctic Survey 2021

with icebreaker Oden

COMPILED BY
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Table of Contents

List of Abbreviations

ACAS	"Arctic climate across scales", a research project on board the SAS-Oden 2021 expedition (WP16)
ASAP	"Adaptive strategies of Arctic prokaryotes at extremely low growth rates", a research project on board the SAS-Oden 2021 expedition (WP5)
AWI	Alfred Wegener Institute, Bremerhaven (Germany)
CAO	The Central Arctic Ocean Large Marine Ecosystem (LME) as defined by the Arctic Council (see *Figure 3.1*). Note that the term "central Arctic Ocean" (lower case "c") may not refer to the same geographical boundaries as those defined for the CAO LME
CATCHEM	"Carbon and tracer chemistry in the Arctic Ocean", a research project on board the SAS-Oden 2021 expedition (WP10)
CDOM	Coloured (or chromophoric) dissolved organic matter
Chl*a*	Chlorophyll-*a* concentration
ChlMax	The chlorophyll maximum in the water column as determined by a fluorometer on the CTD (range 12-45 m, average 28 m of depth during the SAS-Oden 2021 expedition)
CINEA	European Climate, Infrastructure and Environment Executive Agency
CTD	Conductivity, temperature, and depth measuring device
CTD bio SAS	CTD with water sampling mainly for SAS biological parameters and acoustic measurements for WP1 (EFICA), down to 1000 m
CTD ChlMax PICO	CTD taken specifically for WP6 (PICO), down to the chlorophyll maximum
CTD deep SAS	CTD with water sampling mainly for SAS chemical parameters, down to the seafloor
CTD deep VACAO	CTD taken specifically for WP11 (VACAO), down to the seafloor
CTD EK80 SAS	CTD taken specifically for EK80 calibration for WP1 (EFICA) and WP14 (MWA), down to 400 m
CTD omics SAS	CTD with water sampling for SAS RNA and DNA analyses and acoustic measurements for WP1 (EFICA), down to 1000 m
CTD shallow SAS	CTD with water sampling mainly for SAS chemical parameters in surface waters to complement a full-depth profile together with a CTD deep SAS taken immediately after the CTD shallow SAS
CTD test	CTD cast for testing functionality
CTD wbat EFICA	CTD taken specifically for WBAT acoustic measurements for WP1 (EFICA), down to 1000 m, in combination with project-specific water sampling for all projects needing water
CUT	Chalmers University of Technology, Gothenburg (Sweden)
CW	Continuous Wave mode of the EK80 echosounder
DG MARE	Directorate-General for Maritime Affairs and Fisheries of the European Commission
DIC	Dissolved inorganic carbon
DO	Device Operation = a winch operation from the ship or an ice station (listed in *Table 5.3*)
DOC	Dissolved organic carbon
DSL	Deep scattering layer, a layer of living organisms (zooplankton, small fishes) at mesopelagic oceanic depths detected as acoustic backscatter by an echosounder
ECD	Electron capture detector
eDNA	Environmental DNA, i.e., genetic material obtained directly from environmental samples without any obvious signs of biological source material
EEZ	Exclusive Economic Zone
EFICA	European Fisheries Inventory in the Central Arctic Ocean (an EU-financed research consortium) and "Ecosystem mapping in the Central Arctic Ocean during the SAS-Oden Expedition", a research project on board the SAS-Oden 2021 expedition (WP1)
EK80	A wideband scientific echo sounder manufactured by Kongsberg, Norway
EMFF	European Maritime and Fisheries Fund
EU	European Union
FCM	Flow cytometry

FORAM	"Subpolar planktonic foraminifera invaders in the modern Arctic Ocean water column planktonic foraminifera biology", a research project on board the SAS-Oden 2021 expedition (WP9)
FSW	Filtered seawater
GC	Gas chromatograph
GEOMAR	Helmholtz Centre for Ocean Research (Germany)
GU	University of Gothenburg (Sweden)
Heli	Helicopter
Heli station	Sampling station reached by helicopter in the vicinity of the ship (within a radius of ca. 1 nautical mile)
HPLC	High-performance liquid chromatography
HUG	Heidelberg University (Germany)
IB Oden	Icebreaker *Oden*
IASC	International Arctic Scientific Committee
IBCAO	International Bathymetric Chart of the Arctic Ocean
ICES	International Council for Exploration of the Sea
ICSU	International Council for Science
IRMS	Isotope-ratio mass spectrometry
LADCP	Lowered Acoustic Doppler Current Profiler for measuring current speed
LFM Up	Broadband mode of the EK80 echosounder in which the transmitted pulse starts with the lower frequency in the range, and ends with the upper frequency.
LME	Large Marine Ecosystem – a concept used in research and management strategy for living marine resources (Sherman K, 1991: Ecological Applications 1:349-360)
LNU	Linnaeus University, Kalmar (Sweden)
LOKI	Light frame On-site Key species Investigation
MBA	The Marine Biological Association of the United Kingdom
MBES	multibeam echosounder on the hull of IB Oden
MIK net	Midwater Ringnet (ICES standard gear for the sampling of fish larvae)
MIME	"Microbial Metabolism", a research project on board the SAS-Oden 2021 expedition (WP2)
Mini FishCam	Deep-sea camera system deployed by the EFICA scientists during SAS-Oden
MOSAiC	Multidisciplinary drifting Observatory for the Study of Arctic Climate (an international drift expedition with the German research ice-breaker Polarstern in 2019-2020)
MWA	"Midwater acoustics", a research project on board the SAS-Oden 2021 expedition (WP14)
PHYTO	"Responses of pelagic and sympagic primary producers to climate change in the Central Arctic Ocean", a research project on board the SAS-Oden 2021 expedition (WP7)
PI	Principal Investigator = the person who had applied to the SPRS for berths on the SAS-Oden 2021 expedition and is responsible for the project
PICO	"Ecosystem contributions of smallest primary producers in a changing Arctic Ocean", a research project on board the SAS-Oden 2021 expedition (WP6)
PML	Plymouth Marine Laboratory (UK)
POC	Particulate organic carbon
PON	Particulate organic nitrogen
POP	Particulate organic phosphorus
PP	Primary production
ProMis	"Production and export of phytoplankton-derived organic matter in the changing Arctic Ocean – Role of parasites, saprotrophs and mineral ballasting", a research project on board the SAS-Oden 2021 expedition (WP3)
RAID	Redundant array of independent disks, which is a way of storing the same data in different places on multiple hard disks or solid-state drives to protect data in the case of a drive failure
SAS	Synoptic Arctic Survey = a scientist-driven initiative aiming at collecting primary ecosystem data in the Arctic Ocean 2020-2022: Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

SAS-Oden 2021	Swedish Synoptic Arctic Survey expedition with *IB Oden* in 2021 (abbreviation code SO21)
SBP	Sub-bottom Profiler on the hull of *IB Oden*
SLU	Swedish University of Agricultural Sciences
SND	Swedish National Data Service (https://snd.gu.se/en), the data repository where most of the SAS-Oden 2021 expedition data are available. Data not in the SND are available in other data repositories specified in this report
SO21	Abbreviation code for "Swedish Synoptic Arctic Survey expedition with *IB Oden* in 2021"
SO21 omics	The collaboration within the SAS-Oden 2021 expedition on metagenomics and metatranscriptomics
SO21 project	One of the 16 SAS-Oden 2021 research projects
SO21 station	One of the 60 SAS-Oden sampling stations (36 ship stations and 24 helicopter stations)
SO21 WP	SAS-Oden 2021 Work Package (executing the field work for one of the 16 SAS-Oden 2021 projects)
SOP	Standard Operating Procedure
SPRS	Swedish Polar Research Secretariat (www.polar.se)
SU	Stockholm University (Sweden)
TA	Total alkalinity
TempMax	The temperature maximum in the Atlantic Water Layer (range 200-411 m, average 325 m of depth during the SAS-Oden 2021 expedition)
TGB	" Central Arctic Ocean trace gas biogeochemistry", a research project on board the SAS-Oden 2021 expedition (WP13)
TRACE	"Trace gases cycling in the Arctic marine ecosystem", a research project on board the SAS-Oden 2021 expedition (WP12)
TTD	Transit-time distribution
UCPH	University of Copenhagen (Denmark)
UMU	Umeå University (Sweden)
UNH	University of New Hampshire (USA)
US	University of Stirling (UK)
UVP	Underwater Vision Profiler for profiling of particles (including zooplankton) in the water column
VACAO	"Ventilation and Anthropogenic Carbon", a research project on board the SAS-Oden 2021 expedition (WP11)
VIRUS	"Climate change driven effects on the diversity and activity of polar viruses", a research project on board the SAS-Oden 2021 expedition (WP4)
WAOW	"Why is the deep Arctic Ocean Warming?", a research project on board the SAS-Oden 2021 expedition (WP15)
WBAT	Wideband Autonomous Transceiver
WMR	Wageningen Marine Research, IJmuiden (The Netherlands)
WP	Work Package = the field work carried out on the SAS-Oden 2021 expedition for one of the 16 SAS-Oden 2021 projects
ZOO	"Unravelling biodiversity and production in zooplankton in one of the last blind spots of the Arctic Ocean", a research project on board the SAS-Oden 2021 expedition (WP8)

Acknowledgements

1 Executive summary

The SAS-Oden 2021 expedition (SO21) with icebreaker *Oden*[1] (*IB Oden*) is the Swedish contribution to the international scientist-driven initiative "Synoptic Arctic Survey" (SAS)[2]. SAS will collect primary ecosystem data in the Arctic Ocean in 2020-2022 from both icebreaking and non-icebreaking research vessels. The goal of SAS is to generate a comprehensive dataset that allows for an improved characterisation of the Arctic Ocean with respect to its (1) physical oceanography, (2) marine ecosystems and (3) carbon cycle. The complete SAS dataset will provide a unique baseline that will allow for tracking climate change and its impacts as they unfold in the Arctic region over the coming years, decades and centuries.

The marine ecosystems of the Arctic Ocean are experiencing rapid change. This includes the Large Marine Ecosystem (LME) in the middle, the Central Arctic Ocean (CAO) as defined by the Arctic Council[3], i.e., the deep basins and ridges around the North Pole that until recently were permanently covered by 2-3 m thick sea ice. In the past two decades, up to 40% of the 3.3. km[2] large CAO has been ice-free for a short period in September. This reduction in sea-ice coverage of the CAO is transforming a basically inaccessible marine ecosystem into a new type of ecosystem with seasonal changes in sea ice cover. The CAO is a poorly investigated corner of the World Ocean, especially from an ecosystem perspective, i.e., integrating biological, chemical and physical data. Since the SAS-Oden 2021 expedition was designed as a joint ecosystem study with data collection in an integrated way, the results from this expedition will contribute significantly to the knowledge on ecological baselines of the CAO as well as on ecosystem change. The latter is achieved by comparing areas along the expedition route with different types of sea ice, including both steady and heavily melting multi-year ice.

The SAS-Oden 2021 expedition reached further west on the Greenland shelf than any other research expedition has ever done before. Closest was the geological Lomrog III expedition with *IB Oden* in 2007, but then ice-breaking assistance was needed from the accompanying Russian atomic-driven icebreaker "50 Let Pobedy" to get this far west. Between SO21 stations 42 and 53 (*Figure 1.1*), the SAS-Oden 2021 expedition was in a completely unexplored area. This is, a.o., illustrated by the fact that the seabed map of this area now needs revision; in one place we recorded 900 m depth instead of 300 m on the existing map while in another place it was 1200 m shallower than indicated on the map.

We now have the first on-site measurements of physical environment, carbon cycle and nutrients combined with prokaryote, photosynthetic, zooplankton and fish production and diversity, as well as many other ecosystem parameters, from the area between SO21 stations 30 and 53. The SO21 omics samples form the basis for a unique biodiversity dataset, covering water column, ice habitats, and sediments. These samples include the metagenomes and metatranscriptomes of viruses, archaea, bacteria, protists, and eDNA of multicellular organisms such as zooplankton, fish, squid and mammals.

The SO21 joint ecosystem study was carried out by 38 scientists assisted by 15 persons from the Swedish Polar Research Secretariat (SPRS) and the 22-person *IB Oden* crew. Altogether, the expedition visited 60 sampling stations, of which 36 were ship stations and 24 were ice stations accessed by helicopter (*Figure 1.1, Table 1.1*). Since the main research project on board (EFICA) included nine "EFICA Master Stations", lasting for on average 33 hours during which the ship was lying still, there were ample possibilities for ice work from the ship as well.

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[1] Icebreaker *Oden* [https://www.polar.se/en/research-support/icebreaker-oden/]

[2] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

[3] PAME (2013) Large Marine Ecosystems (LMEs) of the Arctic Area: Revision of the Arctic LME Map. PAME International Secretariat, 28nd ed. [https://oaarchive.arctic-council.org/handle/11374/61]

Figure 1.1: The SAS-Oden 2021 expedition route with the 60 sampling stations (SO21 stations 1-60: 36 ship stations and 24 helicopter stations). The circles show the 36 ship stations, blue for the nine EFICA Master Stations and red for all other stations. The white stars indicate the 16 ice stations, and small white dots indicate the 6 box core stations. The first station number is always a ship station, the second and third station numbers are ice stations at the most 1 nautical mile from the ship accessed by helicopter (cf. Table 1.1). The background map was extracted from IBCAO[4].

Table 1.1: Summary of the 60 sampling stations (SO21 stations 1-60: 36 ship stations and 24 helicopter stations) visited during the SAS-Oden 2021 expedition and the 260 successful Device Operations (sampling activities). Ship station = sampling by winch operations from the ship, Heli station = ice station accessed by helicopter in the vicinity of the ship (within a radius of ca. 1 nautical mile). Blue colour indicates the nine EFICA Master Stations and red colour indicates all other stations. Note: on Station 8 the EFICA beam net could not be used due to strong winds and it was therefore deployed at Station 14 instead, together with an EFICA CTD wbat.

Maximum depth	Device operation	Ship/Heli station	Number of stations	Number of device operations
1000 m	CTD bio	SAS	18	18
1000 m	CTD omics	SAS	18	18
1000 m	CTD wbat	EFICA	11	14
Seafloor	CTD deep	SAS	32	32
Seafloor	CTD deep	VACAO	6	6
100-200 m	CTD EK80	SAS	1	1
100-200 m	CTD shallow	SAS	20	20
ChlMax	CTD ChlMax	PICO	3	3
2000 m	NET multi	SAS	16	17
1000 m	NET multi	FORAM	8	8
200 m	NET bongo	SAS	11	13
1000 m	NET bongo	FORAM	3	3
800 m	NET beam	EFICA	9	45
800 m	BET mik	EFICA	2	5
1000 m	LOKI optics	EFICA	11	11
Seafloor	Box core	SAS	6	8
	Ice station next to ship	SAS+proj	13	13
	Ice station helicopter	SAS	4	4
	Ice station helicopter	EFICA	15	16
	Ice station helicopter	ACAS	5	5
Total number of successful device operations				**260**

Column station headers (Ship station): 0, 1, 3, 5, 7, 8, 11, 13, 14, 16, 18, 20, 22, 24, 25, 26, 28, 30, 32, 33, 35, 37, 38, 40, 41, 42, 44, 45, 46, 47, 48, 50, 52, 53, 56, 58, 60

Heli station headers: 2, 4, 6, 9, 12, 15, 17, 19, 21, 23, 27, 29, 31, 34, 36, 39, 43, 49, 51, 54, 57, 59; (third row) 10, 55

Total number of successful device operations per station (0 → 60): 1, 2, 4, 4, 5, 12, 2, 6, 6, 7, 7, 4, 13, 3, 2, 17, 4, 17, 1, 8, 17, 1, 19, 1, 1, 17, 1, 1, 2, 1, 7, 18, 1, 17, 10, 19, 2 — total 260

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4 Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

The SO21 joint ecosystem study was organised by building bridges between the 16 research projects that were accepted for participation in the expedition by the SPRS based on their expected contributions to SAS, including three projects supported by the ARICE Consortium[5]. The pre-cruise organisation pursued that the 16 projects together would cover as many of the internationally stipulated SAS Core Parameters[6] as possible. The result was that the SO21 joint ecosystem study included all stipulated SAS Core Parameters except for studies on birds and mammals, either by direct onboard measurements or by sampling for later on-shore analyses. Each project had its own onboard "Work Package" (WP), i.e., the field work carried out on the SAS-Oden 2021 expedition for the research project. Some WPs were responsible for many of the SAS Core Parameters, such as WP7 (PHYTO) for biological, WP10 (CATCHEM) for chemical, and WPs14+15 (MWA+WAOW) for physical SAS Core Parameters. Other WPs mainly focussed on project-specific measurements and samplings, but these were fully integrated with the SAS core sampling programme so that all SO21 data, including the project-specific data, can be cross-related. When there was overlap between WPs, the field work was integrated. The most integrated data collections on board were the CTD casts (basically all WPs) and the SO21 omics collaboration between the nine biological WPs (WP1-9) in sampling DNA and RNA.

All data collected during the SAS-Oden 2021 expedition can after the expedition easily be coupled to each other through the "Device Operations" (DOs), i.e., a winch operation from the ship (CTD, net, box core) or an ice station. The DOs were recorded in a Logbook on the bridge that has been quality-assured after the expedition and is available as the excel file "SO21_Expedition_Logbook" in the Swedish National Data Service (SND) data repository[7]. Each DO is given a unique number consisting of the expedition abbreviation (SO21 = SAS-Oden 2021), the station number (1-60) and the cast number (a running number for each DO at the same station). Each DO is in the excel file "SO21_Expedition_Logbook" coupled to date, time, geographical position and ocean depth. Ice stations accessed from the ship are in the Logbook classified as DOs at the ship station while ice stations reached by helicopter each have their own station number since their position slightly deviated from that of the ship. A detailed record of the SAS Core Parameters collected at the 16 ice stations is available as the excel file "SO21_Ice_Station_Logbook" in the SND data repository. During the SAS-Oden 2021 expedition, 260 successful DOs were carried out, nine failed and two were short CTD casts for testing functionality of equipment. The latter 11 operations are included in the Expedition Logbook for completeness, which in total contains 271 DOs.

An overview of the scientific data we collected on the CAO ecosystem is given in this report and the accompanying metadata excel files submitted by each WP at the end of the expedition (*Table 1.2*). These metadata represent a detailed account of all onboard measurements made and all samples taken for later analyses, but no measured values. The "SAS-Oden 2021 Research Data Management Policy" of the SPRS (*Appendix A*), signed by the PIs of all research projects participating in the SAS-Oden 2021, is strictly followed. Most of the SAS-Oden 2021 expedition data and metadata are or will be available in the SND data repository. Data not in the SND are or will be available in other data repositories as specified in this report in the respective WP chapters (*8-22*). Our data collections open many opportunities for future scientific collaborations, within the SAS-Oden 2021 scientific party, within the international SAS programme, and with third parties. Data sharing, with fair attribution, is a corner stone for scientific collaboration, not least for the evolvement of interdisciplinary science, and is also the aim of the SO21 joint ecosystem study. Therefore, it is expected that the data owner

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[5] The Arctic Research Icebreaker (ARICE) Consortium is a collaboration of 14 partners from 12 different countries for meeting the needs for marine-based research in the Arctic financed by the EU HORIZON2020 Research and Innovation Action [https://www.europeanpolarboard.org/projects/arice]

[6] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

[7] https://snd.gu.se/en

specified in *Table 7.1* or, if appropriate, the Chief Scientist, is contacted if data or samples collected during the SAS-Oden 2021 expedition are to be used in any type of publication.

Table 1.2: List of the metadata excel files available in the Swedish National Data Service (SND) data repository[8]. This does not include detailed ship data or continuous measurements by specific instruments. These data are available in the SND separately or in another open-access data repository as specified in this report in the respective WP chapters (8-22).

File name	Contents	File delivered by
SO21_Expedition_Logbook.xlsx	Device Operation data	Pauline Snoeijs-Leijonmalm
SO21_Ice_Station_Logbook.xlsx	Ice Station data	Pauline Snoeijs-Leijonmalm
SO21_Metadata_Omics.xlsx	Metadata SO21 omics	Pauline Snoeijs-Leijonmalm
SO21_Metadata_WP1_EFICA.xlsx	Metadata WP1	Pauline Snoeijs-Leijonmalm
SO21_Metadata_WP2_MIME.xlsx	Metadata WP2	Pauline Snoeijs-Leijonmalm
SO21_Metadata_WP3_ProMis.xlsx	Metadata WP3	Birthe Zäncker
SO21_Metadata_WP4_VIRUS.xlsx	Metadata WP4	Janina Rahlff
SO21_Metadata_WP5_ASAP.xlsx	Metadata WP5	Johan Wikner
SO21_Metadata_WP6_PICO.xlsx	Metadata WP6	Hanna Farnelid
SO21_Metadata_WP7_PHYTO.xlsx	Metadata WP7	Hanna Farnelid
SO21_Metadata_WP8_ZOO.xlsx	Metadata WP8	Emma Svahn
SO21_Metadata_WP9_FORAM.xlsx	Metadata WP9	Flor Vermassen
SO21_Metadata_WP10_CATCHEM.xlsx	Metadata WP10	Adam Ulfsbo
SO21_Metadata_WP11_VACAO.xlsx	Metadata WP11	Lennart Gerke
SO21_Metadata_WP12+13_TGB.xlsx	Metadata WP12 and WP13	Ian Brown
SO21_Metadata_WP14_MWA.xlsx	Metadata WP14	Julia Muchowski
SO21_Metadata_WP15_WAOW.xlsx	Metadata WP15	Salar Karam
SO21_Metadata_WP16_ACAS.xlsx	Metadata WP16	John Prytherch
SO21_Metadata_Opportunistic.xlsx	Opportunistic sampling outside WPs	The SO21 participants who sampled
SO21_Route_30_minutes.xlsx	Expedition route 30-min. averages	Pauline Snoeijs-Leijonmalm
Directory with scanned field notes	CTD, ice stations, omics filtrations, etc.	SO21 teams who made the notes
Directory with technical photographs	Individual ice cores, individual box cores	SO21 teams who made the photographs

Standard Operating Procedures (SOPs) used for sampling and onboard measurements during the expedition were adapted as much as possible to methods defined in the international SAS Science and Implementation Plan[9] and other internationally used standard methods. However, for the biological parameters very few internationally agreed SOPs are available. Therefore, some SOPs were designed/adapted especially for the SAS-Oden 2021 expedition (*Table 1.3*). These SO21 SOPs, some simple and others more complicated, are included in the last chapter of this report (*Chapter 24*). These SOPs are written in such a way that all expedition participants could take over tasks from each other in a smooth way when this was necessary during the SAS-Oden 2021 expedition.

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[8] https://snd.gu.se/en

[9] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

Table 1.3: List of the SO21 Standard Operating Procedures (SOPs) used during the SO21 expedition and included in Chapter 24 of this report. These SOPs are written in such a way that all expedition participants could take over tasks from each other in a smooth way when this was necessary during the SAS-Oden 2021 expedition.

Chapter	Name of the SO21 SOP	Contents of the SO21 SOP
24.1	SO21 SOP: box core	Description of the deck work when taking subsamples from a box core sample by the involved WPs
24.2	SO21 SOP: multinet	Description of the deck work when preparing and operating the multinet and collecting the zooplankton samples
24.3	SO21 SOP: surface microlayer	Description of the procedure that was used for sampling the surface microlayer (SML)
24.4	SO21 SOP: acid washing	Description of safe acid washing of bottles and equipment on board
24.5	SO21 SOP: vacuum filtration	Description of the vacuum filtration equipment brought by WP2 with practical guidelines on how to use it
24.6	SO21 SOP: spiking with $^{15}N_2$	Description of how to use the gas regulators brought by WP2 for efficiently spiking experiments with $^{15}N_2$ gas
24.7	SO21 SOP: omics	Description of filtration of DNA and RNA samples on 0.2 μm Sterivex™ filter units for the SO21 omics collaboration with the peristaltic pumps brought by WP2
24.8	SO21 SOP: viromics	Description of the filtration of virus DNA/RNA samples with iron chloride precipitation using the omics Sterivex filtrates
24.9	SO21 SOP: viral isolation	Description of the method used for viral isolation
24.10	SO21 SOP: prokaryotes	Description of the methods used for measuring prokaryotic abundance, prokaryotic biomass growth, and plankton respiration
24.11	SO21 SOP: chlorophyll-*a*	Description of the onboard measurements of chlorophyll-*a* concentration in two size fractions: 0.3-2 μm and 2-200 μm.
24.12	SO21 SOP: flow cytometry	Description of sampling for later on-shore enumeration of viruses (FCM-virus), prokaryotes (FCM-prok), autotrophic phytoplankton (FCM phyto), and heterotrophic nanoflagellates (FCM-pico)
24.13	SO21 SOP: primary production	Description of the onboard simulated in-situ (SIS) incubations with ^{13}C for estimating primary production in natural seawater and ice habitats
24.14	SO21 SOP: phytoplankton community	Description of the procedure for sampling and conserving water samples for later on-shore phytoplankton community composition analyses
24.15	SO21 SOP: zooplankton community	Description of the procedure for sampling and conserving zooplankton samples for later on-shore zooplankton community composition analyses

2 Expedition management and participants

2.1 Expedition management

The Expedition Leader Team consisted of the Master of *IB Oden* Mattias Petersson, the Chief Scientist Pauline Snoeijs-Leijonmalm, and the SPRS Expedition Coordinator Maria Samuelsson (*Figure 2.1*). The total number of expedition participants was 75.

The expedition followed the route and sampling plan in the preparatory "Scope of Work" document as much as possible. Specific requests from the 16 onboard WP Leaders (e.g., requests for large volumes of water from the CTD rosettes for experiments) were discussed with the Chief Scientist and the Expedition Coordinator latest at 16:00 on the day before the requested sampling. The Chief Scientist and the Expedition Coordinator then compiled a preliminary sampling and logistics plan for the next day that was presented to the entire scientific party and SPRS staff during the daily plenary evening briefing (19:00-19:20), and amended if necessary. During this briefing also messages from the *IB Oden* crew were presented. The daily plenary evening briefing was an excellent way to keep everybody informed and avoid misunderstandings, but not an occasion for large discussions – plans were made in preparation of the meeting and problems raised during the meeting were handled afterwards.

The Expedition Leader Team, Chief Officer Mats Wisén and SPRS technician Joachim Gyllestad gathered for a daily morning meeting on the bridge at 07:00 and received a weather briefing by one of the two meteorologists on board. The possibility for helicopter flights was assessed with regard to the weather conditions, especially the probability of fog. The sampling and logistics plan from the evening before was, if necessary, modified during the morning meeting and then posted electronically on the shipboard server with monitors all around the ship as the "Plan of the Day".

Figure 2.1: The SAS-Oden 2021 Expedition Leader Team immediately after reaching the North Pole on 16 August 2021. The Master himself steered IB Oden *the last miles up to the North Pole. From left to right: SPRS Expedition Coordinator Maria Samuelsson, Master of* IB Oden *Mattias Petersson, Chief Scientist Pauline Snoeijs-Leijonmalm. ©SPRS*

2.2 Scientific party

The scientific party consisted of 38 senior scientists, post-docs, PhD-students and technicians in the fields of biology, chemistry and physics, from six countries (Denmark, Germany, The Netherlands, Sweden, UK, USA). Some members of the scientific party participated in several WPs (*Table 2.1*).

Table 2.1: List of the 38 scientists who participated in the SAS-Oden 2021 expedition.

Title	First name	Last name	Expertise	WP(s)	Affiliation	Country
Prof	Pauline	Snoeijs-Leijonmalm	Chief Scientist, marine ecologist	1, 2, 7	SU	Sweden
MSc	Dennis	Amnebrink	Microbial ecologist	5	LNU	Sweden
MSc	Yannis	Arck	Marine chemist	11	HUG	Germany
Dr	Clare	Bird	Molecular biologist	9	Univ. Stirling	UK
Dr	Kimberley	Bird	Microbial ecologist	3	MBA	UK
MSc	Caroline	Bringensparr	Marine geologist	14	SU	Sweden
Dr	Ian	Brown	Marine chemist	12, 13	PML	UK
Dr	Carlos	Castro	Marine geologist	14	SU	Sweden
MSc	Julek	Chawarski	Fishery acoustician	1	SU	Sweden
Dr	Hanna	Farnelid	Microbial ecologist	6, 7	LNU	Sweden
Dr	Hauke	Flores	Marine ecologist	1	AWI	Germany
MSc	Lennart	Gerke	Marine chemist	11	GEOMAR	Germany
Dr	Nicole	Hildebrandt	Marine ecologist	1	AWI	Germany
MSc	Lina	Holthusen	Marine chemist	12, 13	GEOMAR	Germany
MSc	Salar	Karam	Physical oceanographer	15	GU	Sweden
Dr	Christien	Laber	Microbial ecologist	6, 7	LNU	Sweden
Dr	Prune	Leroy	Molecular biologist	2	SU	Sweden
MSc	Anna	Lunde Hermansson	Marine chemist	10	CUT	Sweden
Dr	Frank	Menger	Environmental chemist	1	SLU	Sweden
Dr	Claudia	Morys	Benthic ecologist	2	SU	Sweden
MSc	Julia	Muchowski	Physical oceanographer	1, 14	SU	Sweden
MSc	Sonja	Murto	Atmospheric scientist	16	SU	Sweden
MSc	Amanda	Nylund	Marine chemist	10	CUT	Sweden
Dr	Alexandra	Padilla	Physical oceanographer	14	CCOM	USA
MSc	Clara	Pérez Martínez	Microbial ecologist	2, 7	SU	Sweden
Dr	John	Prytherch	Atmospheric scientist	16	SU	Sweden
Dr	Janina	Rahlff	Microbial ecologist	4	LNU	Sweden
Dr	Serdar	Sakinan	Fishery acoustician	1	WMR	Netherlands
Dr	Marcus	Sundbom	Marine chemist	6, 7, 10	SU	Sweden
MSc	Emma	Svahn	Marine ecologist	8	LNU	Sweden
MSc	Baldvin	Thorvaldsson	Fishery biologist	1	SLU	Sweden
Dr	Adam	Ulfsbo	Marine chemist	10, 11	GU	Sweden
Dr	Javier	Vargas Calle	Molecular ecologist	2, 7	UU	Sweden
Dr	Ashish	Verma	Microbial ecologist	5	UMU	Sweden
Dr	Flor	Vermassen	Marine geologist	9	SU	Sweden
Prof	Johan	Wikner	Microbial ecologist	5	UMU	Sweden
MSc	Lisa	Winberg von Friesen	Microbial ecologist	2, 6, 7	UCPH	Denmark
Dr	Birthe	Zäncker	Microbial ecologist	3	MBA	UK

2.3 Swedish Polar Research Secretariat (SPRS)

The logistics staff from the SPRS consisted of the Expedition Coordinator, seven technicians, a meteorological team, a helicopter team and a medical team (*Table 2.2*). The technicians worked closely together with the ship crew in performing the winch operations and helped the scientists in many ways, such as repairing equipment and solving IT problems throughout the expedition (*Figures 2.2 and 2.3*). The logistics staff also took care of weaponed bear-guarding on the ice, bear-watching from the bridge, and "Ice Control" radio communications. Additionally, the SPRS provided one berth on the ship for a school teacher to disseminate information about the Arctic environment to Swedish school children.

Table 2.2: List of the 15-person SPRS staff who participated in the SAS-Oden 2021 expedition.

Title	First name	Last name	Expertise	Affiliation
Dr	Maria	Samuelsson	Expedition Coordinator	SPRS
Mr	Per	Arnell	Medical doctor	Contractor
Mr	Joachim	Gyllestad	Technician	SPRS
Mr	Hans-Jørgen	Hansen	Technician	Contractor (MacArtney Underwater Technology, DK)
Major	Sara	Johansson	Meteorologist	Contractor (on leave from the Swedish Military)
Mr	Ted	Juliussen	Helicopter pilot	Contractor
Mr	Johan	Kullberg	Nurse	Contractor
Mr	Sven	Lidström	Technician	Contractor
Mr	Per	Lundgren	Meteorologist	Contractor
Mr	Josef	Nilsen	IT technician	Contractor
Mr	Mats	Persson	Helicopter technician	Contractor
Mr	Daniel	Rönn	IT technician	Contractor
Mr	Anton	Sandström	IT technician	SPRS
Dr	Anna	Stiby	School teacher	Nacka Gymnasium, Sweden (SPRS stipend)
Mr	Niklas	Vestin	Technician	Contractor

Figure 2.2: Meeting on the bridge of IB Oden *in preparation for the first EFICA Master Station – a combination of winch and crane operations from the ship and longline and trap deployments by helicopter. From left to right, SPRS meteorologist Sara Johansson, SPRS Expedition Coordinator Maria Samuelsson, Master of* IB Oden *Mattias Petersson, Chief Scientist Pauline Snoeijs-Leijonmalm, SPRS technician Joachim Gyllestad, Chief Officer of* IB Oden *Mats Wisén, SPRS meteorologist Per Lundgren, and SPRS IT-technician Anton Sandström. ©SPRS*

Figure 2.3: SPRS Expedition Coordinator Maria Samuelsson working with scientific Device Operations on the aft of IB Oden *during the SAS-Oden 2021 expedition.* ©Hans-Jørgen Hansen

2.4 Swedish Maritime Administration

IB Oden was during the SAS-Oden 2021 expedition operated by the Master of *IB Oden* Mattias Peterson and his crew of 21 (*Table 2.3*). They also assisted the scientific program with deck operations, a wide variety of technical matters, and expert advice on Arctic conditions.

Table 2.3: List of the 22-person Master and crew of IB Oden *who participated in the SAS-Oden 2021 expedition.*

Title	First name	Last name	Expertise	Affiliation
Captain	Mattias	Petersson	Master of *IB Oden*	Viking Supply Ships
Mr	Johan	Andersson	1st Engineer	Viking Supply Ships
Mr	Patrik	Antonsson	Able seaman	Viking Supply Ships
Mr	Pär-Åke	Bäckström	Fitter	Viking Supply Ships
2nd Officer	Jonas	Danielsson	2nd Officer	Viking Supply Ships
Mr	Daniel	Ernhill	2nd Engineer/Electrician	Viking Supply Ships
Mr	Christopher	Groop	Oiler	Viking Supply Ships
Mr	Martin	Hahne	2nd Engineer	Viking Supply Ships
2nd Officer	Filip	Hansson	2nd Officer/Chief Officer trainee	Viking Supply Ships
Mr	Mats	Hansson	Bosun	Viking Supply Ships
Ms	Tina	Ljung	Cook	Viking Supply Ships
Ms	Johanna	Lundgren	Able seaman	Viking Supply Ships
Ms	Lena	Meyer	Messman	Viking Supply Ships
Mr	Jonas	Nelson	Chief cook	Viking Supply Ships
Mr	Robert	Nielsen	2nd Engineer	Viking Supply Ships
Ms	Ebba	Nyman	Messman	Viking Supply Ships
Mr	Richard	Palm	Oiler	Viking Supply Ships
Mr	Christian	Petersén	Oiler	Viking Supply Ships
Mr	Jörgen	Rundqvist	Chief engineer	Viking Supply Ships
Mr	Oskar	Stenquist	Able seaman	Viking Supply Ships
2nd Officer	Kenneth	Wahlberg	Senior 2nd Officer	Viking Supply Ships
Chief Officer	Mats	Wisén	Chief Officer	Viking Supply Ships

2.5 Scientists with specific operational tasks during the expedition

Overall, the SAS-Oden 2021 expedition was extremely collaborative as a result of the SO21 joint ecosystem study in the spirits of SAS. Nine people had crucial onboard tasks, partly beyond their own WP(s) to the benefit of all.

Pauline Snoeijs-Leijonmalm (WP1,2,7, *Figure 2.4 A*) was Chief Scientist and had coordinated and prepared the scientific contents of the expedition for more than two years in collaboration with the Project PIs. She is the PI of the main research project on board (WP1, EFICA), which financed a substantial part of the ship time of the SAS-Oden 2021 expedition. During the expedition her responsibility was that all 16 onboard projects could execute their sampling programmes as successfully as possible with respect to time constraints, weather conditions, etc., in communication with the other members of the Expedition Leader Team, the Master of *IB Oden* and the SPRS Expedition Coordinator. Pauline also has the main responsibility for the SO21 omics collaboration between WPs 1-9, coordinated by WP2.

Adam Ulfsbo (WP10, *Figure 2.4 B*) was responsible for the CTD water budget on board. Two CTD casts were necessary at all sampling stations deeper than 2000 m to achieve a full-depth profile for the chemical water-column measurements. The reason for this was that only 22 Niskin bottles were available for water sampling on the bow CTD rosette (two places were taken up by the LADCP equipment) while 24 depths were stipulated in international SAS Science and Implementation Plan[4]. Also, at about half of the full-depth casts, deep water was necessary for the SO21 omics collaboration and WP3 (ProMis). Under these circumstances a tailor-made water budget sheet prepared by Adam was needed for each single CTD cast from the bow.
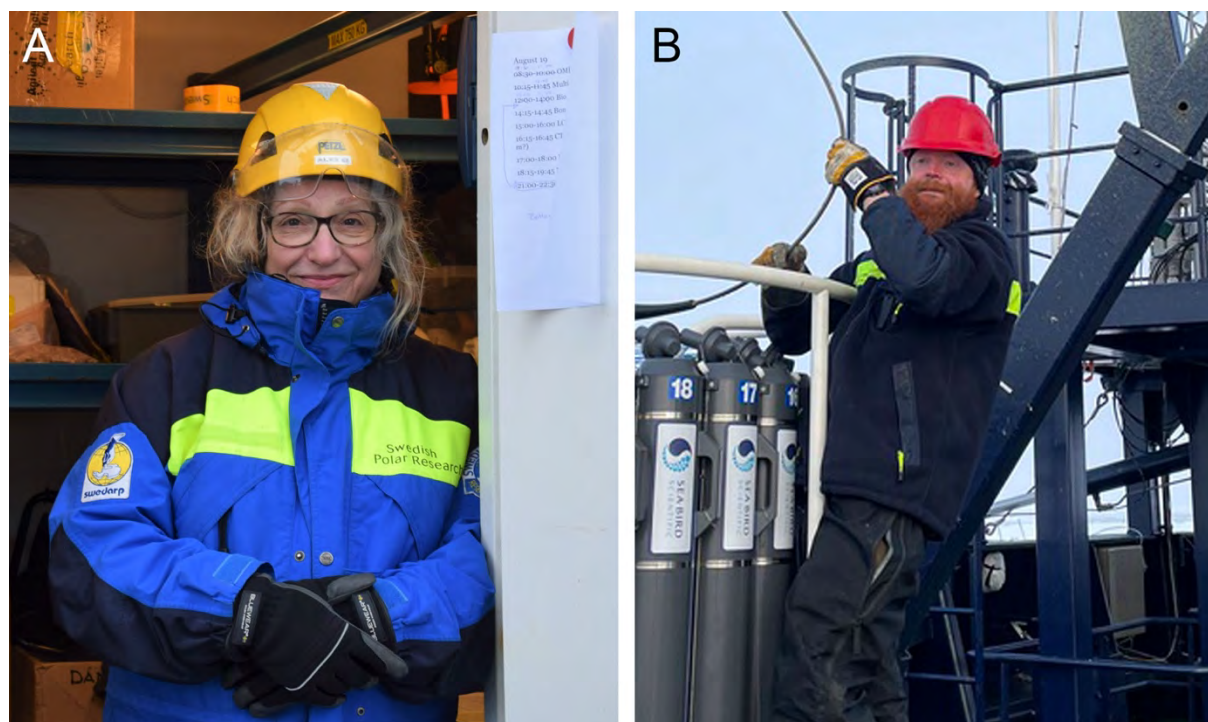
Figure 2.4: Scientists with specific tasks during the SAS-Oden 2021 expedition. (A) Pauline Snoeijs-Leijonmalm, Chief Scientist. (B) Adam Ulfsbo, responsible for the CTD water budget. (A) ©Hans-Jørgen Hansen, (B) ©Lennart Gerke

The CTD team consisted of Julia Muchowski (WP14), Alexandra Padilla (WP14), and Salar Karam (WP15) (*Figure 2.5*). They worked in shifts to coordinate the CTD rosette deck operations and CTD data capture in collaboration with SPRS technicians who drove the winch and scientists from all WPs who helped with, a.o., transports of the CTDs in and out of their containers. These were mainly scientists who captured data from other instruments attached to the CTD or taking water samples.

Responsible for the net casts were Baldvin Thorvaldsson (WP1) and Nicole Hildebrandt (WP1) (*Figure 2.6*). Baldvin coordinated the sampling with the beam net that he had designed and built himself before the expedition at SLU (Sweden), as well as the MIK ring net, in collaboration with SPRS technicians and the *IB Oden* crew. For these large nets, aimed at sampling fish and macrozooplankton, both winch and crane operations were necessary. Nicole Hildebrandt coordinated the multinet, bongo net and LOKI[10] casts given her large experience from zooplankton sampling in the CAO, in collaboration with SPRS technicians. The multinet and LOKI used during the SAS-Oden 2021 expedition were borrowed from Nicole's home institute (AWI, Germany).

Claudia Morys (WP1+2) (*Figure 2.7 A*) coordinated the box core casts given her large experience from sampling bottom fauna. The "giant box corer" used during the SAS-Oden 2021 expedition was borrowed from the AWI (Germany).

Hauke Flores (WP1) (*Figure 2.7 B*) coordinated the ice station sampling given his large experience from both Arctic and Antarctic sea ice work. He also educated many scientists on the SAS-Oden 2021 expedition in how to sample ice cores and on safety aspects of ice work, both in theory during the transit to the CAO and in practice during the first ice stations.
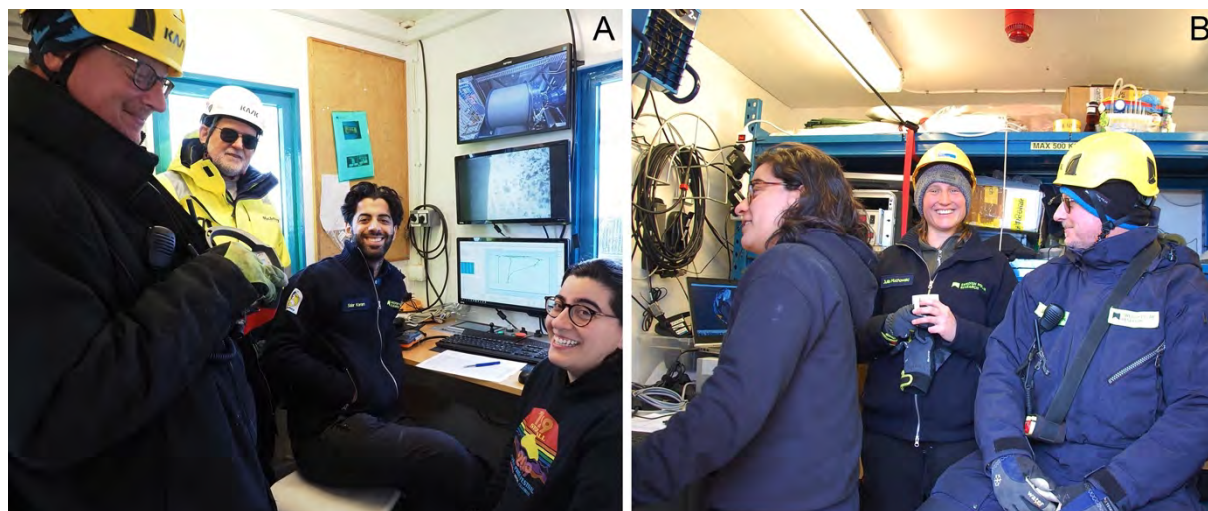
Figure 2.5: Scientists with specific tasks during the SAS-Oden 2021 expedition: the CTD team. (A) The CTD container at the bow with SPRS technicians Joachim Gyllestad and Hans-Jørgen Hansen to the left, and Salar Karam (WP15) and Alexandra Padilla (WP14) to the right. (B) The CTD container at the stern with Alexandra Padilla (WP14), Julia Muchowski (WP14) and SPRS technician Joachim Gyllestad. (A)©Anna Lunde Hermansson, (B) ©SPRS

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[10] Schulz J, et al. (2009) Lightframe On-sight Key species Investigation (LOKI). IEEE OCEANS 2009-EUROPE [http://doi.org/10.1109/OCEANSE.2009.5278252]

Figure 2.6: Scientists with specific tasks during the SAS-Oden 2021 expedition: net casts. (A) Baldvin Thorvaldsson, responsible for the beam net operations, during a beam cast at the aft. (B) Nicole Hildebrandt, responsible for the zooplankton net operations, with the multinet steering unit. (A) ©SPRS, (B) ©Pauline Snoeijs-Leijonmalm

*Figure 2.7: Scientists with specific tasks during the SAS-Oden 2021 expedition. (A) Claudia Morys, responsible for the box core casts, subsampling a box core sample at the aft of IB Oden. (B) Hauke Flores, responsible for coordinating ice station sampling, showing the SAS-Oden 2021 expedition's first fish catch: a polar cod (*Boreogadus saida*) caught in a trap. (A) ©Pauline Snoeijs-Leijonmalm, (B) ©SPRS*

3 Synoptic Arctic Survey (SAS)

3.1 Scientific motivation of SAS

The international SAS collaboration[11] seeks to define the present state of the Arctic Ocean and to understand the major ongoing transformations, with an emphasis on water masses, the marine ecosystems and the carbon cycle. It will not be possible to assess either the consequences or the range of the ongoing changes unless necessary empirical data are collected, analysed and understood in concert with each other for all parts of the Arctic Ocean.

A fundamental premise for approaching, sampling and understanding the far-reaching changes in the Arctic Ocean is thus that the international SAS collaboration should be synoptic across the Arctic marine ecosystems, i.e., including both the deep central basins and the shallower seas on the continental shelves, and both areas within the Exclusive Economic Zones (EEZs) of the coastal states and the High Seas area outside national jurisdictions (*Figure 3.1*). Such an effort is beyond the scope of any single nation. Collecting empirical data on a pan-Arctic scale includes the involvement of many research vessels, a set of defined SAS Core Parameters, shared protocols and the usage of the best available technology. The original SAS objective was a multi-national coordinated engagement of research vessels in the summer of 2020. However, due to the Covid19 pandemic the various expeditions had to take place over a three-year period (2020-2022).

Within the international SAS collaboration, the SAS-Oden 2021 expedition was tasked with covering an area of the CAO LME with the thickest Arctic sea-ice cover (*Figure 3.1*). Most of the SAS-Oden 2021 expedition route was situated within the High Seas area. Other SAS expeditions that cover parts of the High Sea area of the CAO are *RV Araon* in 2020, 2021 (South-Korea: Chukchi Plateau), *RV Mirai* in 2020, 2021 (Japan: Chukchi Plateau), CCGS Louis S St. Laurent 2020, 2021 (Canada: Beaufort Gyre, Canada Basin), *RV Kronprins Haakon* 2021, 2022 (Norway: Nansen and Amundsen Basins), and the *USCGC Healy* expedition in 2022 (USA: Canada and Makarov Basins). The Chukchi Plateau belongs to the High Seas area, but does not fall within the CAO LME as defined by the Arctic Council (*Figure 3.1*). Other ships participating in SAS (a.o., Denmark, Italy, Canada, Norway, USA, Russia) have operated or are planned to operate in the Arctic shelf seas and the Fram Strait and Bering strait Arctic gateway areas in 2020-2022.

The common SAS goal is to generate an unmatched dataset that allows for a complete characterization of Arctic Ocean hydrography and circulation, organismal and ecosystem functioning and productivity, and carbon uptake and ocean acidification. By comparison to historical data the SAS observations will also enable detection of change. However, the possibilities for doing so are clearly limited by the insufficient temporal and spatial coverage of existing data, in particular for the state of the ecosystem. In this respect, the comprehensive dataset from SAS will provide a unique and critically needed baseline for future studies as it will allow us to track climate change and its impacts as they unfold in the Arctic over the coming years, decades and centuries. It also will inform and better constrain biogeochemical modelling efforts that similarly seek to understand, detect, and predict change. The SAS vision is that the 2020-2022 expeditions will be the first of several decadal efforts to assess the state of the Arctic ecosystem and carbon cycle, in concert with the physical system, that will lead to a better understanding of the specific questions posed in the international SAS Science and Implementation Plan[4].

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[11] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]
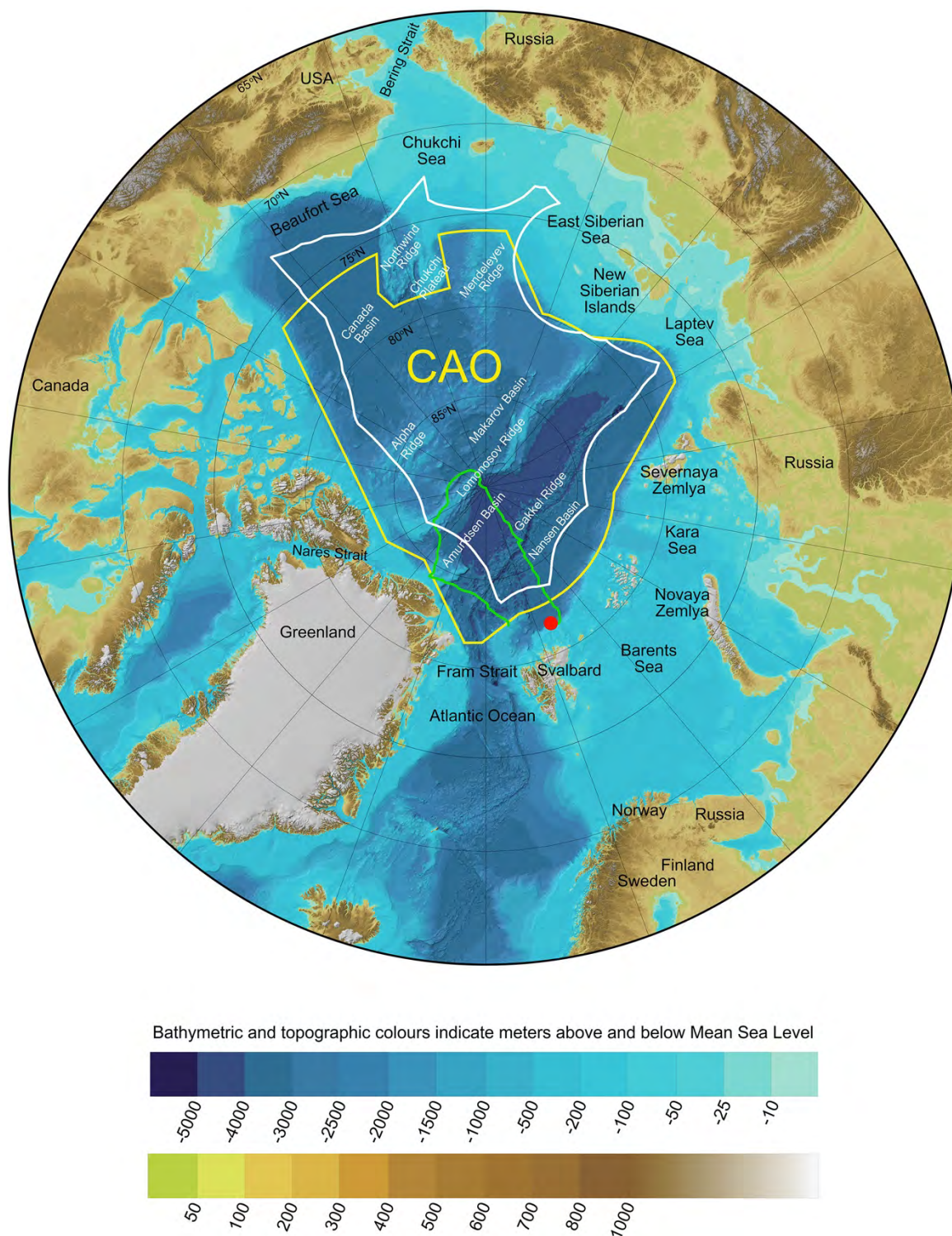
Figure 3.1: The SAS-Oden 2021 expedition route (green line) in relation to the Central Arctic Ocean Large Marine Ecosystem as defined by the Arctic Council[12] (CAO, yellow line) and the High Seas portion of the Arctic Ocean outside national jurisdictions (white line). The red dot indicates the starting point of the SAS-Oden 2021 expedition route. The background map was extracted from IBCAO[13].

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[12] PAME (2013) Large Marine Ecosystems (LMEs) of the Arctic Area: Revision of the Arctic LME Map. PAME International Secretariat, 28nd ed. [https://oaarchive.arctic-council.org/handle/11374/61]

[13] Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

Some of the suggested SAS sections cover regions that have only rarely or never been characterised, while others cover the regions that have been more frequently sampled. SAS is envisioned to take place during the summer months, not only because the Arctic Ocean is most accessible in this season but also since most previous work has been conducted during those periods. Altogether, this spatial and temporal sampling strategy enables detection of change for those characteristics and regions where previous information is available, in addition to providing the comprehensive ecological characterization of today's Arctic Ocean.

3.2 Key scientific questions of SAS

The nine key scientific questions of the international SAS Science and Implementation Plan[14] are subdivided into three categories, with three research questions each:

Physical Drivers
   (1) How are Arctic Ocean water masses and circulation responding to changes in sea ice properties, and atmospheric, advective and freshwater forcing?
   (2) What are the states of, and changes in, heat and freshwater budgets in the Arctic region?
   (3) What are the changes in water mass sources, sinks and transformations?

Ecosystem Response
   (4) How does primary production and associated availability of nutrients vary between Arctic regions?
   (5) Does northward range expansion of subarctic species vary regionally and are any of these species likely to establish permanent populations in Arctic regions?
   (6) How does carbon flow vary across regional ecosystems of the Arctic?

Carbon Cycle and Ocean Acidification
   (7) What is the contribution of the Arctic Ocean to maintaining the global ocean carbon dioxide reservoir and uptake?
   (8) What are the input and fate of terrestrial and subsea carbon to the Arctic Ocean?
   (9) What are the magnitude, drivers, and impacts of ocean acidification in the different regions of the Arctic?

3.3 SAS Core Parameters

The set of SAS Core Parameters as defined in the international SAS Science and Implementation Plan (*Table 3.1*) contain selected physical, chemical and biological measurements made directly on the sea ice or on board during the SAS expeditions, or later in land-based laboratories from samples taken during the SAS expeditions. During the SAS-Oden 2021 expedition all SAS Core Parameters (except for birds and mammals) were measured, and many of them both in the water column and in sea-ice habitats. This was possible because the 16 research projects selected for participation in the expedition were chosen according to their contributions in covering the SAS Core Parameters, alone or in collaboration with other projects participating in the SO21 joint ecosystem study. Thus, the expedition had the overall mission to jointly collect the physical, chemical and biological ecosystem parameters stipulated by the international SAS collaboration while also project-specific work was carried out within the expeditions' 16 research projects. The project-specific work often consisted of incubation experiments while field measurements and field sampling often constituted SAS Core Parameters. Metadata and measured data values are to be deposited in the SND data repository or another open-

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[14] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

access data repository latest by 1 October 2023 according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*, see also *Chapter 7*).

Table 3.1: Recommended set of SAS Core Parameters[15].

Parameter	Sampling [Target accuracy if applicable]	Relevance
Physical and chemical measurements		
Pressure	CTD [3 ± 0.5 dbar]	Depth
Temperature	CTD [0.002 ± 0.0005°C]	Atlantic and Pacific water inflows Stratification, mixing Brine formation
Salinity	CTD + Niskin [0.002 ± 0.001 g kg^{-1}]	Atlantic and Pacific water inflows Stratification, mixing Brine formation
Dissolved oxygen	CTD + Niskin [± 1%]	Oxidation of terrestrial DOC and POC to CO_2 Oxidation of methane
Nutrients (NO_3/NO_2, PO_4, SiO_3)	Niskin [1-3 ± 0.2%]	Ecosystem productivity
CFCs and SF_6	Niskin [1-2 ± 1%]	Rates of ventilation Anthropogenic carbon storage Biogeochemical transformation rates
Dissolved inorganic carbon (DIC)	Niskin [± 2 μmol kg^{-1}]	CO_2 chemistry, ocean acidification
Total alkalinity	Niskin [± 3 μmol kg^{-1}]	CO_2 chemistry, ocean acidification
pH	Niskin [± 0.005]	CO_2 chemistry, ocean acidification
$\delta^{18}O$ of H_2O	Niskin	Water mass mixing
Methane	Niskin	Release of subsea methane Outgassing of methane
Dissolved organic carbon (DOC)	Niskin	Terrestrial organic material (shelves) Export to the global ocean (gateways)
Particulate organic carbon (POC)	Niskin	Terrestrial organic material (shelves) Remineralisation length scales (deep basins)
Water column ecosystem measurements		
Chlorophyll	Niskin	Phytoplankton standing stock Ecosystem productivity
Primary production	Incubation	Phytoplankton production Ecosystem productivity Carbon flux Uptake or release of CO_2 from surface waters
Viruses	Niskin	Biodiversity, ecosystem functioning
Prokaryotes	Niskin	Biodiversity, ecosystem functioning
Phytoplankton composition	Niskin	Biodiversity, ecosystem functioning
Microzooplankton	Niskin	Biodiversity, ecosystem functioning
Meso- and macrozooplankton	Bongo net, multinet, optical instruments, acoustics	Biodiversity, ecosystem functioning
Ichthyoplankton	Aluette or tucker trawls, acoustics	Biodiversity, ecosystem functioning
Fish	Trawls, acoustics	Biodiversity, ecosystem functioning
Marine mammals	Passive acoustics, visual observations	Biodiversity, ecosystem functioning
Other carbon transformation rates	Selected process studies (e.g., grazing, reproduction, sinking, respiration)	Ecosystem functioning
Benthic measurements		
Meio- and macrofauna	Box core, multicore or other corers	Biodiversity, ecosystem functioning
Epifauna	Benthic camera, beam trawl	Biodiversity, ecosystem functioning
Other carbon transformation rates	Selected process studies (e.g., grazing, reproduction, sinking, respiration)	Ecosystem functioning
Other		
Epontic communities[16]	Under-ice imaging, ice cores, sub-ice sampling	Biodiversity, ecosystem functioning
Seabirds	Visual observations	Biodiversity, ecosystem functioning

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[15] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]
[16] Epontic = sympagic = ice-associated

4 The SAS-Oden 2021 expedition

4.1 Preparations

A group of Swedish scientists proposed to the SPRS that a SAS expedition would be organised with *IB Oden* and this was approved by the SPRS in 2018. The expedition was organised by the SPRS in collaboration with the participating scientists, and the infrastructure (ship time) was financially supported by the SPRS, the EFICA Consortium[17] and the ARICE Consortium[18]. Within the international SAS collaboration, *IB Oden* would cover an area of the CAO with challenging ice conditions (*Figure 3.1*). The expedition was originally planned for summer 2020, but in in April 2020 it was postponed to summer 2021 due to the Covid19 pandemic.

In 2017-2021, ten workshops and on-line meetings were held among Swedish scientists during which the scientific programme and its logistic requirements were evolving. The scientists applied for national research grants for specific project costs (wages for scientists, equipment, consumables, transports, etc.), and the SPRS dedicated six berths to the ARICE programme. A preparatory "Scope of Work" document was drafted by the SPRS in collaboration with the scientists. This document was central in the organisational work for more than two years, being used as an evolving document combining the scientific and logistic planning of the expedition. This procedure proved to be utterly beneficial for communication between science and logistics and was crucial for a successful SAS-Oden 2021 expedition.

By spring 2021, the "Scope of Work" had developed into an operational expedition plan. Since the research projects that would collect data during the SAS-Oden 2021 expedition were selected based on their expected contributions to measuring as many of the SAS Core Parameters as possible within the projects, the SAS-Oden 2021 expedition could cover almost all of these parameters. Sampling depths and Standard Operating Procedures (SOPs) used during the expedition were adapted as much as possible to those defined in the international SAS Science and Implementation Plan (*Table 3.1*). However, for the biological parameters very few internationally agreed SOPs were available and some were especially adapted/designed for the SAS-Oden 2021 expedition (*Table 1.3, Chapter 24*).

Since part of the expedition was outside the High Seas, and some scientists wanted to take underway samples on the way home to Helsingborg, special permissions for measuring and sampling within Exclusive Economic Zones (EEZs) were applied for and granted by Greenland (Kingdom of Denmark) and Norway. This implies that reports accompanied with expedition data of the work inside their EEZs are to be delivered after the expedition to Greenland and Norway, respectively. Since the SAS-Oden 2021 expedition partly overlapped in time and space with the Nansen Legacy JC2-2 expedition (a Norwegian contribution to SAS) with icebreaker *RV Kronprins Haakon* (Norway) communication about possible "cross-over" stations and other collaboration was established.

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[17] The European Fisheries Inventory in the Central Arctic Ocean (EFICA) Consortium is a collaboration between eight European research institutes financed by the European Commission through Framework Contract FWC: EASME/EMFF/2018/003 to collect ecosystem data in the CAO for supporting the Joint Program of Scientific Research and Monitoring of the international "Agreement to Prevent Unregulated High Seas fisheries in the Central Arctic Ocean" that entered into force on 25 June 2021. The EFICA Consortium is coordinated by Pauline Snoeijs-Leijonmalm, Stockholm University, Sweden.

[18] The Arctic Research Icebreaker (ARICE) Consortium is a collaboration of 14 partners from 12 different countries for meeting the needs for marine-based research in the Arctic financed by the EU HORIZON2020 Research and Innovation Action. [https://www.europeanpolarboard.org/projects/arice]

4.2 Time schedule

Before the expedition could start, strict Covid19 quarantine measures were absolutely necessary because an onboard outbreak of Covid19 could adventure the whole expedition. From 16 until 24 July all expedition participants endured individual eight-day quarantine in the Scandic Malmö City Hotel (*Table 4.1*). Nobody was allowed to leave the hotel room. The quarantine time was mainly used for organisational meetings for all participants, and smaller meetings within the 16 WPs. All 75 expedition participants tested negative for Covid19 infection twice and were on 24 July transported by bus from the hotel in Malmö to *IB Oden* anchored off Råå (Helsingborg, Sweden). The expedition was carried out as planned, except that the scientific field work was carried out during 41 days (2 August - 11 September) instead of 43 days. It was planned to last until the evening of 13 August, but, due to a bad weather report for the transit, the Master of *IB Oden* decided to start the transit back to Sweden already in the evening of 11 August.

Table 4.1: Time schedule of the SAS-Oden 2021 expedition.

Date(s)	Activity
June 7	Heavy mobilization, Helsingborg, Sweden
June 9-11	Mobilization of scientific equipment, Helsingborg, Sweden
July 16-24	Individual quarantine (single room, hotel Scandic Malmö City Hotel, Sweden) PCR tests for Covid19 on July 16 and July 23
July 24-25	Preparations, safety courses on board *IB Oden*, Helsingborg, Sweden
July 25	ETD *IB Oden*, Helsingborg, Sweden
July 25 - August 2	Transit to the Survey area
August 2 - September 11	Synoptic Arctic Survey field work (41 days)
September 11-20	Transit back to Sweden
September 20	ETA *IB Oden* Helsingborg, Sweden
October 5-7	Demobilization, Helsingborg, Sweden

4.3 Transit to the marginal ice zone

During the transit from Helsingborg to the marginal ice zone north of Svalbard, the expedition participants discussed the coming field work and started up the practical preparations. In the first place this involved packing up the scientific equipment sent to *IB Oden* during the expedition mobilization in the beginning of June and installing this equipment in containers, labs and on deck. This included the "beam net", a newly designed net for targeting pelagic fish and zooplankton larger than 1 cm under the sea ice, consisting of a 10 m steel beam and a 30 m long fishing net. This also included the "giant box corer" that was used for taking deep sea-bottom samples. The acoustic equipment was calibrated on 1 August close to the marginal ice zone, where the ship was more stable than in open water.

On *IB Oden* the scientists disposed of the ship's chemical/biological laboratories ("Main Lab" and "Triple Lab"), as well as 12 laboratory and 5 storage containers on deck. The SPRS disposed of CTD, winch and storage containers on deck, as well as a large storage hall below the helicopter deck. The acoustic instruments (EK80 hydroacoustics, multibeam, sub-bottom profiler) were operated from the bridge. The bridge was always open for office work or leisure by scientists and SPRS staff, but they should not disturb the crew or eat/drink at the bridge.

4.4 Expedition route and the nine EFICA Master Stations

The SAS-Oden scientific programme was carried out during 41 days. The first sampling station with winch operations from the ship was performed on 2 August (Station 1, *Figure 1.1*). The last winch operation from the ship was performed on 11 September on the Yermak Plateau (Station 60, *Figure 1.1*).

The SAS-Oden 2021 expedition reached further west on the Greenland shelf than any other research expedition has ever done before because the ice conditions in summer 2021 allowed us to get this far west. Closest was the geological Lomrog I expedition with *IB Oden* in 2007[19], but then ice-breaking assistance was needed from the accompanying Russian atomic-driven icebreaker "50 Let Pobedy" ("50 years of Victory") to get this far west (*Figure 4.1*).

Figure 4.1: IB Oden *and the Russian ice-breaker "50 Let Pobedy" in the difficult ice conditions on the Lomonosov Ridge north of Greenland during the LOMROG I expedition. ©Martin Jakobsson*
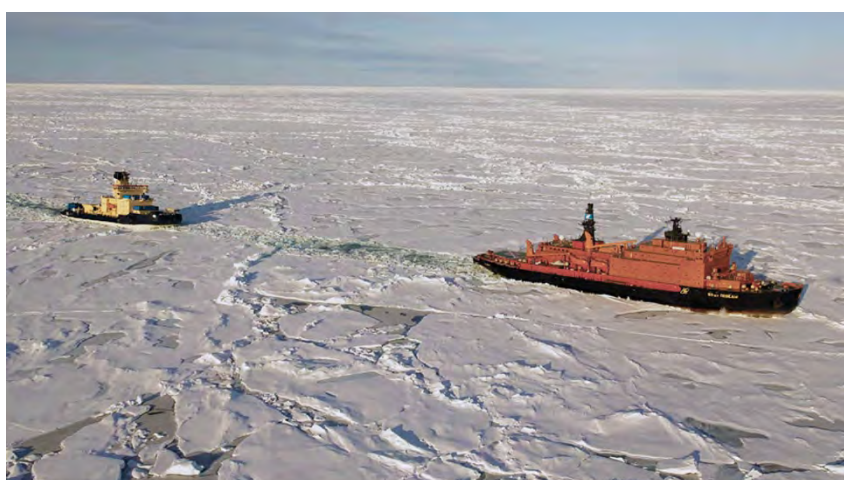
The SAS-Oden 2021 expedition was prospected with regard to previous studies with *IB Oden*, the international MOSAiC expedition in 2019-2020, and the Norwegian Nansen Legacy SAS expedition with RV Kronprins Haakon in 2021. Along the planned route (*Figure 4.2 A*), nine EFICA Master Stations (originally named A-I) were included for WP1 (EFICA). In the rest of this report the nine EFICA Master Station names A-I are not used, and these stations are referred to by their station numbers along the realized route according to the Expedition Logbook (*Figure 4.2 B*).

During the expedition, *IB Oden* crossed the Nansen Basin, the Gakkel Ridge, the Amundsen Basin, the North Pole, the Lomonosov Ridge, a small corner of the Makarov Basin, the western Amundsen Basin, and then back over the Gakkel Ridge to the Yermak Plateau (*Figure 4.2 B*). When the course of the ship was fundamentally changed after a station, a new leg started. The originally planned route was followed as much as possible. However, Station A (SO21 Station 8) could not be completely realized because of strong winds preventing the deployment of the EFICA beam net when *IB Oden* passed the Gakkel Ridge. The beam net was deployed at SO21 Station 14 instead (*Figure 4.2*). Since the pack ice west and east of the route between Stations F (SO21 station 38) and H (SO21 station 50) was too massive to be forced by *IB Oden*, and remaining sampling time was limited, the route needed to be adapted in the end of the expedition with the aim to get as close as possible to Greenland in the shortest possible time. Due to the latter route changes in the end of the expedition, the realized number of expedition legs during SAS-Oden 2021 was seven instead of eight (*Figure 4.2*).

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[19] Jakobsson M, Marcussen C, and LOMROG Scientific Party (2008) Lomonosov Ridge off Greenland 2007 (LOMROG) – Cruise Report, 122 pp. Special Publication Geological Survey of Denmark and Greenland, Copenhagen, Denmark [https://epic.awi.de/id/eprint/37756/1/cr_lomrog-i.pdf]

Planned route SAS-Oden 2021   Realized route SAS-Oden 2021
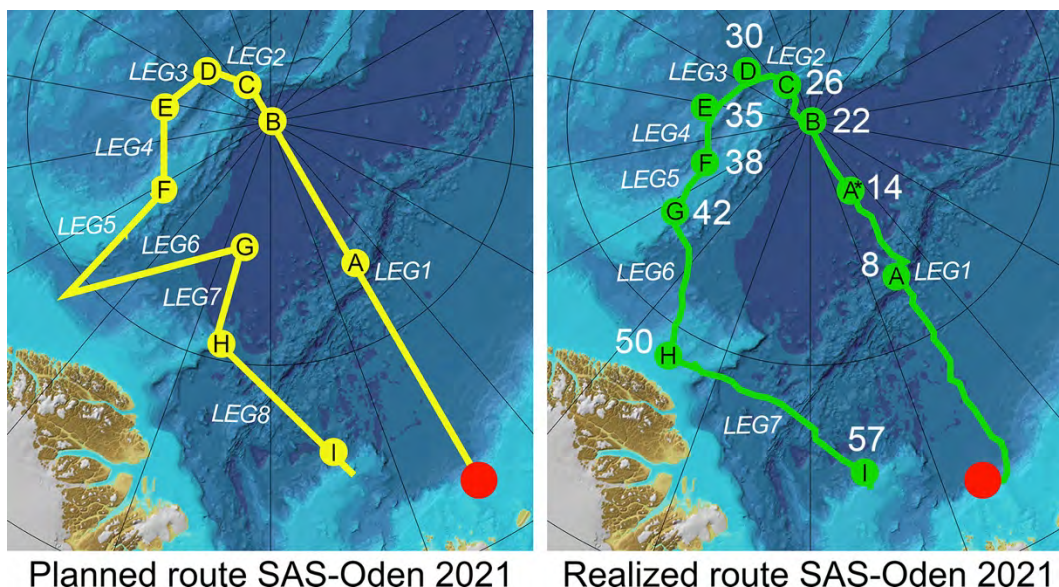
Figure 4.2: The planned (yellow) and realized (green) expedition routes of the SAS-Oden 2021 expedition with the original EFICA Master Stations A-I for beam net operations. All EFICA sampling activities and a SAS ice station, were performed near the originally planned position of Station A (SO21 Station 8). However, the first deployment of the EFICA beam net was performed more north, at Station A* (SO21 Station 14), because of strong winds when IB Oden *passed the Gakkel Ridge. Stations G and H needed to be adapted due to the heavy ice situation around their original positions in combination with the time schedule of the expedition. The background map was extracted from IBCAO[20].*

4.5 Sea ice conditions

During the SAS-Oden 2021 expedition the ice was very thick (up to 3.2 m) around the North Pole, but we found a surprisingly large area with open water north of Greenland (*Figures 4.3 and 4.4*). Generally, the ice was highly dynamic over large areas, and changing significantly from day to day (*Figure 4.5*). Because of this, many fast decisions on how to adapt the expedition route needed to be taken by the Expedition Leader Team to avoid that we would get stuck in the ice.
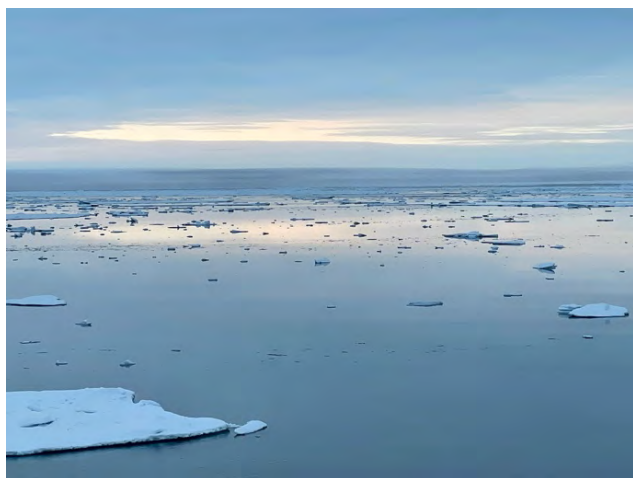
Figure 4.3: Open water north of Greenland during the SAS-Oden 2021 expedition. ©Pauline Snoeijs-Leijonmalm

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[20] Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

Figure 4.4: Satellite image showing the large open-water area north of Greenland that we passed during the SAS-Oden 2021 expedition. ©European Space Agency

Figure 4.5: Ice charts on four days close in time. The sea ice was highly dynamic, see, e.g., the difference between 28 August when IB Oden *was at point "F" with relatively low ice cover (yellow) whereas on 31 August the ice was completely closed in this geographical area (purple). These fast ice dynamics are largely regulated by winds. ©ARTIST Sea Ice (ASI), AMSR2 satellite*

4.6 The SAS-Oden 2021 Research Projects and Work Packages

Altogether, 16 research projects participated in the SAS-Oden 2021 expedition (*Table 4.2*). The onboard work was carried out within 16 work packages (WPs) corresponding to the field work required for each of the research projects as described in the preparatory "Scope of Work" document.

Each research project has one PI or two co-PIs, i.e., the person(s) who had applied to the SPRS for berths on the expedition. For each project there is also one WP Leader, i.e., the scientist responsible for the implementation of the field work for the project and onboard communication with the Chief Scientist.

For all measurements and samples collected during the SAS-Oden 2021 expedition, metadata are available in the SND data repository (*Table 1.2*), and the final results of the post-cruise analyses should be submitted to the SND latest by 1 October 2023. The SPRS and the SAS-Oden 2021 expedition must be acknowledged in any publication of the data as described in *Chapter 7*.

Table 4.2: The 16 research projects and work packages that participated in the SAS-Oden 2021 expedition, their PIs and onboard WP Leaders. Full project titles and scientific descriptions are given in the respective WP Chapters (8-22). nob = PI not on board

WP	Project acronym	Subject	Research project PI/co-PIs	WP Leader on board *IB Oden*
WP1	EFICA	Ecology: nekton and zooplankton	Pauline Snoeijs-Leijonmalm (SU)	Pauline Snoeijs-Leijonmalm (SU)
WP2	MIME	Ecology: microbial metabolism	Pauline Snoeijs-Leijonmalm (SU)	Pauline Snoeijs-Leijonmalm (SU)
WP3	ProMis	Ecology: fungi	Birthe Zäncker (MBA)	Birthe Zäncker (MBA)
WP4	VIRUS	Ecology: viruses	Karin Holmfeldt (LNU) - nob	Janina Rahlff (LNU)
WP5	ASAP	Ecology: prokaryotes	Johan Wikner (UMU)	Johan Wikner (UMU)
WP6	PICO	Ecology: picophytoplankton	Hanna Farnelid (LNU)	Hanna Farnelid (LNU)
WP7	PHYTO	Ecology: phytoplankton	Hanna Farnelid (LNU) Pauline Snoeijs-Leijonmalm (SU)	Hanna Farnelid (LNU)
WP8	ZOO	Ecology: zooplankton	Samuel Hylander (LNU) - nob	Emma Svahn (LNU)
WP9	FORAM	(Palaeo)biology: foraminifers	Helen Coxall (SU) - nob	Flor Vermassen (SU)
WP10	CATCHEM	Chemistry: carbon and nutrient chemistry	Adam Ulfsbo (GU)	Adam Ulfsbo (GU)
WP11	VACAO	Chemistry: tracer chemistry	Toste Tanhua (GEOMAR) - nob	Lennart Gerke (GEOMAR)
WP12	TRACE	Chemistry: nitrous oxide	Damian L. Arévalo-Martínez (GEOMAR) - nob	Ian Brown (PML)
WP13	TGB	Chemistry: methane	Brett Thornton (SU) - nob	Ian Brown (PML)
WP14	MWA	Oceanography: midwater acoustics	Christian Stranne (SU) - nob	Julia Muchowski (SU)
WP15	WAOW	Oceanography: deep-water hydrography	Céline Heuzé (GU) - nob	Salar Karam (GU)
WP16	ACAS	Arctic climate across scales	Michael Tjernström (SU) - nob	John Prytherch (SU)

4.7 WP Leaders during the expedition

The 13 WP Leaders on board (*Table 4.2, Figures 4.6-4.9*) were responsible for carrying out the field work for the 16 onboard research projects (*Chapters 8-22*) in the best possible way. The WP Leaders communicated with the Chief Scientist if there were specific wishes, e.g., for water, ice or sediment samples from the various Device Operations, or any if any problems occurred. The Chief Scientist communicated with the SPRS Expedition Coordinator about the possibilities to perform the field work in the best possible way, and the SPRS Expedition Coordinator communicated with her staff and the Master of *IB Oden*, who communicated with his crew.
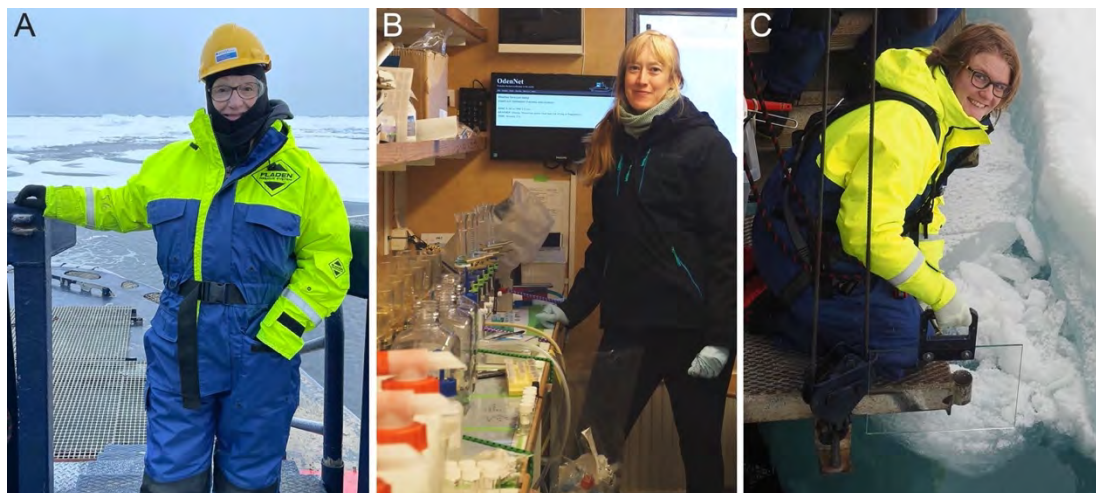
Figure 4.6: WP Leaders of the biological WPs 1-4 during the SAS-Oden 2021 expedition. (A) Pauline Snoeijs-Leijonmalm (Stockholm University, Sweden), WP Leader and PI of WP1 (EFICA) and WP2 (MIME), and co-PI of WP7 (PHYTO) during winch operations from the stern. (B) Birthe Zäncker (Marine Biological Association of the United Kingdom), WP Leader and PI of WP3 (ProMis) working with filtrations in the WP3 laboratory container. (C) Janina Rahlff (Linnaeus University, Sweden), WP Leader of WP4 (VIRUS) taking samples from the surface microlayer with the glass plate method. (A) ©Pauline Snoeijs-Leijonmalm, (B,C) ©SPRS

Figure 4.7: WP Leaders of the biological WPs 5-8 and the (palaeo)biological WP9 during the SAS-Oden 2021 expedition. (A) Johan Wikner (Umeå University, Sweden), WP Leader and PI of WP5 (ASAP), working in the "Triple Lab" of IB Oden. (B) Hanna Farnelid (Linnaeus University, Sweden), WP Leader and PI of WP6 (PICO) and WP Leader and co-PI of WP7 (PHYTO), working in the "Main Lab" of IB Oden. (C) Emma Svahn (Linnaeus University, Sweden), WP Leader of WP8 (ZOO), ready to start sampling with the multinet. (D) Flor Vermassen, WP Leader of WP9 (FORAM), elaborating sediment samples on deck. (A) ©Ashish Verma, (B) ©Pauline Snoeijs-Leijonmalm, (C) ©Janina Rahlff, (D) ©Carlos Castro

Figure 4.8: WP Leaders of the chemical WPs 10-13 during the SAS-Oden 2021 expedition. (A) Adam Ulfsbo (University of Gothenburg, Sweden), WP Leader and PI of WP10 (CATCHEM), studying an oxygen sample. (B) Lennart Gerke (GEOMAR, Germany), WP Leader of WP11 (VACAO), working in the "Main Lab" of IB Oden. (C) Ian Brow (Plymouth Marine Laboratory, UK), WP Leader of WP12 (TRACE) and WP13 (TGB), after successfully taking water samples from the CTD for onboard methane and nitrous oxide analyses. (A, C) ©SPRS, (B) ©Anna Lunde Hermansson
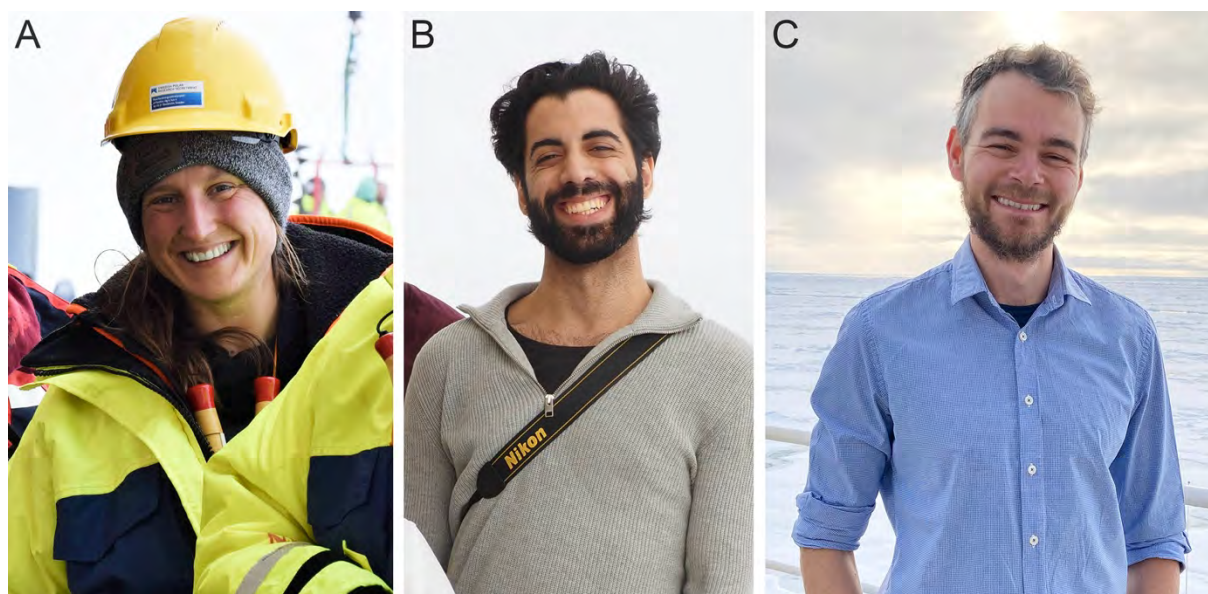
Figure 4.9: WP Leaders of the physical WPs 14-16 during the SAS-Oden 2021 expedition. (A) Oceanographer and acoustician Julia Muchowski (Stockholm University, Sweden), WP Leader of WP14 (MWA). (B) Oceanographer Salar Karam (University of Gothenburg, Sweden), WP Leader of WP15 (WAOW). (C) Meteorologist John Prytherch (Stockholm University, Sweden), WP Leader of WP16 (ACAS). (A) ©Hans-Jørgen Hansen, (B) ©SPRS, (C) ©Sonja Murto

4.8 Opportunistic sampling

So-called "opportunistic samples" were taken during the SAS-Oden 2021 expedition by some of the expedition participants for projects without berths onboard *IB Oden* (*Table 4.3, Chapter 23*). The condition for opportunistic sampling was that these samples should in no way compete with the scientific aims of any of the 16 SO21 projects on board. Like for all other samples taken during the expedition, metadata are available, the final results of the analyses must be submitted to the SND data repository latest by 1 October 2023, and the SPRS and the SAS-Oden 2021 expedition must be acknowledged in any publication of the data as described in *Chapter 7*.

Table 4.3: List of opportunistic sampling during the SAS-Oden 2021 expedition. See Chapter 23 for short project descriptions.

Project and type of samples	PI and affiliation	Person(s) sampling onboard *IB Oden*
Aerosol omics (*Chapter 23.1*) Air, surface water and fog samples for (meta)genomic analyses	Caroline Leck, SU (Sweden) lina@misu.su.se	Pauline Snoeijs-Leijonmalm (WP1+2+7) Frank Menger (WP1) John Prytherch (WP16) Sonja Murto (WP16) Maria Samuelsson (SPRS)
Quantification and isolation of microbes from sediments (*Chapter 23.2*) Sediment samples for viruses and prokaryotes enumeration	Mathias Middelboe, University of Copenhagen (Denmark) mmiddelboe@bio.ku.dk	Hanna Farnelid (WP6+7) Lisa Winberg von Friesen (WP7)
Deep-sea benthic nitrogen fixation (*Chapter 23.3*) Sediment samples for investigation of nitrogen fixation and diazotrophs	Lisa Winberg von Friesen, UCPH (Denmark) lisa.vonfriesen@bio.ku.dk	Hanna Farnelid (WP6+7) Lisa Winberg von Friesen (WP7)
Ice-algal aggregates (*Chapter 23.4*) Algal aggregates found in association with sea ice	Lisa Winberg von Friesen, UCPH (Denmark) lisa.vonfriesen@bio.ku.dk	Lisa Winberg von Friesen (WP7)
Dissolved TRC (*Chapter 23.5*) Water samples for measuring dissolved thiamine-related compounds (TRC)	Lasse Riemann, UCPH (Denmark) lriemann@bio.ku.dk	Hanna Farnelid (WP6+7) Lisa Winberg von Friesen (WP7)
TCHO and TAA (*Chapter 23.6*) Water and ice-habitat samples for total carbohydrates (TCHO) and total amino acids (TAA)	Anja Engel, GEOMAR (Kiel, Germany) aengel@geomar.de	Birthe Zäncker (WP3)
PFAS (*Chapter 23.7*) Water and ice-habitat samples for analyses of per- and polyfluoroalkyl substances (PFAS)	Frank Menger, SLU (Sweden) frank.menger@slu.se	Frank Menger (WP1)

5 The SAS-Oden 2021 Expedition Logbook

5.1 Definition of the SO21 Device Operations

For the SAS-Oden 2021 expedition a "Device Operation" (DO) is defined as either a winch operation from the ship or an ice station (*Table 5.1*). Each DO is coupled to date, time, geographical position and ocean depth in the excel file "SO21_Expedition_Logbook" in the SND data repository[21]. Every single measurement and sample taken during the SAS-Oden 2021 expedition is coupled to a specific DO. For the 16 ice stations, a detailed record of the SAS Core Parameters collected, including basic data measured on the ice, is given in the excel file "SO21_Ice_Station_Logbook" in the SND.

The ice stations next to the ship were accessed by the gangway (*Figure 5.1 A*) or the personnel transfer basket (*Figure 5.1 B*), and are in the Expedition Logbook classified as DOs taking place at the ship station. An ice station reached by helicopter has its own station number since its geographical positions differed from that of the ship. This organization of the field work enables smooth coupling of physical, chemical and biological measurements from the same sampling station to the ship data and to each other, both for the SAS Core Parameters and the project-specific parameters.

Table 5.1: The different types of Device Operations (DOs) performed during the SAS-Oden 2021 expedition, the number of stations covered by each DO type, and the total number of successful DOs. The principal purpose of the DO type is indicated, but project-specific seawater/ice-habitat sampling also took place during most DOs.

Device Operation type	Deployed from	Standard depth	Principal purpose of the Device Operation (besides physical environment)	Nr of stations	Nr of DOs
CTD EK80 SAS	Bow	400 m	EK80 calibration for WP1 and WP14	1	1
CTD deep SAS	Bow	Seafloor	SAS Core Parameters chemistry + deep DNA/RNA	32	32
CTD shallow SAS	Bow	100 m	SAS Core Parameters chemistry	20	20
CTD bio SAS	Stern	1000 m	SAS Core Parameters biology	18	18
CTD omics SAS	Stern	1000 m	SAS Core Parameters RNA/DNA	18	18
CTD wbat EFICA	Stern	1000 m	Project-specific for WP1 (nekton + zooplankton)	11	14
CTD ChlMax PICO	Stern	ChlMax	Project-specific for WP6 (experimental water)	3	3
CTD deep VACAO	Bow	Seafloor	Project-specific for WP11 (chemistry)	6	6
NET multi SAS	Stern	2000 m	SAS Core Parameter mesozooplankton	16	17
NET bongo SAS	Stern	200 m	SAS Core Parameter microzooplankton	11	13
NET beam EFICA	Stern	800 m	Project-specific for WP1 (nekton + zooplankton)	9	45
NET mik EFICA	Stern	800 m	Project-specific for WP1 (nekton + zooplankton)	2	5
LOKI optics EFICA	Stern	1000 m	Project-specific for WP1 (optics zooplankton)	11	11
NET multi FORAM	Stern	1000 m	Project-specific for WP9 (sampling foraminifers)	8	8
NET bongo FORAM	Stern	1000 m	Project-specific for WP9 (sampling foraminifers)	3	3
Box corer SAS	Stern	Seafloor	SAS Core Parameters benthic fauna + DNA/RNA	6	8
Ice station SHIP	Gangway	-	SAS Core Parameters ice habitats	13	14
Ice station HELI	Helicopter	-	SAS Core Parameters ice habitats	3	3
Ice station EFICA	Helicopter	-	Project-specific for WP1 (nekton + zooplankton)	16	16
Ice station ACAS	Helicopter	-	Project-specific for WP16 (gas flux measurements)	5	5
Total number of successful Device Operations					**260**

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[21] https://snd.gu.se/en
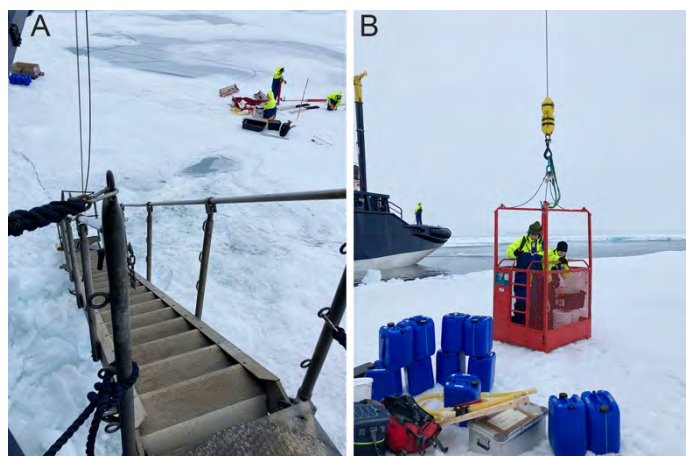
Figure 5.1: Accessing an ice station next to the ship during the SAS-Oden 2021 expedition. (A) Access via the gangway. (B) Access via the personnel transfer basket. (A) ©SPRS, (B) ©Julia Muchowski

5.2 Recording the SO21 Device Operations

Device Operations (DOs) were performed at 60 sampling stations: 36 ship stations and 24 helicopter stations (*Figure 1.1, Table 1.1*). Altogether, 260 successful DOs were carried out during the SAS-Oden 2021 expedition, nine failed due to winch problems (2× CTD deep, 1× multinet 1× LOKI, 1× MIK net, 1× beam net) or stones at the seafloor (3× box core), and two were short CTD casts for testing functionality. The latter 11 DOs are also included in the Expedition Logbook for completion because they had at least started and were given a running number in the log file. Altogether, the Expedition Logbook thus includes 271 DOs. In the Expedition Logbook, each DO number consists of the expedition abbreviation (SO21 = SAS-Oden 2021), the station number (1-60) and the cast number = a running number for each DO that was carried out at the same station. This provides a unique code for each DO that was accomplished. For example, SO21_14-02 denotes the second DO at Station 14 of the SAS-Oden 2021 expedition.

The winch casts from the ship were logged by the seafloor acoustics team of WP14: Carlos Castro (day) and Caroline Bringensparr (night). The helicopter stations were logged by the SPRS meteorological team: Sara Johansson and Per Lundgren, and the ice stations were logged by the Chief Scientist. After the expedition all logs were double-checked by the Chief Scientist and for each DO the average geographical position and average water-column depth were calculated since the ship was drifting with the ice during the DOs. The final Expedition Logbook includes comprehensive information on all DOs, times, positions, station depth, etc. The excel file "SO21_Expedition_Logbook" consists of six sheets (*Table 5.2*). All 271 DOs are listed in *Table 5.3*.

Table 5.2: Organisation of the excel file "SO21_Expedition_Logbook" in the SND data repository.

Sheet name in the excel file	Contents of the sheet
1. Explanations	Explanations of abbreviations, definitions, methods, etc., used in the SO21 Expedition Logbook file
2. All 60 stations	Summary tables of the types and numbers of Device Operations at each station + station map (*Table 1.1* and *Figure 1.1*)
3. All 271 Device Operations	Extended *Table 5.3* with more details
4. Ship station times	Dates and times for the scientific activities at the ship stations (start first Device Operation and end last Device Operation)
5. Details helicopter flights	Dates, times and people on all helicopter flights carried out for scientific activities
6. Notes for acoustics	Notes made on the bridge about the times and geographical positions when acoustic instruments were turned on and off, and other observations relevant for the EK80 hydroacoustics, multibeam and sub-bottom profiler

Table 5.3: All 271 Device Operations (DOs) performed during the SAS-Oden 2021 expedition. The 11 DOs indicated with * were unsuccessful. For explanations of the different DO types, see Table 5.1. UTC = Coordinated Universal Time, DM = Degrees Minutes. Detailed information for each DO is given in the excel file "SO21_Expedition_Logbook" in the SND data repository[22].

Leg	DO number	DO type	Start date	Start time (UTC)	DO Length (min.)	Average longitude (DM)	Average latitude (DM)	Average depth (m)
0	SO21_00-01	CTD EK80 SAS	2021-08-01	21:36	40	18°29.175′E	81°13.738′N	-450
1	SO21_01-01	CTD deep SAS	2021-08-02	09:19	19	26°36.683′E	81°30.156′N	-1190
1	SO21_02-01	ICE station EFICA	2021-08-02	09:33	286	26°36.825′E	81°30.123′N	-1221
1	SO21_03-01	CTD omics SAS	2021-08-03	11:51	60	30°22.070′E	82°01.289′N	-3235
1	SO21_03-02	CTD bio SAS	2021-08-03	16:03	94	30°26.788′E	82°03.809′N	-3235
1	SO21_03-03	NET multi SAS	2021-08-03	18:31	158	30°29.071′E	82°04.204′N	-3224
1	SO21_03-04	CTD TEST *	2021-08-03	21:42	3	30°26.052′E	82°05.005′N	-3224
1	SO21_04-01	ICE station EFICA	2021-08-03	12:22	271	30°22.900′E	82°02.900N	-3243
1	SO21_05-01	CTD shallow SAS	2021-08-04	10:12	24	29°25.608′E	83°05.169′N	-3851
1	SO21_05-02	CTD deep SAS	2021-08-04	11:50	243	29°21.825′E	83°05.751′N	-3892
1	SO21_05-03	CTD deep VACAO	2021-08-04	17:29	189	29°28.859′E	83°07.168′N	-3926
1	SO21_06-01	ICE station HELI	2021-08-04	10:07	359	29°25.464′E	83°05.148′N	-3881
1	SO21_07-01	CTD omics SAS	2021-08-05	09:05	82	30°36.847′E	84°16.610′N	-4000
1	SO21_07-02	NET bongo FORAM	2021-08-05	10:42	63	30°29.555′E	84°16.807′N	-3752
1	SO21_07-03	CTD bio SAS	2021-08-05	12:32	83	30°21.534′E	84°17.308′N	-4001
1	SO21_07-04	CTD shallow SAS	2021-08-05	14:46	39	30°15.270′E	84°18.007′N	-4001
1	SO21_07-05	CTD deep SAS	2021-08-05	16:22	198	30°12.708′E	84°18.459′N	-4001
1	SO21_08-01	CTD ChlMax PICO	2021-08-07	13:21	12	30°11.400′E	86°30.810′N	-2674
1	SO21_08-02	CTD shallow SAS	2021-08-07	15:01	38	30°11.600′E	86°30.810′N	-2581
1	SO21_08-03	CTD deep SAS	2021-08-07	20:18	146	30°38.143′E	86°20.470′N	-2368
1	SO21_08-04	CTD omics SAS	2021-08-08	08:43	83	31°46.849′E	86°23.173′N	-2992
1	SO21_08-05	NET multi SAS	2021-08-08	10:39	133	32°03.770′E	86°23.564′N	-3075
1	SO21_08-06	CTD bio SAS	2021-08-08	13:37	84	32°20.597′E	86°23.762′N	-2616
1	SO21_08-07	NET bongo SAS	2021-08-08	15:15	14	32°25.035′E	86°24.117′N	-2883
1	SO21_08-08	LOKI optics EFICA	2021-08-08	15:51	63	32°26.615′E	86°24.362′N	-2952
1	SO21_08-09	CTD deep VACAO	2021-08-08	21:53	153	33°23.954′E	86°26.851′N	-3179
1	SO21_08-10	ICE station SHIP	2021-08-07	14:20	730	30°11.400′E	86°30.810′N	-2829
1	SO21_09-01	ICE station EFICA	2021-08-07	12:59	1729	30°52.356′E	86°17.988′N	-2821
1	SO21_10-01	ICE station EFICA	2021-08-07	16:12	1684	30°52.704′E	86°18.324′N	-2966
1	SO21_11-01	CTD shallow SAS	2021-08-09	10:53	26	30°46.193′E	87°02.982′N	-3926
1	SO21_11-02	CTD deep SAS *	2021-08-09	13:06	106	31°02.532′E	87°03.141′N	-3647
1	SO21_11-03	CTD TEST *	2021-08-09	16:48	13	31°23.449′E	87°03.261′N	-3722
1	SO21_12-01	ICE station ACAS	2021-08-09	13:17	150	31°02.568′E	87°03.132′N	-3656
1	SO21_13-01	CTD omics SAS	2021-08-10	08:52	68	29°21.175′E	88°02.003′N	-4294
1	SO21_13-02	NET multi SAS	2021-08-10	10:27	127	29°27.381′E	88°02.080′N	-4357
1	SO21_13-03	CTD bio SAS	2021-08-10	13:50	86	29°31.585′E	88°02.249′N	-4252
1	SO21_13-04	CTD shallow SAS	2021-08-10	17:38	50	29°32.452′E	88°02.715′N	-4059
1	SO21_13-05	CTD deep SAS	2021-08-10	19:30	190	29°34.992′E	88°02.960′N	-4061
1	SO21_13-06	ICE station SHIP	2021-08-10	08:30	540	29°21.175′E	88°02.003′N	-4146
1	SO21_14-01	NET beam EFICA	2021-08-11	07:47	65	29°13.911′E	88°30.121′N	-4354
1	SO21_14-02	NET beam EFICA	2021-08-11	09:45	208	29°09.397′E	88°30.145′N	-4354
1	SO21_14-03	NET beam EFICA	2021-08-11	14:27	158	28°58.297′E	88°29.782′N	-4360
1	SO21_14-04	NET beam EFICA	2021-08-11	18:00	70	28°42.360′E	88°29.445′E	-4360
1	SO21_14-05	CTD wbat EFICA	2021-08-11	20:23	91	28°32.608′E	88°29.253′N	-4366
1	SO21_15-01	ICE station EFICA	2021-08-11	15:13	154	28°54.948′E	88°29.712′N	-4360
1	SO21_16-01	CTD omics SAS	2021-08-12	08:37	97	25°05.932′E	88°59.796′N	-4332
1	SO21_16-02	NET multi SAS	2021-08-12	10:34	137	24°50.122′E	88°59.383′N	-4332
1	SO21_16-03	CTD bio SAS	2021-08-12	13:13	105	24°23.936′E	88°58.594′N	-4333
1	SO21_16-04	CTD shallow SAS	2021-08-12	15:38	47	23°53.903′E	88°57.820′N	-4334
1	SO21_16-05	CTD deep SAS	2021-08-12	17:24	206	23°26.775′E	88°57.360′N	-4335
1	SO21_16-06	CTD deep VACAO	2021-08-12	21:59	193	22°43.842′E	88°56.690′N	-4336
1	SO21_17-01	ICE station ACAS	2021-08-12	09:24	116	25°05.490′E	88°59.604′N	-4332
1	SO21_18-01	CTD omics SAS	2021-08-13	08:44	71	23°14.735′E	89°10.752′N	-4301
1	SO21_18-02	NET bongo FORAM	2021-08-13	10:12	62	23°13.238′E	89°10.468′N	-4301
1	SO21_18-03	CTD bio SAS	2021-08-13	11:43	110	23°19.136′E	89°10.120′N	-4309
1	SO21_18-04	CTD shallow SAS	2021-08-13	13:43	37	23°37.915′E	89°09.583′N	-4309
1	SO21_18-05	CTD deep SAS	2021-08-13	15:13	198	23°58.442′E	89°09.113′N	-4318
1	SO21_18-06	ICE station SHIP	2021-08-13	09:03	483	23°14.735′E	89°10.752′N	-4310

1	SO21_19-01	ICE station EFICA	2021-08-13	09:56	270	23°09.996′E	89°10.422′N	-4309
1	SO21_20-01	CTD shallow SAS	2021-08-14	09:09	40	16°59.618′E	89°36.793′N	-4249
1	SO21_20-02	CTD deep SAS	2021-08-14	11:33	226	16°45.809′E	89°36.672′N	-4247
1	SO21_20-03	CTD deep VACAO	2021-08-14	16:32	196	17°00.896′E	89°36.116′N	-4246
1	SO21_21-01	ICE station ACAS	2021-08-14	09:09	126	16°54.804′E	89°36.930′N	-4248
1	SO21_22-01	NET beam EFICA	2021-08-15	07:16	53	35°54.631′E	89°54.924′N	-4241
1	SO21_22-02	NET beam EFICA	2021-08-15	09:58	66	38°09.069′E	89°54.990′N	-4241
1	SO21_22-03	CTD wbat EFICA	2021-08-15	13:50	90	45°29.938′E	89°54.985′N	-4241
1	SO21_22-04	LOKI optics EFICA	2021-08-15	15:37	63	48°24.629′E	89°54.935′N	-4241
1	SO21_22-05	NET bongo SAS	2021-08-15	16:55	25	49°55.241′E	89°54.918′N	-4241
1	SO21_22-06	NET multi SAS	2021-08-15	18:18	264	52°06.313′E	89°54.970′N	-4241
1	SO21_22-07	NET multi SAS *	2021-08-15	23:20	22	58°53.556′E	89°55.199′N	-4241
1	SO21_22-08	CTD shallow SAS	2021-08-16	00:43	42	60°45.290′E	89°55.227′N	-4241
1	SO21_22-09	CTD deep SAS	2021-08-16	03:42	227	62°12.554′E	89°55.173′N	-4241
1	SO21_22-10	CTD omics SAS	2021-08-16	08:44	70	64°48.713′E	89°55.326′N	-4241
1	SO21_22-11	CTD bio SAS	2021-08-16	10:46	108	66°36.549′E	89°55.377′N	-4241
1	SO21_22-12	NET beam EFICA *	2021-08-16	14:23	78	67°21.539′E	89°55.297′N	-4241
1	SO21_22-13	ICE station SHIP	2021-08-16	08:51	579	64°48.713′E	89°55.326′N	-4241
1	SO21_23-01	ICE station EFICA	2021-08-15	09:35	1570	40°10.800′E	89°54.786′N	-4241
1	SO21_24-01	CTD ChlMax PICO	2021-08-18	03:05	48	145°50.963′W	89°30.877′N	-4156
1	SO21_24-02	CTD shallow SAS	2021-08-18	04:35	20	145°51.484′W	89°30.861′N	-4156
1	SO21_24-03	CTD deep SAS	2021-08-18	05:40	206	145°50.960′W	89°30.877′N	-4156
1	SO21_25-01	CTD shallow SAS	2021-08-18	15:47	28	147°59.402′W	89°19.036′N	-3318
1	SO21_25-02	CTD deep SAS	2021-08-18	17:08	169	147°52.294′W	89°19.297′N	-3379
1	SO21_26-01	CTD shallow SAS	2021-08-19	01:21	25	149°58.376′W	89°06.654′N	-1313
1	SO21_26-02	CTD deep SAS	2021-08-19	02:38	87	150°02.487′W	89°06.944′N	-1313
1	SO21_26-03	CTD omics SAS	2021-08-19	09:23	72	149°28.445′W	89°05.836′N	-1319
1	SO21_26-04	NET multi SAS	2021-08-19	11:11	89	149°38.685′W	89°06.074′N	-1328
1	SO21_26-05	CTD bio SAS	2021-08-19	13:11	129	149°53.656′W	89°06.445′N	-1333
1	SO21_26-06	Box corer SAS	2021-08-19	15:55	109	150°06.423′W	89°06.930′N	-1319
1	SO21_26-07	LOKI optics EFICA	2021-08-19	18:50	66	150°15.034′W	89°07.282′N	-1321
1	SO21_26-08	NET bongo SAS	2021-08-19	20:12	14	150°21.419′W	89°07.375′N	-1331
1	SO21_26-09	NET bongo FORAM	2021-08-19	20:45	62	150°24.774′W	89°07.423′N	-1342
1	SO21_26-10	NET multi FORAM	2021-08-19	22:15	82	150°35.605′W	89°07.577′N	-1343
1	SO21_26-11	CTD wbat EFICA	2021-08-20	04:09	109	151°04.785′W	89°08.276′N	-1355
1	SO21_26-12	NET beam EFICA	2021-08-20	07:28	97	151°05.472′W	89°08.397′N	-1359
1	SO21_26-13	NET beam EFICA	2021-08-20	09:26	65	151°17.458′W	89°08.452′N	-1361
1	SO21_26-14	NET beam EFICA	2021-08-20	11:11	69	151°28.458′W	89°08.548′N	-1371
1	SO21_26-15	NET beam EFICA	2021-08-20	13:35	64	151°42.208′W	89°08.698′N	-1385
1	SO21_26-16	ICE station SHIP	2021-08-19	10:09	391	149°28.445′W	89°05.836′N	-1315
1	SO21_27-01	ICE station EFICA	2021-08-19	08:37	1762	149°12.288′W	89°05.586′N	-1346
2	SO21_28-01	CTD shallow SAS	2021-08-21	05:25	49	136°27.121′W	88°44.668′N	-3949
2	SO21_28-02	CTD deep SAS	2021-08-21	07:09	181	136°31.071′W	88°44.583′N	-3949
2	SO21_28-03	CTD deep VACAO	2021-08-21	11:08	169	136°54.079′W	88°44.375′N	-3952
2	SO21_29-01	ICE station ACAS	2021-08-21	13:20	100	137°11.688′W	88°44.526′N	-3949
2	SO21_30-01	CTD shallow SAS	2021-08-21	22:04	44	129°48.264′W	88°30.435′N	-3846
2	SO21_30-02	CTD deep SAS	2021-08-21	23:44	171	129°46.158′W	88°30.640′N	-3943
2	SO21_30-03	CTD wbat EFICA	2021-08-22	04:13	84	129°25.214′W	88°31.351′N	-3936
2	SO21_30-04	NET beam EFICA	2021-08-22	08:48	76	129°18.947′W	88°31.039′N	-3919
2	SO21_30-05	NET beam EFICA	2021-08-22	10:29	64	129°17.130′W	88°31.132′N	-3930
2	SO21_30-06	NET beam EFICA	2021-08-22	12:35	54	129°13.288′W	88°31.240′N	-3930
2	SO21_30-07	NET beam EFICA	2021-08-22	14:05	62	129°08.488′W	88°31.343′N	-3931
2	SO21_30-08	NET beam EFICA	2021-08-22	16:34	69	128°45.668′W	88°31.511′N	-3936
2	SO21_30-09	NET multi SAS	2021-08-22	19:10	274	128°29.510′W	88°31.639′N	-3940
2	SO21_30-10	NET multi SAS	2021-08-23	00:15	34	127°45.984′W	88°33.463′N	-3943
2	SO21_30-11	CTD omics SAS	2021-08-23	08:38	88	127°36.914′W	88°35.371′N	-3942
2	SO21_30-12	LOKI optics EFICA *	2021-08-23	10:26	14	128°06.589′W	88°36.026′N	-3942
2	SO21_30-13	CTD bio SAS	2021-08-23	13:46	113	129°04.905′W	88°37.953′N	-3944
2	SO21_30-14	LOKI optics EFICA	2021-08-23	15:54	63	129°17.720′W	88°38.803′N	-3944
2	SO21_30-15	NET bongo SAS	2021-08-23	17:11	15	129°21.533′W	88°39.234′N	-3944
2	SO21_30-16	CTD wbat EFICA	2021-08-23	19:04	62	129°27.188′W	88°39.538′N	-3944
2	SO21_30-17	ICE station SHIP	2021-08-23	09:00	480	128°46.074′W	88°37.398′N	-3944
2	SO21_30-18	ICE station SHIP	2021-08-23	13:29	619	128°46.074′W	88°37.398′N	-3946
2	SO21_31-01	ICE station EFICA	2021-08-23	08:31	152	128°03.882′W	88°35.586′N	-3942
3	SO21_32-01	CTD deep SAS *	2021-08-24	10:57	65	115°28.015′W	88°22.017′N	-2979
3	SO21_32-02	CTD deep SAS	2021-08-24	13:14	148	115°41.493′W	88°23.195′N	-2889
3	SO21_33-01	CTD shallow SAS	2021-08-25	02:24	43	100°36.257′W	88°05.883′N	-2948
3	SO21_33-02	CTD deep SAS	2021-08-25	04:10	160	100°44.832′W	88°06.014′N	-2964
3	SO21_33-03	CTD omics SAS	2021-08-25	09:04	73	101°48.578′W	88°06.737′N	-3049
3	SO21_33-04	NET multi SAS	2021-08-25	10:37	128	102°00.482′W	88°07.389′N	-3018
3	SO21_33-05	CTD bio SAS	2021-08-25	13:13	109	102°00.896′W	88°08.326′N	-2987

3	SO21_33-06	NET multi FORAM	2021-08-25	15:23	64	101°54.542′W	88°08.579′N	-2987
3	SO21_33-07	LOKI optics EFICA	2021-08-25	16:59	65	101°57.940′W	88°08.560′N	-2987
3	SO21_34-01	ICE station HELI	2021-08-25	08:58	458	101°47.010′W	88°06.690′N	-3015
3	SO21_35-01	NET beam EFICA	2021-08-26	06:53	57	87°24.622′W	87°52.510′N	-1492
3	SO21_35-02	NET beam EFICA	2021-08-26	08:25	75	87°20.358′W	87°52.445′N	-1500
3	SO21_35-03	NET beam EFICA	2021-08-26	10:06	67	87°12.344′W	87°52.307′N	-1495
3	SO21_35-04	NET beam EFICA	2021-08-26	11:30	64	87°05.140′W	87°52.151′N	-1489
3	SO21_35-05	NET beam EFICA	2021-08-26	13:15	58	86°55.165′W	87°51.827′N	-1480
3	SO21_35-06	NET beam EFICA	2021-08-26	15:18	70	86°45.673′W	87°51.379′N	-1472
3	SO21_35-07	NET beam EFICA	2021-08-26	16:52	53	86°42.210′W	87°51.114′N	-1471
3	SO21_35-08	CTD wbat EFICA	2021-08-26	20:10	82	86°34.127′W	87°50.843′N	-1456
3	SO21_35-09	NET multi SAS	2021-08-26	21:51	88	86°24.644′W	87°50.727′N	-1440
3	SO21_35-10	LOKI optics EFICA	2021-08-26	23:38	65	86°13.889′W	87°50.562′N	-1430
3	SO21_35-11	CTD shallow SAS	2021-08-27	00:50	33	86°07.006′W	87°50.423′N	-1426
3	SO21_35-12	CTD deep SAS	2021-08-27	02:19	89	86°00.691′W	87°50.269′N	-1412
3	SO21_35-13	CTD omics SAS	2021-08-27	08:31	68	85°39.676′W	87°50.043′N	-1388
3	SO21_35-14	NET bongo SAS	2021-08-27	09:52	14	85°33.976′W	87°50.027′N	-1383
3	SO21_35-15	CTD bio SAS	2021-08-27	10:48	106	85°29.556′W	87°50.035′N	-1389
3	SO21_35-16	ICE station SHIP	2021-08-27	06:00	630	87°24.622′W	87°52.510′N	-1432
3	SO21_36-01	ICE station EFICA	2021-08-26	09:23	1581	87°12.654′W	87°52.008′N	-1444
4	SO21_37-01	CTD deep SAS	2021-08-27	20:00	142	76°32.885′W	87°47.376′N	-2559
4	SO21_38-01	NET beam EFICA	2021-08-28	07:03	60	67°13.685′W	87°44.426′N	-1162
4	SO21_38-02	NET beam EFICA	2021-08-28	08:32	53	67°09.616′W	87°44.314′N	-1162
4	SO21_38-03	NET beam EFICA	2021-08-28	10:02	47	67°07.200′W	87°44.230′N	-1162
4	SO21_38-04	NET beam EFICA	2021-08-28	11:20	54	67°05.297′W	87°44.233′N	-1162
4	SO21_38-05	NET beam EFICA	2021-08-28	12:57	38	67°03.054′W	87°44.281′N	-1162
4	SO21_38-06	NET beam EFICA	2021-08-28	14:05	52	66°57.972′W	87°44.402′N	-1170
4	SO21_38-07	NET beam EFICA	2021-08-28	15:26	53	66°52.899′W	87°44.499′N	-1178
4	SO21_38-08	CTD wbat EFICA	2021-08-28	17:38	97	66°42.746′W	87°44.601′N	-1178
4	SO21_38-09	NET multi SAS	2021-08-28	19:32	72	66°35.467′W	87°44.649′N	-1178
4	SO21_38-10	LOKI optics EFICA	2021-08-28	21:04	66	66°31.559′W	87°44.704′N	-1179
4	SO21_38-11	NET multi FORAM	2021-08-28	22:24	64	66°29.301′W	87°44.800′N	-1181
4	SO21_38-12	Box corer SAS	2021-08-29	00:15	81	66°25.691′W	87°45.013′N	-1187
4	SO21_38-13	CTD shallow SAS	2021-08-29	03:00	28	66°17.203′W	87°45.516′N	-1186
4	SO21_38-14	CTD deep SAS	2021-08-29	04:15	91	66°09.581′W	87°45.876′N	-1187
4	SO21_38-15	CTD omics SAS	2021-08-29	08:34	75	65°50.086′W	87°46.518′N	-1201
4	SO21_38-16	NET bongo SAS	2021-08-29	09:59	13	65°44.663′W	87°46.706′N	-1201
4	SO21_38-17	CTD bio SAS	2021-08-29	10:51	98	65°41.240′W	87°46.824′N	-1198
4	SO21_38-18	ICE station SHIP	2021-08-29	06:00	360	67°13.685′W	87°44.426′N	-1194
4	SO21_39-01	ICE station EFICA	2021-08-28	08:49	1574	67°16.098′W	87°44.430′N	-1162
5	SO21_40-01	CTD deep SAS	2021-08-29	20:36	96	63°16.784′W	87°20.559′N	-1169
5	SO21_41-01	CTD deep SAS	2021-08-30	06:25	94	59°16.942′W	86°55.934′N	-1140
5	SO21_42-01	CTD deep SAS	2021-08-30	18:05	69	57°23.484′W	86°30.876′N	-562
5	SO21_42-02	CTD wbat EFICA	2021-08-30	19:42	58	57°20.241′W	86°31.011′N	-595
5	SO21_42-03	NET multi SAS	2021-08-30	20:55	35	57°17.054′W	86°31.110′N	-600
5	SO21_42-04	LOKI optics EFICA	2021-08-30	21:45	39	57°15.805′W	86°31.125′N	-610
5	SO21_42-05	NET multi FORAM	2021-08-30	22:30	39	57°13.941′W	86°31.147′N	-618
5	SO21_42-06	CTD omics SAS	2021-08-30	23:27	47	57°11.410′W	86°31.167′N	-631
5	SO21_42-07	NET bongo SAS	2021-08-31	00:30	18	57°08.133′W	86°31.207′N	-660
5	SO21_42-08	CTD bio SAS	2021-08-31	01:19	75	57°06.000′W	86°31.250′N	-660
5	SO21_42-09	NET beam EFICA	2021-08-31	07:22	42	56°36.683′W	86°31.016′N	-695
5	SO21_42-10	NET beam EFICA	2021-08-31	08:35	44	56°30.984′W	86°30.744′N	-695
5	SO21_42-11	NET beam EFICA	2021-08-31	09:36	52	56°26.594′W	86°30.455′N	-695
5	SO21_42-12	NET beam EFICA	2021-08-31	10:59	43	56°21.477′W	86°30.058′N	-695
5	SO21_42-13	NET beam EFICA	2021-08-31	12:15	55	56°16.589′W	86°29.669′N	-695
5	SO21_42-14	NET beam EFICA	2021-08-31	13:35	47	56°13.146′W	86°29.472′N	-695
5	SO21_42-15	NET beam EFICA	2021-08-31	14:54	45	56°08.557′W	86°29.313′N	-700
5	SO21_42-16	Box corer SAS *	2021-08-31	19:16	31	55°47.773′W	86°29.153′N	-705
5	SO21_42-17	Box corer SAS *	2021-08-31	20:01	30	55°43.824′W	86°29.083′N	-699
5	SO21_42-18	ICE station SHIP	2021-08-30	20:00	360	57°20.241′W	86°31.011′N	-628
5	SO21_43-01	ICE station EFICA	2021-08-30	18:56	1297	57°27.546′W	86°30.666′N	-634
6	SO21_44-01	CTD deep SAS	2021-09-01	11:24	170	43°09.776′W	86°17.070′N	-3310
6	SO21_45-01	CTD deep SAS	2021-09-01	19:54	166	39°58.988′W	86°02.781′N	-3161
6	SO21_46-01	CTD deep SAS	2021-09-02	05:03	161	38°28.831′W	85°50.769′N	-3253
6	SO21_46-02	CTD deep VACAO	2021-09-02	08:52	144	38°25.171′W	85°50.914′N	-3253
6	SO21_47-01	CTD deep SAS	2021-09-02	18:50	149	35°40.597′W	85°28.695′N	-2716
6	SO21_48-01	CTD deep SAS	2021-09-03	09:24	95	33°27.496′W	84°56.537′N	-1496
6	SO21_48-02	CTD omics SAS	2021-09-03	13:00	69	33°29.757′W	84°55.516′N	-1547
6	SO21_48-03	NET multi SAS	2021-09-03	14:24	102	33°28.536′W	84°55.516′N	-1552
6	SO21_48-04	CTD bio SAS	2021-09-03	16:24	100	33°28.724′W	84°55.495′N	-1554
6	SO21_48-05	NET multi FORAM	2021-09-03	18:29	61	33°29.936′W	84°55.553′N	-1548

6	SO21_48-06	Box corer SAS	2021-09-03	20:03	62	33°30.345′W	84°55.654′N	-1544
6	SO21_49-01	ICE station HELI	2021-09-03	10:20	490	33°26.246′W	84°56.562′N	-1527
6	SO21_50-01	NET beam EFICA	2021-09-04	10:10	49	32°02.270′W	84°09.389′N	-893
6	SO21_50-02	NET beam EFICA	2021-09-04	11:32	47	32°02.029′W	84°09.130′N	-887
6	SO21_50-03	NET beam EFICA	2021-09-04	12:50	54	32°03.765′W	84°08.981′N	-884
6	SO21_50-04	NET beam EFICA	2021-09-04	14:11	58	32°06.776′W	84°08.932′N	-883
6	SO21_50-05	NET beam EFICA	2021-09-04	15:35	65	32°10.232′W	84°08.919′N	-886
6	SO21_50-06	CTD wbat EFICA	2021-09-04	17:37	102	32°15.508′W	84°09.100′N	-895
6	SO21_50-07	NET multi SAS	2021-09-04	20:09	54	32°19.146′W	84°09.506′N	-896
6	SO21_50-08	LOKI optics EFICA	2021-09-04	21:16	57	32°19.244′W	84°09.708′N	-891
6	SO21_50-09	NET bongo SAS	2021-09-04	22:27	12	32°19.113′W	84°09.724′N	-890
6	SO21_50-10	NET bongo SAS	2021-09-04	23:04	9	32°19.075′W	84°09.722′N	-891
6	SO21_50-11	CTD omics SAS	2021-09-04	23:27	53	32°19.139′W	84°09.710′N	-890
6	SO21_50-12	NET multi FORAM	2021-09-05	00:39	42	32°19.882′W	84°09.644′N	-889
6	SO21_50-13	CTD bio SAS	2021-09-05	01:43	96	32°21.323′W	84°09.566′N	-889
6	SO21_50-14	Box corer SAS	2021-09-05	03:45	44	32°25.393′W	84°09.603′N	-889
6	SO21_50-15	Box corer SAS	2021-09-05	06:03	39	32°28.786′W	84°10.031′N	-899
6	SO21_50-16	CTD deep SAS	2021-09-05	08:13	81	32°23.466′W	84°09.649′N	-892
6	SO21_50-17	ICE station SHIP	2021-09-04	13:30	330	32°05.719′W	84°08.932′N	-892
6	SO21_51-01	ICE station EFICA	2021-09-04	08:41	1329	32°06.504′W	84°09.336′N	-893
7	SO21_52-01	CTD deep SAS	2021-09-05	13:55	89	27°15.576′W	84°16.084′N	-1054
7	SO21_53-01	NET multi SAS	2021-09-05	19:59	70	23°56.034′W	84°26.456′N	-996
7	SO21_53-02	LOKI optics EFICA	2021-09-05	21:22	57	23°57.117′W	84°26.729′N	-996
7	SO21_53-03	NET bongo SAS	2021-09-05	22:34	12	23°58.906′W	84°27.430′N	-996
7	SO21_53-04	NET multi FORAM	2021-09-05	23:02	75	23°59.171′W	84°27.712′N	-996
7	SO21_53-05	CTD wbat EFICA	2021-09-06	00:26	84	23°59.420′W	84°28.413′N	-1250
7	SO21_53-06	CTD wbat EFICA	2021-09-06	01:51	84	23°59.420′W	84°28.413′N	-1250
7	SO21_53-07	CTD wbat EFICA	2021-09-06	03:16	78	23°59.358′W	84°28.766′N	-1250
7	SO21_53-08	CTD omics SAS	2021-09-06	10:55	29	24°17.239′W	84°29.872′N	-1351
7	SO21_53-09	CTD bio SAS	2021-09-06	12:30	97	24°23.569′W	84°30.919′N	-1351
7	SO21_53-10	Box corer SAS *	2021-09-06	14:30	60	24°27.123′W	84°31.259′N	-1353
7	SO21_53-11	Box corer SAS	2021-09-06	16:56	61	24°31.712′W	84°31.204′N	-1364
7	SO21_53-12	NET mik EFICA	2021-09-06	18:51	62	24°40.649′W	84°31.473′N	-1364
7	SO21_53-13	NET mik EFICA	2021-09-06	20:20	64	24°40.649′W	84°31.473′N	-1364
7	SO21_53-14	CTD shallow SAS	2021-09-06	22:10	25	24°42.356′W	84°31.745′N	-1364
7	SO21_53-15	CTD deep SAS	2021-09-06	22:59	98	24°44.026′W	84°31.814′N	-1391
7	SO21_53-16	ICE station SHIP	2021-09-06	10:00	420	24°11.884′W	84°29.459′N	-1364
7	SO21_54-01	ICE station EFICA	2021-09-05	20:05	1578	23°54.414′W	84°25.794′N	-1180
7	SO21_55-01	ICE station ACAS	2021-09-06	16:07	130	24°30.666′W	84°31.236′N	-1360
7	SO21_56-01	CTD omics SAS	2021-09-08	12:53	66	2°30.378′W	83°52.135′N	-2650
7	SO21_56-02	NET bongo SAS	2021-09-08	14:17	14	2°32.376′W	83°51.758′N	-2652
7	SO21_56-03	CTD bio SAS	2021-09-08	15:15	112	2°34.176′W	83°51.426′N	-2654
7	SO21_56-04	NET multi SAS	2021-09-08	17:23	136	2°41.033′W	83°50.638′N	-2654
7	SO21_56-05	CTD wbat EFICA	2021-09-08	20:00	93	2°53.705′W	83°49.956′N	-2654
7	SO21_56-06	CTD ChlMax PICO	2021-09-08	22:14	7	3°01.514′W	83°49.704′N	-3028
7	SO21_56-07	CTD shallow SAS	2021-09-08	23:16	27	3°03.916′W	83°49.569′N	-3028
7	SO21_56-08	CTD deep SAS	2021-09-09	00:18	149	3°05.429′W	83°49.408′N	-3008
7	SO21_56-09	ICE station SHIP	2021-09-08	13:00	300	02°30.541′W	83°52.109′N	-2652
7	SO21_57-01	ICE station EFICA	2021-09-08	13:13	633	02°31.752′W	83°52.296′N	-2839
7	SO21_58-01	NET beam EFICA	2021-09-10	08:56	52	9°26.760′E	82°29.311′N	-1162
7	SO21_58-02	NET beam EFICA	2021-09-10	10:32	52	9°22.828′E	82°28.520′N	-1162
7	SO21_58-03	NET beam EFICA	2021-09-10	12:15	61	9°14.351′E	82°27.752′N	-1215
7	SO21_58-04	NET beam EFICA	2021-09-10	13:45	54	9°10.988′E	82°27.573′N	-1215
7	SO21_58-05	NET mik EFICA *	2021-09-10	16:28	48	8°57.611′E	82°27.880′N	-1268
7	SO21_58-06	NET mik EFICA	2021-09-10	17:20	69	8°53.282′E	82°28.182′N	-1268
7	SO21_58-07	NET mik EFICA	2021-09-10	18:50	71	8°49.579′E	82°28.373′N	-1378
7	SO21_58-08	NET mik EFICA	2021-09-10	20:19	70	8°45.943′E	82°28.497′N	-1497
7	SO21_58-09	CTD omics SAS	2021-09-10	21:54	79	8°42.821′E	82°28.438′N	-1506
7	SO21_58-10	NET bongo SAS	2021-09-10	23:24	16	8°41.569′E	82°28.146′N	-1506
7	SO21_58-11	NET bongo SAS	2021-09-10	23:57	12	8°41.353′E	82°28.064′N	-1506
7	SO21_58-12	CTD bio SAS	2021-09-11	00:53	111	8°42.161′E	82°27.749′N	-1274
7	SO21_58-13	NET multi SAS	2021-09-11	02:58	89	8°45.029′E	82°26.660′N	-1259
7	SO21_58-14	LOKI optics EFICA	2021-09-11	04:50	64	8°46.467′E	82°25.653′N	-1209
7	SO21_58-15	CTD wbat EFICA	2021-09-11	06:25	117	8°45.285′E	82°24.272′N	-1168
7	SO21_58-16	NET multi FORAM	2021-09-11	09:32	70	8°35.168′E	82°22.534′N	-982
7	SO21_58-17	CTD shallow SAS	2021-09-11	11:40	22	8°23.950′E	82°22.117′N	-983
7	SO21_58-18	CTD deep SAS	2021-09-11	12:42	88	8°19.027′E	82°22.121′N	-1015
7	SO21_58-19	ICE station SHIP	2021-09-10	10:00	1410	9°24.702′E	82°28.809′N	-1215
7	SO21_59-01	ICE station EFICA	2021-09-10	09:45	1448	9°25.770′E	82°29.010′N	-1072
7	SO21_60-01	Box corer SAS	2021-09-11	17:09	45	9°07.970′E	82°15.549′N	-717
7	SO21_60-02	Box corer SAS	2021-09-11	18:31	38	9°08.725′E	82°15.762′N	-713

5.3 Planned and realized sampling

The SAS-Oden 2021 expedition was the first *IB Oden* expedition with a fully integrated ecological (biological, chemical, physical) approach in the SO21 joint ecosystem study. The original sampling plan as described in the "Scope of Work" was quite over-ambitious and some crucial factors that increased station time, and thereby decreased the number of ship stations, were not realized at the time of planning.

The original sampling plan included 60 ship stations and 57 expedition days: 14 days transit time between Sweden and the CAO, 23.5 days station time for sampling in the CAO, and 19.5 days steaming time between stations (*Table 5.4*). Only 36 ship stations of the original plan could be realized. The transit from Sweden to the marginal ice zone took 6.5 days and on 1 August the ship was drifting for calibration of the acoustic equipment and repacking of laboratory equipment that could not be performed before the expedition because of Covid19 or during transit because of waves.

The early transit back to Sweden due to weather conditions caused 48 hours loss of sampling time whereas the steaming time between stations fitted very well the calculations that were made prior to the expedition. Ice station work always took place simultaneous with winch operations from the ship, either by helicopter or by access to the ice from the ship. Only on two occasions the ice station ended later than the winch operations and the total expedition time loss because of this was less than three hours. Looking back, we calculated that the expedition should have been ca. 10 days longer in the CAO to cover 60 ship stations instead of the 36 ship stations realized.

Table 5.4: Planned and realized time for sampling stations and steaming during the SAS-Oden 2021 expedition.

Activity	Planned in the "Scope of Work" (days)	Realized SAS-Oden 2021 (days)	Difference
Transit Sweden - CAO	7	7	
Station time	23.5	21.4	- 2 days
Steaming between stations	19.5	19.6	
Transit CAO - Sweden	7	9	+2 days
Total days	**57**	**57**	

Besides the early transit home, the discrepancy between the number of planned and realized Device Operations (*Table 5.5*) was mainly due to the assumption during expedition planning that winch operations from the bow and stern could alternate so that winch time would be as efficient as possible. However, for winch operations from the bow the ship needed to be stable in the ice with the stern, and for winch operations from the stern the ship needed to be stable in the ice with the bow (*Figure 5.2*). Time for repositioning the ship at a station was not accounted for in the preparatory "Scope of Work" document. It could take up to three hours before a stable ship position was achieved, both when arriving at a new station and when turning the ship at a sampling station in-between winch operations from bow and stern. Since we could not work from the bow and the stern without turning the ship, time was also lost at the bow for emptying the Niskin bottles from the CTD shallow SAS before it could be deployed for the second time (CTD deep SAS). At the stern the discrepancy of emptying the Niskin bottles was partly solved by alternating between CTD and net sampling, but the exchange of sampling gear on the winch also took time that was not foreseen because we had planned to exchange gear at the stern while the bow CTD was down. Altogether, this costed 4-5 days of expedition time. This was the first time many winch operations were carried out at many stations both from the bow and the stern of *IB Oden*, and for coming expeditions it should be considered what is most efficient time-wise for each specific expedition, i.e., winch operations from both bow and stern or only from the stern.

Figure 5.2: IB Oden *in sampling position for winch operations from the stern for which it was required that the bow was fixed stable in the ice and vice versa. ©Julia Muchowski*

Another reason for loss of sampling time (*Table 5.5*) was that the EFICA (WP1) beam net could only be deployed for max. 12 hours at one station and not for 16 hours as planned. Instead, 14 extra WP1 CTDs were deployed to obtain more acoustic data from the EFICA WBAT.

Additionally, eight hours were lost due to winch casts that failed, but in reality more time was lost due to attempts to repair the winch while the ship was waiting. It appeared that the ship's "North Sea winch" could not be repaired due to a break-down of a motherboard, and after SO21 Station 8 all sampling from the stern was performed with a spare winch. WP1 also lost equipment due to a motherboard crash (one of the EFICA WBATs). This emphasizes that motherboards are among the weakest links on scientific expeditions to remote places and that equipment/motherboard back-ups can be crucial for success.

Table 5.5: Planned and realized Device Operations by winch from the ship during the SAS-Oden 2021 expedition.

Device Operation type	Nr planned	Nr realized	Difference	% Realized
Calibration EK80, incl. CTD	1	1	0	100%
CTD deep SAS	60	32	-28	53%
CTD shallow SAS	46	20	-26	43%
CTD bio SAS	30	18	-12	60%
CTD omics SAS	30	18	-12	60%
CTD wbat EFICA	0	14	14	Extra (instead of beam net)
CTD ChlMax PICO	0	3	3	100%
CTD deep VACAO	6	6	0	100%
NET multi SAS	30	17	-13	57%
NET bongo SAS	12	13	1	108%
NET beam EFICA	90	45	-45	50%
NET mik EFICA	0	5	5	Extra (instead of beam net)
LOKI optics EFICA	12	11	-1	92%
NET FORAM (multinet/bongo net)	15	11	3	73%
Box corer SAS	5	6	3	120%

6 Descriptions of the Device Operations

6.1 General information about the SO21 Device Operations

This chapter describes all Device Operations, i.e., winch operations from the ship and SAS ice station work carried out during the SAS-Oden 2021 expedition. Project-specific field work not involving winch operations, i.e., ice work outside the SAS programme, are described in the respective WP chapters (*8-22*).

Most of the winch operations took place after breakfast (8:15) and before dinner (17:45) and, unless the ship was in transit, evening/night work occurred as well. Very often the daily planning had to be revised due to weather conditions. The main reason for cancelled winch operations, especially deployment of the large beam net, was high wind speed. Other reasons were repositioning of the ship and in a few cases technical problems with a winch. The main reasons for breaking off ice stations and resuming them later were fog and (rarely) polar bears. Since the winch operations at the ice stations took much longer time than the ice work, resuming ice stations delayed the expedition only marginally: on two occasions the ice station ended later than the winch operations and the total expedition time loss because of this was altogether less than three hours.

Sampling for biological parameters was as much as possible carried out at specific times of day to avoid the effect of possible diel migration even if there was 24 hours of sunlight on every expedition day. The CTD bio and the CTD omics were usually taken around noon, and the net operations were usually performed during the day (beam and MIK net) or in the evening (plankton nets).

6.2 CTD at the bow

The CTD + rosette at the bow (*Figure 6.1*) was used to achieve full-depth profiles of oceanographic, chemical, and biological (virus and prokaryote abundances) parameters from the 24 SAS standard depths defined in the international SAS Science and Implementation Plan (*Table 6.1*). Since two Niskin bottle spaces were used for LADCP equipment by WP14 (MWA) and often deep-water Niskin-bottle water was needed for the SO21 omics collaboration (*Chapter 7.4*) and WP3 (ProMis), water sampling was divided over two CTD casts for water depth deeper than 2000 m: "CTD shallow SAS" with sampling in the upper 100-200 m and "CTD deep SAS" with sampling between the seafloor and 100-200 m. Six deep "CTD deep VACAO" were taken specifically for WP11 (VACAO). When water depth at a station was less than 2000 m, one CTD cast was sufficient to cover all the water samples needed by all projects.

The bow CTD was a standard SeaBird SBE911 plus system provided by the SPRS with dual sensors to measure temperature, salinity and single sensors measuring pressure (depth) and oxygen. The bow CTD was further equipped with an altimeter (SPRS) to measure the distance to the seafloor to avoid that the CTD would hit the seafloor. In the beginning of the expedition also a chlorophyll fluorometer (SPRS) was mounted on the bow CTD, but was later moved to the stern CTD. In the end of the expedition a turbidity sensor (SPRS) was moved from the stern CTD to the bow CTD. A detailed account of the different CTD configurations is provided in *Chapter 21*. The bow CTD was also equipped by the SPRS with two LADCPs (Lowered Acoustic Doppler Current Profiler) targeting current speed, a CDOM sensor (Cyclops-6K 6000m version targeting coloured dissolved organic matter), and a PAR sensor (Photosynthetically Active Radiation). Additionally, WP1 (EFICA) mounted a stand-alone battery-driven UVP (Underwater Vision Profiler, UVP6-HF) on the bow CTD.

The water budget sheet of each bow CTD cast was prepared by Adam Ulfsbo (WP10). The order of sampling was determined by the risk of contamination, i.e., starting with CFC-12 and SF_6 (WP11), O_2 (WP10), CH_4/N_2O (WP12+13), DIC, pH/TA, nutrients, $\delta^{18}O$ of H_2O, DOC, and ending with POC (WP10), prokaryote abundance (WP2) and viral abundance (WP4). The deck operations for the bow CTD were coordinated by Julia Muchowski (day shift), Alexandra Padilla (day shift), and Salar Karam (night shift). During deployment and recovery, other scientists helped to move the CTD to and from the CTD container. The winch was operated by Joachim Gyllestad, Hans-Jørgen Hansen, Niklas Vestin, Anton Sandström and/or Sven Lidström (SPRS). Altogether, 62 successful CTD casts were made from the bow (*Table 5.3*), i.e., all 20 CTD shallow SAS, all 32 CTD deep SAS, all six CTD deep VACAO, two CTD ChlMax PICO (SO21_08-01, SO21_24-01), one CTD omics SAS (SO21_03-01), and one CTD EK80 SAS (SO21_00-01) for calibration of the EK80 echosounder. The SO21 projects involved in further analyses of water samples and sensor data from the bow CTD are listed in *Table 6.2*.

*Table 6.1: The 24 SAS standard depths for sampling of the SAS Core Parameters[23] by the bow CTD during the SAS-Oden 2021 expedition. * = SAS standard depths of the deep samples for the SO21 omics collaboration.*

Niskin number	Depth (m)	Niskin number	Depth (m)	Niskin number	Depth (m)	Niskin number	Depth (m)
24	10	18	100	12	400	6	2500
23	20	17	125	11	500	5	3000*
22	30	16	150	10	700	4	3500
21	40	15	200	9	1000	3	4000
20	50	14	250	8	1500	2	bottom-50
19	75	13	300	7	2000*	1	bottom*
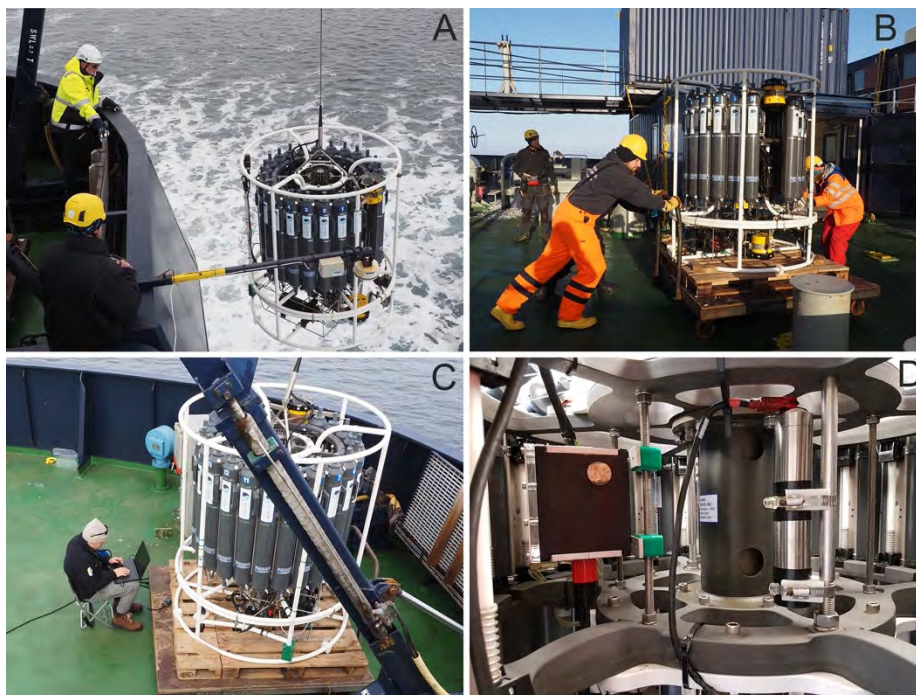
Figure 6.1: Sampling with the bow CTD during the SAS-Oden 2021 expedition. (A) SPRS technicians Joachim Gyllestad and Hans-Jørgen Hansen launching the CTD. (B) CTD retrieval by Anna Lunde Hermansson (WP10) and Lennart Gerke (WP11). (C) Julek Chawarski (WP1) downloading UVP data. (D) Inner view of the bow CTD showing. a.o., the IMP magnetometer and accelerometer. (A,B,C) ©SPRS, (D) ©Yannis Arck

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[23] Synoptic Arctic Survey - a pan-Arctic Research Program. Science and Implementation Plan (2017) [https://synopticarcticsurvey.w.uib.no]

Table 6.2: Projects involved in further analyses of sensor data and water samples from the bow CTD + rosette. These further analyses are described in the respective WP chapters (8-21) in this report.

WP	Project	Scientific purpose	Sensor data required by project
WP1-9	SO21 omics	Metagenomics and metatranscriptomics (*Chapter 7.4*)	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP1	EFICA	Ecology: nekton and zooplankton	CTD, PAR, CHL, CDOM, UVP, oxygen, turbidity
WP2	MIME	Ecology: microbes	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP3	ProMis	Ecology: fungi	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP4	VIRUS	Ecology: viruses	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP6	PICO	Ecology: picophytoplankton	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP7	PHYTO	Ecology: phytoplankton	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP8	ZOO	Ecology: zooplankton	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP10	CATCHEM	Chemistry: carbon and nutrient chemistry	CTD, PAR, CHL, CDOM, oxygen, turbidity
WP11	VACAO	Chemistry: tracer chemistry	CTD, PAR, oxygen, turbidity
WP12/13	TRACE/TGB	Chemistry: methane and nitrous oxide chemistry	CTD, PAR, oxygen, turbidity
WP14	MWA	Oceanography: midwater acoustics	CTD, LADCP, UVP, turbidity
WP15	WAOW	Oceanography: deep-water hydrography	CTD, turbidity

6.3 CTD at the stern

The CTD + rosette at the stern (*Figure 6.2*) was used to achieve oceanographic, chemical and biological profiles down to 1000 m. The water samples were taken at selected depths according to the international SAS Science and Implementation Plan (*Table 6.1*), except that the biologically relevant variable depth of the chlorophyll maximum (ChlMax) as determined by a fluorometer on the CTD, and the variable depth of the temperature maximum in the Atlantic Water Layer (TempMax) as measured by the CTD, were included for sampling and some other depths were left out. If more than one mesopelagic temperature peak occurred, the deepest peak was selected as the TempMax. During the SAS-Oden 2021 expedition, the ChlMax ranged between 12 and 45 m of depth (average 28 m) and the TempMax ranged between 200 and 411 m of depth (average 325 m) (*Table 6.3*).

Water sampling for the SAS Core Parameters was divided over two CTD casts: "CTD bio SAS" with two Niskin bottles taken from 12 depths (10 m, ChlMax, 30 m, 40 m, 50 m, 75 m, 100 m, 125, 150 m, 200 m, TempMax, 500 m) for biological and biogeochemical parameters (WP2, WP5, WP6, WP7), and "CTD omics SAS" with six Niskin bottles taken from four depths (ChlMax, 100 m, TempMax, 1000 m) for DNA and RNA sampling within the SO21 omics collaboration (*Chapter 7.4*). Water for project-specific experimental work by WP2 (MIME) and WP5 (ASAP) was taken from the CTD bio or CTD omics, and when larger volumes were required, water was taken from the "CTD wbat casts" for WP1 (EFICA) hydroacoustic measurements. The SO21 projects involved in further analyses of water samples and sensor data from the bow CTD are listed in *Table 6.4*.

The stern CTD was a standard SeaBird SBE911 plus system provided by the SPRS with dual sensors to measure temperature, salinity and single sensors measuring pressure (depth) and oxygen. A difference with the rosette at the stern was the closing mechanism of the Niskin bottles; the bow bottles had external metal springs and the stern bottles had internal rubber bands. The latter closing mechanisms were unfortunately prone to leakage which made water sampling from the rosette at the stern problematic. For most of the expedition the stern CTD was further equipped with a chlorophyll fluorometer and a turbidity sensor. However, the chlorophyll fluorometer (SPRS) was mounted on the bow CTD in the beginning of the expedition, and the turbidity sensor (SPRS) was mounted on the bow CTD in the end of the expedition. A detailed account of these different CTD configurations is provided

in *Chapter 21*. Additionally, WP1 (EFICA) equipped the stern CTD with stand-alone equipment: light sensor (TDR-Mk9-404A tag), deep-sea video camera targeting nekton and zooplankton (FishCam), and WBAT (Wideband Autonomous Transceiver) with two frequencies (38 kHz targeting nekton, 333 kHz targeting zooplankton). These frequencies were alternated between CTD casts (one frequency/cast).

Table 6.3: Depths at which water samples from the chlorophyll maximum in the surface water layer (ChlMax) and the temperature maximum in the Atlantic Water Layer (TempMax) were taken for the biological SAS Core Parameters and project-specific work. The sampling depths were decided by the CTD operator as measured by the CTD (chlorophyll fluorometer maximum, lowest temperature peak) when the CTD was on its way down. UTC = Coordinated Universal Time. CTD depth = according to the deck sheet, the exact depth should still be verified by the results of the CTD measurements. EK80 = average station depth as recorded by the EK80 echosounder on the bridge (cf. Table 5.3).

Leg	Device Operation	Description	Start date	Start time (UTC)	Duration (min)	ChlMax (m)	TempMax (m)	CTD depth (m)	EK80 depth (m)
1	SO21_03-01	CTD omics SAS	2021-08-03	11:51	60	-12	-200	-500	-3235
1	SO21_03-02	CTD bio SAS	2021-08-03	16:03	94	-12	-200	-500	-3235
1	SO21_07-01	CTD omics SAS	2021-08-05	09:05	82	-12	-205	-1000	-4000
1	SO21_07-03	CTD bio SAS	2021-08-05	12:32	83	-12	-200	-1000	-4001
1	SO21_08-01	CTD ChlMax PICO	2021-08-07	13:21	12	-24	No sample	-24	-2674
1	SO21_08-04	CTD omics SAS	2021-08-08	08:43	83	-27	-351	-1000	-2992
1	SO21_08-06	CTD bio SAS	2021-08-08	13:37	84	-27	-350	-1000	-2616
1	SO21_13-01	CTD omics SAS	2021-08-10	08:52	68	-30	-359	-1000	-4294
1	SO21_13-03	CTD bio SAS	2021-08-10	13:50	86	-30	-360	-1000	-4252
1	SO21_16-01	CTD omics SAS	2021-08-12	08:37	97	-19	-370	-1000	-4332
1	SO21_16-03	CTD bio SAS	2021-08-12	13:13	105	-19	-380	-1000	-4333
1	SO21_18-01	CTD omics SAS	2021-08-13	08:44	71	-22	-280	-1000	-4301
1	SO21_18-03	CTD bio SAS	2021-08-13	11:43	110	-25	-280	-1000	-4309
1	SO21_22-10	CTD omics SAS	2021-08-16	08:44	70	-18	-255	-1000	-4241
1	SO21_22-11	CTD bio SAS	2021-08-16	10:46	108	-20	-251	-1000	-4241
1	SO21_24-01	CTD ChlMax PICO	2021-08-18	03:05	48	-17.5	No sample	-17.5	-4156
1	SO21_26-03	CTD omics SAS	2021-08-19	09:23	72	-30	-250	-1000	-1319
1	SO21_26-05	CTD bio SAS	2021-08-19	13:11	129	-30	-250	-1000	-1333
1	SO21_26-11	CTD wbat EFICA	2021-08-20	04:09	109	-25	-270	-1000	-1355
2	SO21_30-03	CTD wbat EFICA	2021-08-22	04:13	84	-35	-410	-800	-3936
2	SO21_30-11	CTD omics SAS	2021-08-23	08:38	88	-37	-410	-1000	-3942
2	SO21_30-13	CTD bio SAS	2021-08-23	13:46	113	-35	-411	-1000	-3944
3	SO21_33-03	CTD omics SAS	2021-08-25	09:04	73	-35	-410	-1000	-3049
3	SO21_33-05	CTD bio SAS	2021-08-25	13:13	109	-43	-370	-1000	-2987
3	SO21_35-13	CTD omics SAS	2021-08-27	08:31	68	-41	-410	-1000	-1388
3	SO21_35-15	CTD bio SAS	2021-08-27	10:48	106	-43	-410	-1000	-1389
4	SO21_38-15	CTD omics SAS	2021-08-29	08:34	75	-31	-380	-1000	-1201
4	SO21_38-17	CTD bio SAS	2021-08-29	10:51	98	-32	-386	-1000	-1198
5	SO21_42-02	CTD wbat EFICA	2021-08-30	19:42	58	-45	-325	-500	-595
5	SO21_42-06	CTD omics SAS	2021-08-30	23:27	47	-45	-320	-580	-631
5	SO21_42-08	CTD bio SAS	2021-08-31	01:19	75	-45	-320	-500	-660
6	SO21_48-02	CTD omics SAS	2021-09-03	13:00	69	-36	-342	-1000	-1547
6	SO21_48-04	CTD bio SAS	2021-09-03	16:24	100	-33	-335	-1000	-1554
6	SO21_50-06	CTD wbat EFICA	2021-09-04	17:37	102	-28	-323	-865	-895
6	SO21_50-11	CTD omics SAS	2021-09-04	23:27	53	-27	-345	-850	-890
6	SO21_50-13	CTD bio SAS	2021-09-05	01:43	96	-27	-350	-850	-889
7	SO21_53-07	CTD wbat EFICA	2021-09-06	03:16	78	-35	-350	-1000	-1250
7	SO21_53-08	CTD omics SAS	2021-09-06	10:55	29	-36	-350	-1000	-1351
7	SO21_53-09	CTD bio SAS	2021-09-06	12:30	97	-32	-355	-1000	-1351
7	SO21_56-01	CTD omics SAS	2021-09-08	12:53	66	-20	-285	-1000	-2650
7	SO21_56-03	CTD bio SAS	2021-09-08	15:15	112	-20	-380	-1000	-2654
7	SO21_56-05	CTD wbat EFICA	2021-09-08	20:00	93	-15	No sample	-1000	-2654
7	SO21_56-06	CTD ChlMax PICO	2021-09-08	22:14	7	-17.4	No sample	-17.4	-3028
7	SO21_58-09	CTD omics SAS	2021-09-10	21:54	79	-20	-275	-1000	-1506
7	SO21_58-12	CTD bio SAS	2021-09-11	00:53	111	-15	-270	-1000	-1274
Minimum depth						**-12 m**	**-200 m**		
Maximum depth						**-45 m**	**-411 m**		
Average depth						**-28 m**	**-325 m**		

Figure 6.2: Preparations and deployment of the stern CTD. (A) The blue 20-L carboys for sampling the SO21 omics water are brought to the CTD container at the stern. (B) Carboys waiting for sampling the SO21 omics water. (C) The CTD is taken out of the container. (D) The CTD is prepared for deployment. (E) The CTD is ready for deployment. (F) The CTD is being deployed. (A) ©Serdar Sakinan, (B,C,D,E,F) ©Pauline Snoeijs-Leijonmalm

The deck operations for the stern CTD were coordinated by Julia Muchowski (day shift), Alexandra Padilla (day shift), and Salar Karam (night shift). During deployment and recovery, other scientists helped to move the CTD to and from the CTD container. The winch was operated by Joachim Gyllestad, Hans-Jørgen Hansen, Niklas Vestin, Anton Sandström and/or Sven Lidström (SPRS). Altogether, 50 successful CTD casts were made from the stern (*Table 5.3*), i.e., all 18 CTD bio SAS, 17 CTD omics SAS, all 14 CTD wbat EFICA, one CTD ChlMax PICO (SO21_56-06).

Table 6.4: Projects involved in further analyses of sensor data and water samples from the stern CTD + rosette. These further analyses are described in the respective WP chapters (8-21) in this report.

WP	Project	Scientific purpose	Sensor data required by project
WP1-9	SO21 omics	Metagenomics and metatranscriptomics (*Chapter 7.4*)	CTD, CHL, oxygen, turbidity
WP1	EFICA	Ecology: nekton and zooplankton	CTD, CHL, WBAT, TDR, FishCam, oxygen, turbidity
WP2	MIME	Ecology: microbes	CTD, CHL, oxygen, turbidity
WP4	VIRUS	Ecology: viruses	CTD, CHL, oxygen, turbidity
WP5	ASAP	Ecology: prokaryotes	CTD, CHL, oxygen, turbidity
WP6	PICO	Ecology: picophytoplankton	CTD, CHL, oxygen, turbidity
WP7	PHYTO	Ecology: phytoplankton	CTD, CHL, oxygen, turbidity
WP8	ZOO	Ecology: zooplankton	CTD, CHL, oxygen, turbidity
WP9	FORAM	(Palaeo)biology: foraminifers	CTD, CHL, oxygen, turbidity
WP12/13	TRACE/TGB	Chemistry: methane and nitrous oxide chemistry	CTD, oxygen, turbidity
WP14	MWA	Oceanography: midwater acoustics	CTD, turbidity
WP15	WAOW	Oceanography: deep-water hydrography	CTD, turbidity

6.4   Net sampling of nekton and macrozooplankton

Since mesopelagic trawling is not possible in an ice-covered ocean, the EFICA beam net was designed especially for the SAS-Oden 2021 expedition by SLU (Lysekil, Sweden). This net targeted nekton (actively swimming organisms, i.e., fish and squid) and macrozooplankton (>20 mm) in the water column. The beam net is a vertically towed net with a 10 m long steel beam in the middle to achieve maximum opening in the cross-track direction, two aluminium otter boards (trawl doors) of 1.5 m² each and kites attached to the headrope to open the net (*Figures 6.3-6.5*). A 30 meter-long net is attached to the beam in the ends of the steel beam and also to the otter boards. On the beam there are four attachment points which are connected to one dyneema line, five meters above the steel beam. From there it goes another 15 meters and meets the two dyneema lines from the otter boards, where the tree lines are connected to the main winch. The net is subdivided into three sections. The top section has 35 mm mesh, the middle section 16 mm, and the cod end 10 mm. The cod end is equipped with two throats to avoid the escape of fish after capture. To the lower part of the cod end we attached four weights with a total mass of 70 kg.

Attached to the centre of the beam was a Simrad PX TrawlEye with Simrad TV 80 software with portable hydrophone on board the ship. With this equipment the opening on one side of the beam net could be observed and an echogram, roll and pitch (the rotation of the beam along the transverse and longitudinal axes) and opening height were registered. Attached to the beam was also a Simrad PX Universal sensor that recorded depth and temperature.

The EFICA beam net was deployed from the stern with the ship crane. We connected the towing wire of the winch and lifted the beam net from the deck (*Figures 6.3-6.5*). The A-frame was leaned backwards, and then the net was launched with the weights until it was fully stretched. After that we paid out the wire to 800 m depth at a speed of 0.3 m s⁻¹. After reaching the target depth, the net was hauled up at a speed of 1.0 m s⁻¹. This was the optimal speed to obtain an as large net opening as possible. At the surface, we pulled the rope attached to the end of the beam net and leads down to the cod end to lift it on deck and empty it (*Figure 6.5*). After the first two beam net stations, the motherboard of the main winch of *IB Oden* (the "North Sea winch") broke. After that, we used the spare winch for beam net sampling. The latter winch did not have the same capacity as the North sea winch, and we could only haul it up with max. 0.87 m s⁻¹ instead of 1.0 m s⁻¹.

Altogether, we performed 45 successful hauls with the beam net at nine stations (*Tables 1.1 and 5.3*). At the first beam net station (Station 14) the aimed haul depth was 700-0 m and at all other stations 800-0 m, except for Station 42 where the bottom depth was only 600-700 m. At some stations (e.g., Station 8), beam net deployments could not be carried out because of strong wind or failed due strong ice drift.

Since the beam net opening was much larger, but less precise, than that of a ring net with a fixed opening, we performed five net hauls with a MIK net provided by SLU (*Figure 6.6*, *Table 5.3*) to be able to compare the catches of the beam and MIK nets quantitatively. The MIK net is the standard gear for the sampling of fish larvae adopted by the International Council for Exploration of the Sea (ICES). It has a strong and robust construction. The MIK net consists of a 2-m diameter ring frame to which the net is attached. The 13-m long black net has a mesh size of 1.6 mm and is strengthened by canvas straps. The last meter of the MIK net consists of a 500 µm mesh net, and in the bottom we attached a cod-end bucket for sample collection. Underneath, a weight of 30 kg enabled the cod end to sink fast enough to avoid entanglement during the lowering of the net. We attached a flow meter (Hydrobios) for determination of the volume of water filtered and a Star Oddi TD probe for monitoring net depth and water temperature.
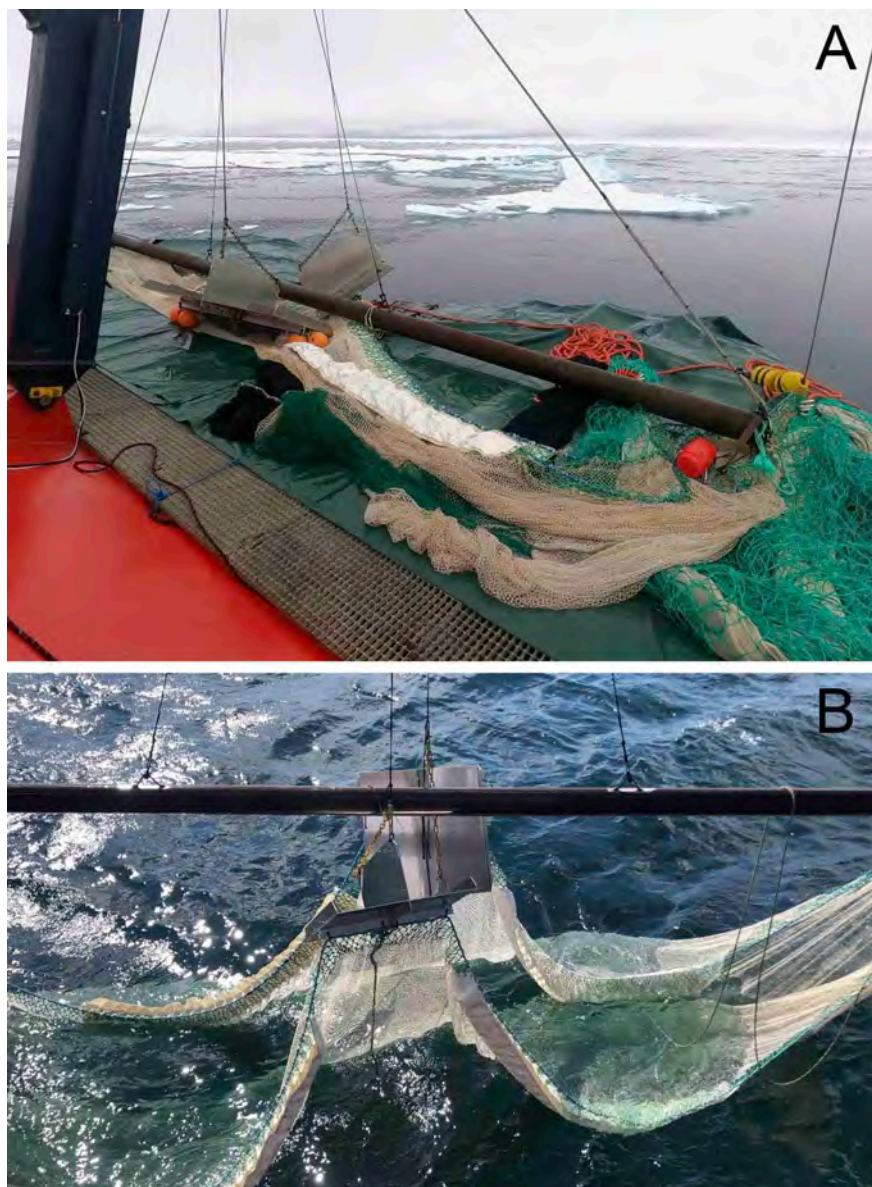
Figure 6.3: The EFICA beam net. (A) Lying on the aft deck of IB Oden *during the SAS-Oden 2021 expedition. (B) During a test deployment in Swedish waters. ©Baldvin Thorvaldsson*
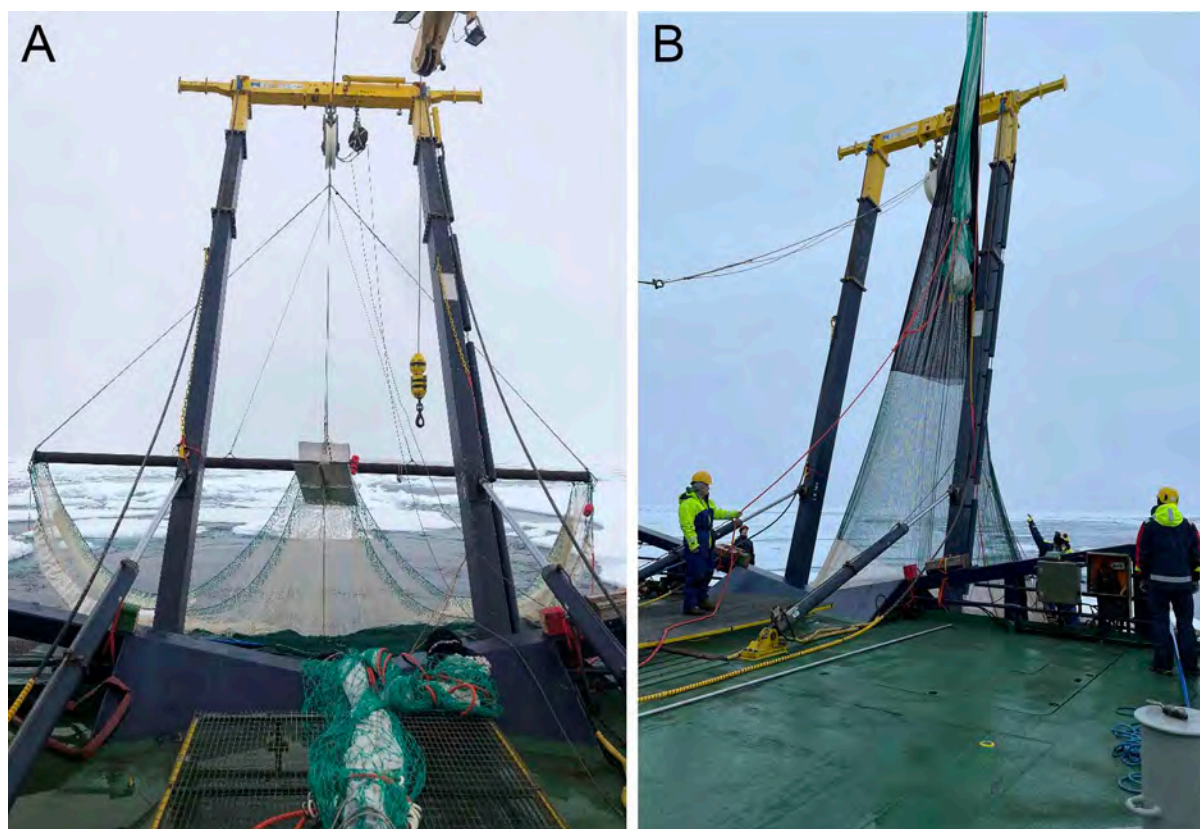
Figure 6.4: The EFICA beam net connected to the towing wire during the SAS-Oden 2021 expedition. (A) The beam net before deployment. (B) Retrieval of the beam net. (A) ©Baldvin Thorvaldsson, (B) ©Pauline Snoeijs-Leijonmalm
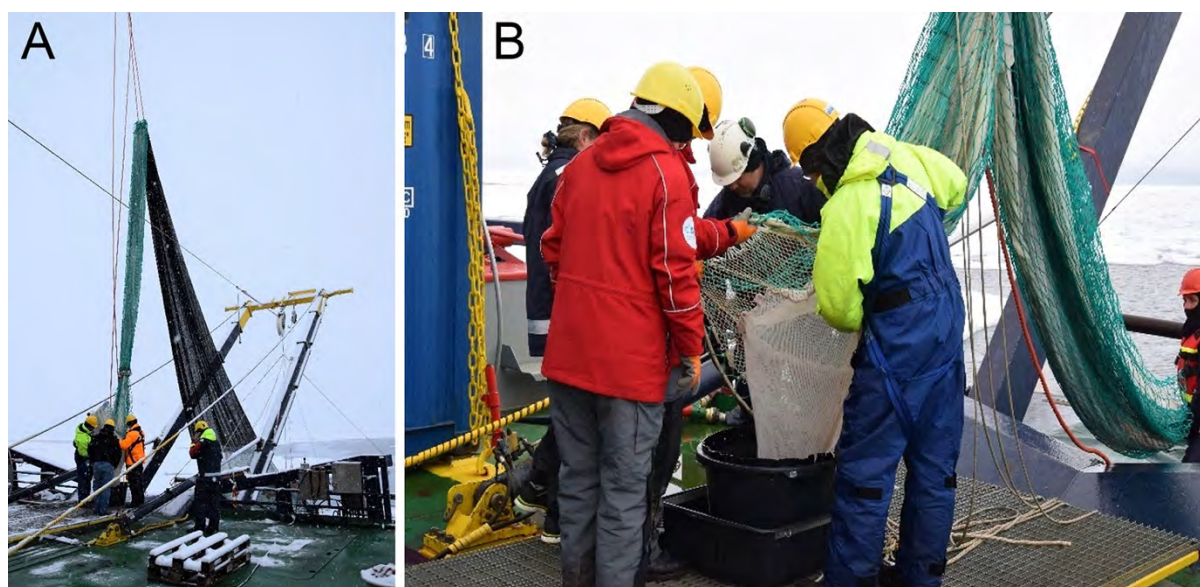
Figure 6.5: Emptying the cod end of the EFICA beam net during the SAS-Oden 2021 expedition. (A) Overview of the aft deck after retrieval of the beam net. (B) Collecting the catch. (A,B) ©Hans-Jørgen Hansen

For deployment of the MIK net, we connected the ring to the winch wire, leaned the A-frame backwards and lifted up the ring as high as possible. Then we launched the 30-kg weights and the net until it was stretched downward in the water (*Figure 6.6 B*). After that, we paid out the wire to the desired depth (800 m) at a speed of 0.3 m s^{-1}. After reaching the desired depth, we hauled the net upwards at a speed of 0.67 m s^{-1}. When the MIK net came out of the water, we lifted it out of the water as high as possible with the A-frame and then put a strap around the net to lift the cod end on deck with the ship crane.

The EFICA beam and MIK net deck operations were coordinated by Baldvin Thorvaldsson and other WP1 participants assisted. The winch was mainly operated by Joachim Gyllestad, Hans-Jørgen Hansen, Niklas Vestin, Anton Sandström and/or Sven Lidström (SPRS). The beam net required additional assistance on deck from at least four *IB Oden* crew under the command of Chief Officer Mats Wisén, including bosun Mats Hansson, crane operator and two additional seamen. The WPs involved in further analyses of (sub)samples taken from the net samples are summarized in *Table 6.5*.
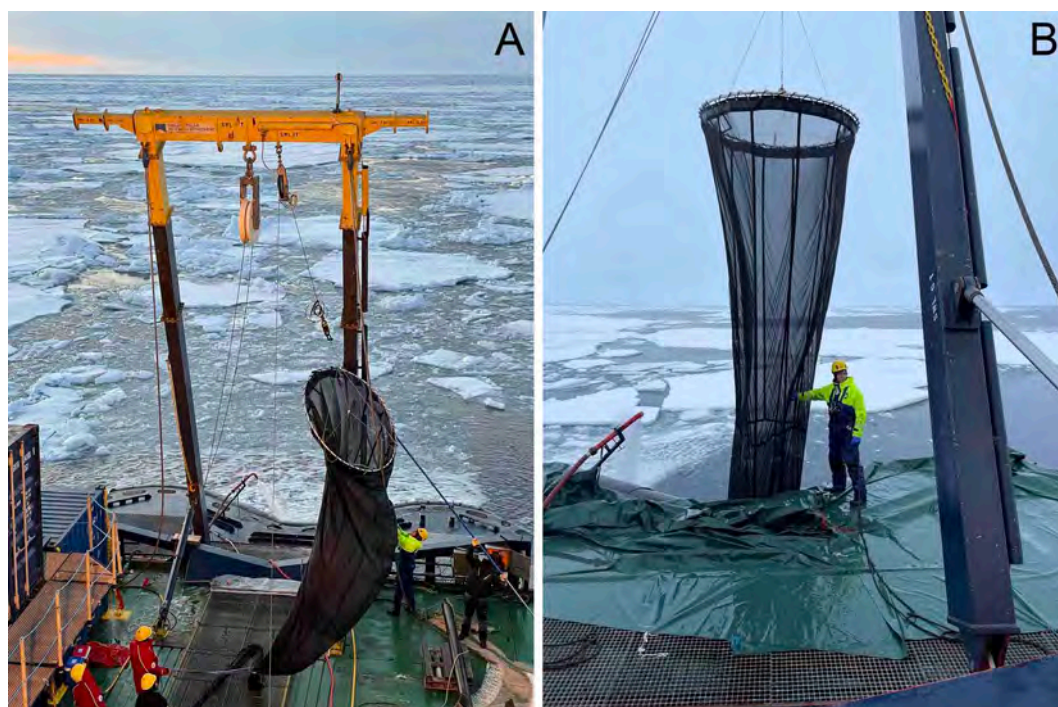
Figure 6.6: The MIK ring net used during the SAS-Oden 2021 expedition. (A) Overview of the aft deck before deployment of the MIK net. (B) The weights have been launched and the MIK net is stretched downward in the water. (A) ©Pauline Snoeijs-Leijonmalm, (B) ©Nicole Hildebrandt

6.5 Net sampling of meso- and microzooplankton and LOKI

Multinet

A multiple plankton sampler net (multinet) Midi provided by the AWI was used at 16 ship stations (*Tables 1.1 and 5.3*). The multinet had five 150-µm mesh nets to sample mesozooplankton for analysing the SAS Core Parameters zooplankton abundance, community composition, and depth distribution, which were measured jointly by WP1 (EFICA) and WP8 (ZOO) (*Figure 6.7, Table 6.5*). The multinet collected five samples per cast from the five depth intervals 2000-1000-500-200-50-0

m, i.e., the standard depths for sampling mesozooplankton in the CAO as applied during the MOSAiC expedition 2019-2020 and other previous studies, see "SO21 SOP: multinet" (*Chapter 24*). On two occasions (at Stations 22 and 30) the multinet covered the full water column and two casts were made to cover the seven depth intervals bottom-3000-2000-1000-500-200-50-0 m. When the depth at the station was less than 2000 m, the maximum sampling depth was approximately 20 m above the seafloor as monitored by the deck unit. The multinet had two nets on the side: one "bicycle net" that collected zooplankton for species sorting by WP1 (EFICA) and WP8 (ZOO), and a smaller "gypsum net" to collect gypsum samples for WP3 (ProMis). At eight SO21 stations the same multinet equipment was used with 55 µm mesh nets to sample foraminifers for WP9 (FORAM) (*Tables 1.1 and 5.3*).
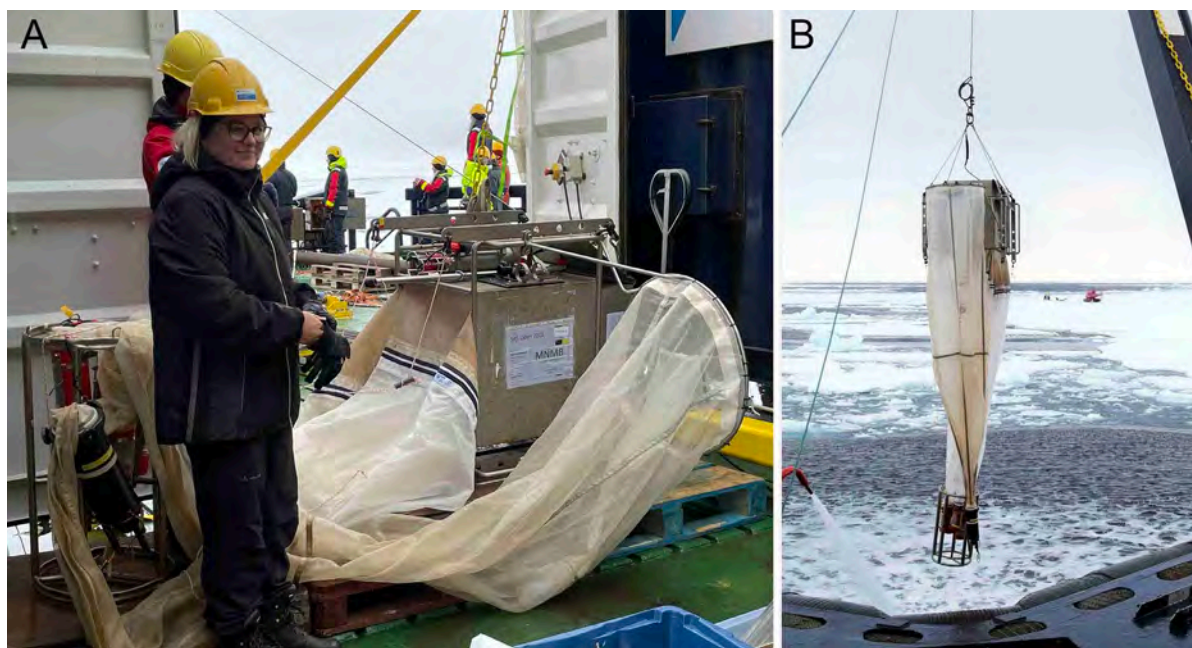
Figure 6.7: Deployment of the multinet during the SAS-Oden 2021 expedition. (A) Preparation of the multinet by Emma Svahn (WP8) and Nicole Hildebrandt (WP1) (behind Emma). (B) The multinet is winched into the water; the water hose is used to keep the ice open. (A) ©Pauline Snoeijs-Leijonmalm, (B) ©Hans-Jørgen Hansen

Bongo net

At 11 ship stations (*Table 5.3*) a bongo net provided by SU was hauled from 200 m to the surface. The bongo net consisted of two ring nets, both with an upper diameter of 60 cm (*Figure 6.8*). One net had a mesh size of 100 or 150 µm, and the other one had a mesh size of 50 µm. The bongo net was used to sample microzooplankton (the 50 µm mesh net) for analysing the SAS Core Parameter microzooplankton abundance and community composition in surface water which is measured jointly by WP1 (EFICA) and WP8 (ZOO). From both bongo ring nets extra mesozooplankton individuals were taken for food-web parameters (^{13}C/^{15}N, fatty acids) by WP1 (EFICA), genetic (CO1 amplicon) analyses by WP1 (EFICA) and for incubation experiments by WP8 (ZOO). At three stations (*Table 5.3*) the same bongo net was hauled 1000-0 m to collect foraminifers for WP9 (FORAM) from the 50 µm mesh ring net and the other net was used to sample extra mesozooplankton individuals for WP1 (EFICA) and WP8 (ZOO).
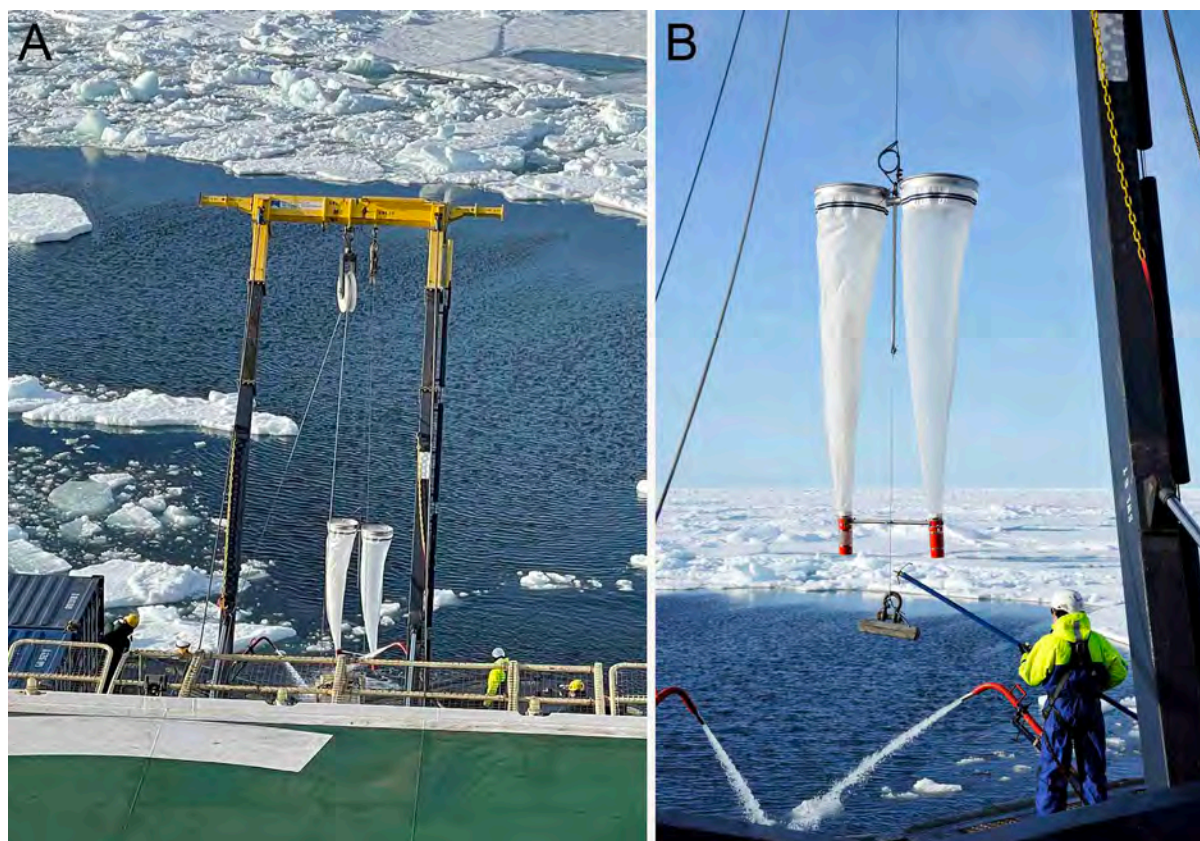
Figure 6.8: Deployment of the bongo net during the SAS-Oden 2021 expedition. (A) ©Pauline Snoeijs-Leijonmalm, (B) ©Flor Vermassen

LOKI

The Lightframe On-sight Key species Investigation system[24] (LOKI) provided by the AWI was deployed at 11 stations (*Table 5.3*) to collect optical zooplankton profiles for WP1 (EFICA) (*Figure 6.9*). This plankton recorder, manufactured by iSiTEC, Germany, provides high-resolution photographs of mesozooplankton organisms and particles taken directly in the water column during vertical hauls from 1000 m water depth to the surface. These photographs often allow the identification of genera/species or even (for copepods) developmental stages. A built-in image analysis automatically recognizes objects in the pictures. In addition to the camera system, the LOKI carries sensors for measuring depth, salinity, temperature, oxygen concentration and fluorescence. This allows the study of small-scale zooplankton distribution patterns in relation to environmental conditions.

During the SAS-Oden 2021 expedition, the LOKI was used with a winch speed of 0.5 m s^{-1}, and the standard sampling depth was 0-1000 m. The LOKI was equipped with a 150 μm plankton net with a net opening of 0.28 m². A net with a mesh size of 1 cm covered the net opening prevented larger animals from entering and clogging the system. A flow meter with back run stop was used to calculate the amount of filtered water. At the outflow of the net, within a narrow flow-through chamber, a 6.1 megapixels digital camera took pictures at a max. frame rate of 19.8 pictures sec^{-1}. A built-in computer unit processed the pictures immediately by scanning them for objects, which were then cut out and stored on the internal hard drive. To simultaneously measure the environmental parameters, we

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[24] Schulz J, et al. (2009) Lightframe On-sight Key species Investigation (LOKI). IEEE OCEANS 2009-EUROPE [http://doi.org/10.1109/OCEANSE.2009.5278252]

mounted a Sea-Bird SBE19plus V2 SeaCAT Profiler CTD (Sea-Bird Electronics Inc., USA) to the side of the LOKI frame. The CTD was equipped with additional sensors for measuring oxygen and fluorescence. Pictures and environmental data can later be matched via a time stamp.

Operation of nets and LOKI

The zooplankton nets and LOKI deck operations were performed from the stern and coordinated by Nicole Hildebrandt (WP1) assisted by Emma Svahn (WP8) and/or Flor Vermassen/Clare Bird (WP9). The winch was operated by Joachim Gyllestad, Hans-Jørgen Hansen, Niklas Vestin, Anton Sandström and/or Sven Lidström (SPRS). The WPs involved in further analyses of (sub)samples taken from the net samples and LOKI data are summarized in *Table 6.5*.
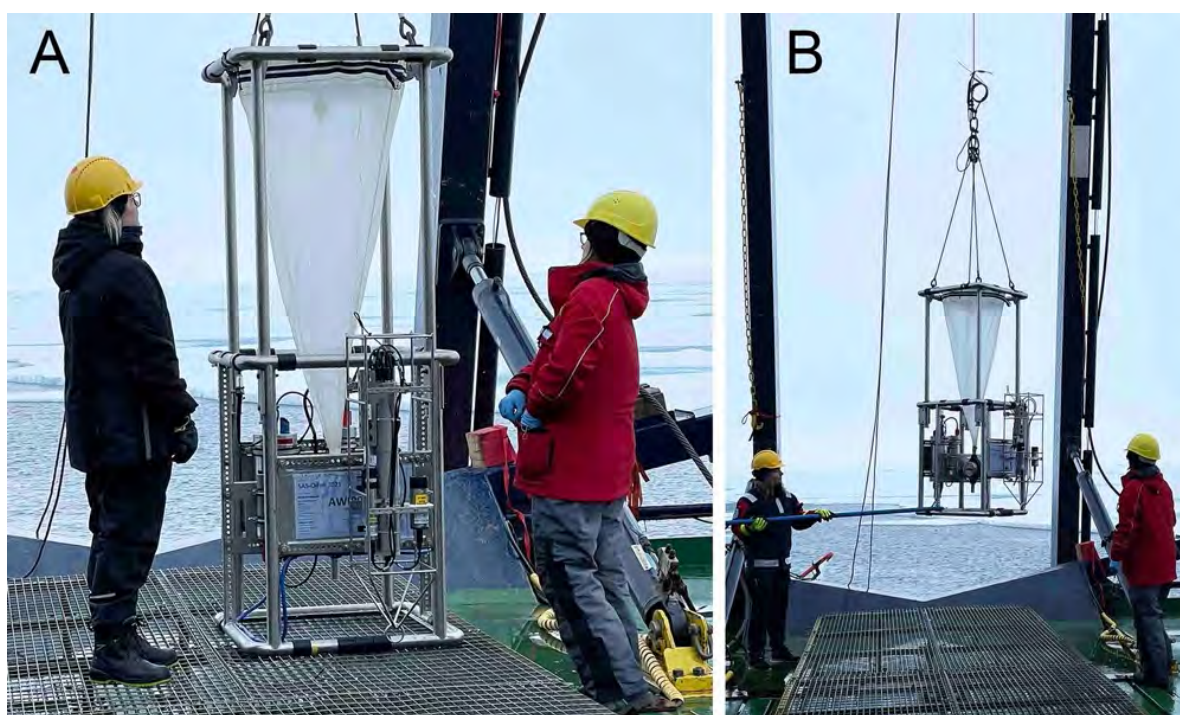
Figure 6.9: Deployment of the Lightframe On-sight Key species Investigation system (LOKI) during the SAS-Oden 2021 expedition. (A) Preparations on deck. (B) Winching the LOKI into the water. ©Pauline Snoeijs-Leijonmalm

Table 6.5: Projects involved in further analyses of (sub)samples taken from the NET + LOKI Device Operations. These further analyses are described in the respective WP chapters (8, 10, 15, 16) in this report.

WP	Project	Scientific purpose
WP1+8	EFICA+ZOO	Meso- and microzooplankton abundance, community composition, and depth distributions
WP1	EFICA	Macrozooplankton abundance and community composition (NET beam and NET mik)
WP1	EFICA	Food-web parameters ($^{13}C/^{15}N$, fatty acids), genetic (CO1 amplicon) analyses
WP1	EFICA	Optical profiles of zooplankton depth distributions (LOKI)
WP3	ProMis	Gypsum net sample analyses
WP8	ZOO	Incubation experiments, genetic analyses of stomach contents
WP9	FORAM	Abundance, species composition, and water-column depth distributions of foraminifers

6.6 Box core sampling

For box core sampling a "giant box corer" provided by the AWI was used at six sampling stations (*Figure 6.10*). This box corer samples a comparatively large area of the sediment surface (50 × 50 cm) with minimal disturbance and collects a large block of sediment up to max. 60 cm below the seafloor. The square box is fixed to a head with a column that is connected to a frame by a cardanic (gimballed) suspension. This allows vertical penetration of the box into the sediment. A crank with spade including rubber sealed plate is attached to the head of the box. The column is filled with lead weights to aid penetration and the whole construction weighs 900 kg. During lowering to the seafloor and sampling, two flaps at the top of the head remain open to allow a free flow of water. This prevents pressure build-up and following disturbance of the sediment surface. When the box has reached the seafloor, the box is triggered by a trip as the column passes through its frame. While pulling the corer out of the sediment the flaps at the head are closed and the spade is drawn down into vertical position so that the bottom of the box is closed and the sample is secured. The square boxes are equipped with a removable front plate for access the sediment sample from the side, to obtain undisturbed subsamples of the near-surface sediments.
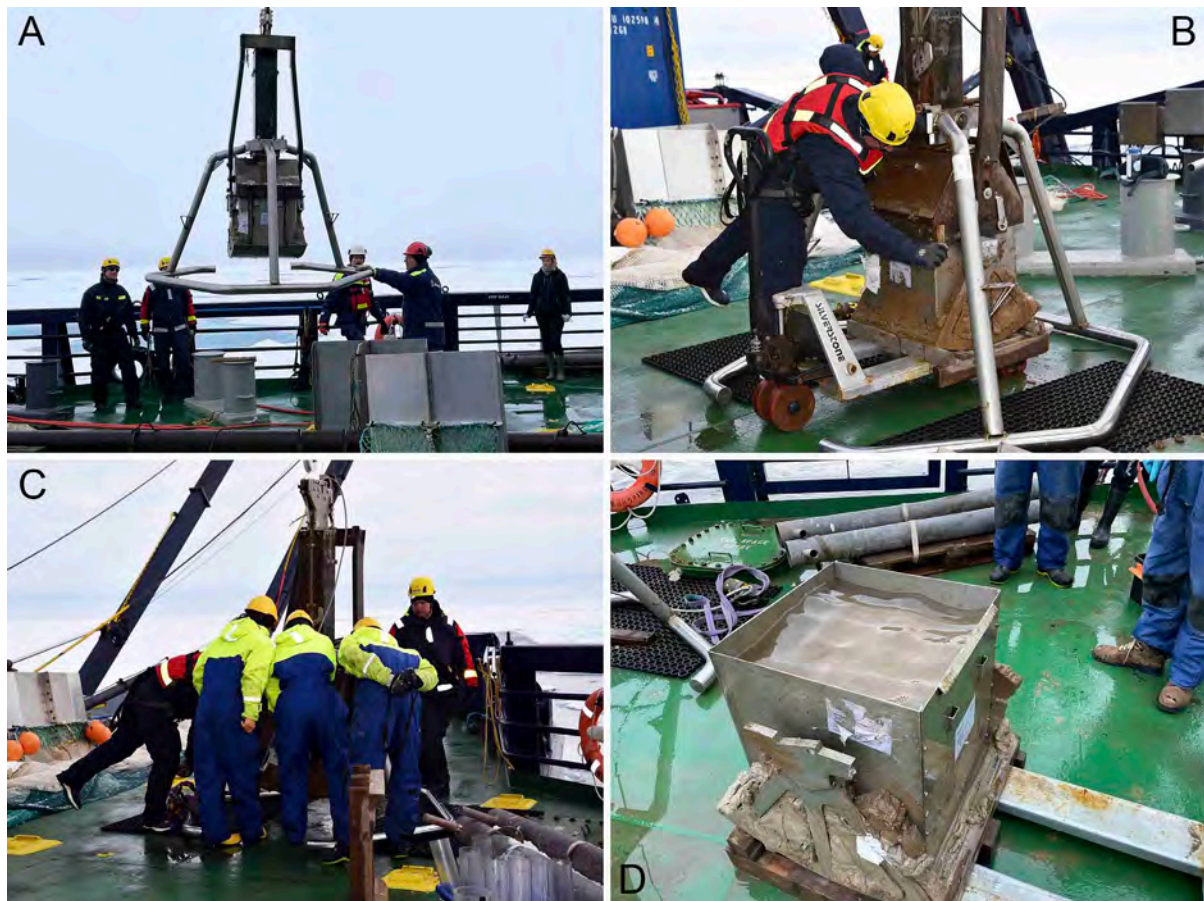
Figure 6.10: Retrieval of the "giant box corer" during the SAS-Oden 2021 expedition. (A) The box corer is lifted on the aft deck with a crane. (B) The box is detached from the frame. (C) Eager scientists inspect the sample. (D) The box with the sediment sample covered by bottom water. ©Pauline Snoeijs-Leijonmalm

Figure 6.11: Subsampling from a box core sample during the SAS-Oden 2021 expedition. (A) The subsampling tubes of WP1 (EFICA), WP9 (FORAM) and the SO21 omics collaboration were gently pushed into the sample. When there was some resistance, this was most easily done with two persons and a piece of wood. (B) Subsampling tubes for the different projects in the box core sample. (C) Removal of the front plate. (D) Subsampling tubes with bulk sample in-between. (E) Digging out the cores and collecting the bulk sample. (F) Slicing the upper darker (presumably Holocene) layer from each core into 1-cm sections and combining the same sections from the replicate subsamples for WP1. (G) Sieving sediment over a 300-μm sieve for sampling otoliths. (A,B,C,D,E) ©Pauline Snoeijs-Leijonmalm, (F) ©Anna Hermanssen, (G) ©SPRS

Once in the desired general area, the specific location of box core stations was considered in dialogue with WP14 (MWA, geophysical mapping). WP14 identified regions of level, soft sediments from the multibeam and sub-bottom profiler data: regions of relatively flat topography, or small bathymetric "highs", characterised by coherent acoustic layering were targeted. Steep slopes and acoustically chaotic sediment piles were avoided.

When the box corer had returned on board the ship after sampling, the box with the sample was detached from the frame for subsampling of the sediment (*Figures 6.10 and 6.11*). Each box core was individually photographed. All box core photographs are stored in the SO21 directory of technical photographs on the SND data repository. The subsamples for the different projects were taken according to "SO21 SOP: box core" (*Chapter 24*).

The box core deck operations were performed from the stern and coordinated by Claudia Morys (WP1) assisted by Julek Chawarski (WP1), Pauline Snoeijs-Leijonmalm (WP1), and Flor Vermassen (WP9). The winch was operated by Joachim Gyllestad, Hans-Jørgen Hansen, Niklas Vestin, Anton Sandström and/or Sven Lidström (SPRS). Box core samples were taken at six SO21 stations (*Table 5.3*). At two stations an extra box core was available for bulk sampling of otoliths due to obliquely sampled sediment during the first cast, which was not ideal for the other parameters. The WPs involved in further analyses of (sub)samples taken from the box core samples are summarized in *Table 6.6*.

Table 6.6: Projects involved in sampling and further analyses of subsamples taken from the box core Device Operations. These further analyses are described in the respective WP chapters (8 and 16) in this report.

WP	Project	Scientific purpose
WP1-9	SO21 omics	Collaboration on metagenomics and metatranscriptomics deep-sea sediments (*Chapter 7.4*)
WP1+9	EFICA+FORAM	Sediment characteristics
WP1	EFICA	Macro- and meiofauna, fish otoliths
WP9	FORAM	Abundance, species composition, and sediment depth distributions of foraminifers

6.7 SAS Ice Stations

More than 70% of the scientists on board the SAS-Oden 2021 expedition contributed to the sampling of ice cores, snow and ice-habitat water (melt ponds, brackish brine, ice-seawater interface) at the 16 SAS Ice Stations. For most biological SAS Core Parameters large amounts of melted ice and snow, as well as ice-habitat water, was combined into bulk samples to allow for direct comparisons between the different parameters (*Table 6.7*, *Figure 6.12*).

Subsamples were taken from these bulk samples for later land-based analyses within the SO21 omics collaboration (WP1-9), prokaryote cell density (WP2), viral cell density (WP4), picophytoplankton density and chlorophyll-*a* (2-200 μm) (WP6), $^{13}C/^{15}N$, POC, PON, POP, primary production, chlorophyll-*a* (2-200 μm), HPLC pigments, total primary producer cell density, and phytoplankton community composition by microscopy (WP7). Separate ice cores were taken for temperature and salinity measurements (WP2+7), inorganic nutrients, TOC, DIC and TA (WP10), the gases NO_2 and NH_4 (WP12+13), and meiofauna (WP1). Lists of all SAS ice cores and other ice-habitat samples and notes taken during the expedition and how they were processed are provided in the excel file "SO21_Ice_Station_Logbook" in the SND data repository (*Table 6.7*).
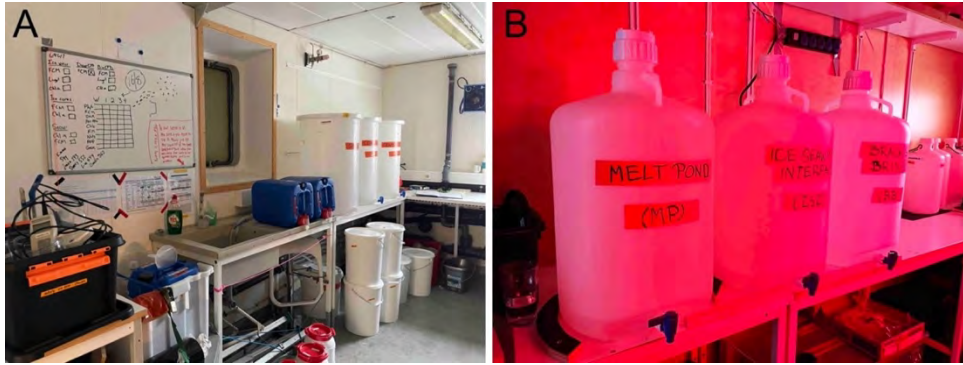
Figure 6.12: Handling of the bulk ice-habitat samples during the SAS-Oden 2021 expedition. (A) Six white 20-L buckets for sampling snow, three 115-L tanks with taps for melting snow, centre-top ice (variable length), and centre-bottom ice (variable length), 5-L containers with red locks for sampling and melting top ice (10 cm) and bottom ice (10 cm), and two blue 20-L carboys with taps for combining melted top ice and melted bottom ice, respectively. (B) 50-L tanks with taps for combining water from melt ponds, ice-seawater interface, and brackish brine, respectively. ©Pauline Snoeijs-Leijonmalm

Table 6.7: Organisation of the excel file "SO21_Ice_Station_Logbook" in the SND data repository.

Sheet name in the excel file	Contents of the sheet
1. Explanations	Explanations of abbreviations, definitions, methods, etc., used in the Ice Station Logbook file
2. All SO21 Ice Stations	Summary tables of the numbers of all SO21 Ice Stations + station map: (A) next to ship – SAS + project-specific sampling. (B) helicopter – SAS, (C) helicopter – EFICA, (D) helicopter - ACAS
3. SAS Ice Stations - DATA	List of the 16 SAS Ice Stations with geographical positions in Decimal Degrees (DD) and Degrees Minutes (DM), station depth, etc.
4. SAS Ice Stations - Helicopter	Dates, times and people on the helicopter flights carried out for the SAS Ice Stations
5. SAS Ice Stations - Notes	Notes made in the field about the general conditions at the SAS Ice Stations: ice coverage, presence of ridges, ice type, air, snow and now/ice interface temperature
6. All 316 ice cores - DATA	Ice coring notes made in the field for each ice core taken: time, ice thickness, freeboard, core length, snow depth
7. All 316 ice cores - STAT	Basic statistics of ice thickness, freeboard, core length, and snow depth for each SAS Ice Station
8. Temperature + salinity - DATA	Measurements of temperature and salinity in one ice core for each SAS Ice Station
9. Ice-habitat water - DATA	Sampling data for brackish brine water, ice-seawater interface water and melt pond water: date, position, volume sampled, temperature measured in the field and preliminary salinity. Note: these salinity measurements still need to be verified from frozen water samples and will be added to the file later.
10. Snow - DATA	Sampling data for snow: date, position, volume sampled and melted volume
11. Ice FWC additions - DATA	Sampling data for ice sections: ice core length melted, volume of filtered seawater (FSW; 0.2 µm Sterivex™ filter units) added = 50 mL per 10 cm ice core length, final melted volume and dilution factor to be used for the calculations of concentrations of the SAS Core Parameters in the bulk samples.
12. Ice FWC additions - STAT	Basic statistics of the dilution process when melting the ice core sections.
13. Ice FWC additions - REC	Reconstruction of dilution data for Stations 6 and 8 because the final volume (after melting) was not noted.

The SAS Ice Stations were selected either by observation from the bridge or from the helicopter. The aim was to sample from ice floes that were representative of a wider area around the ship as observed during the past 3-6 hours of steaming and the availability of melt ponds. It was necessary for *IB Oden* to hold position at the ice station without destroying the ice floe, which implied that relatively thin ice floes (<1.2 m) could not be selected. When the position of the ice station was decided, this also became the station for winch operations from the ship.

The ice stations next to the ship were accessed either via the gangway or with a personnel transfer basket (*Figure 6.13*), and the sampling sites were not situated further than 300 m away from the ship. The maximum allowed distance between a sampling site and the ship depended on visibility, and was decided by the SPRS bear guards on the ice and the bear watch on the bridge so that polar bears could be detected soon enough to guarantee the safety of the scientists. Before choosing the site for the ice coring plot, several potential sites were investigated with ice thickness drills. This inspection was necessary to ensure safety and to choose an ice thickness representative of the floe. The situation of the ice coring plot was also selected in such a way that the impact from the ship was minimized.

The ice stations reached by helicopter were situated within visible distance, less than ca. 1 nautical mile (1.85 km), from the ship. The final selection of a helicopter ice station was made after polar bear survey, test landing and local inspection of the potential sampling site. Twelve of the 16 SAS Ice Stations were situated next to the ship, three were accessed by helicopter. One SAS Ice Station started next to the ship, was interrupted by fog, then the ship and the ice coring plot drifted apart ca. 1 nautical mile, the station was resumed by helicopter, was again interrupted by fog, and finally the sampling was resumed and concluded after the ship had moved to the ice coring plot. The latter station (Station 30) is therefore subdivided into two Device Operations (*Table 5.3*).

All ice station work for the SAS Core Parameters followed the same design (*Figures 6.13 and 6.14*). A square 5×5 m ice coring plot was identified. Cross-wise walk ways allowed access to the inner area of the ice coring plot. The outer corners of the ice coring plot and the walking path between ship or helicopter and the ice coring plot were marked with red poles to ensure recognition in foggy conditions. This was especially important when the ice floe needed to be temporarily abandoned for safety reasons. After the ice coring plot was established, a suitable site for sampling melt pond water was identified. Before ice core sampling was started, the general conditions at the ice station in terms of ice coverage, presence of ridges, ice type, air, snow and now/ice interface temperature, were noted.

For ice coring, teams of up to five people worked with up to three ice corers in parallel. Ice cores were sampled with 9-cm diameter Kovacs ice corers operated by battery-driven drilling engines. Before coring, the snow depth above each individual ice core was measured. After the ice core was removed, we measured ice thickness and freeboard (the difference between the surface of the ice and the water level in the borehole) using an ice-thickness gauge. Each ice core was placed in a cradle and individually photographed before further processing (*Figure 6.14 C*). Ice core photographs are stored in the SO21 directory of technical photographs on the SND data repository. As a standard, one ice core was taken for temperature and salinity measurements (WP2+7) every 10 cm, and another one for nutrient analyses + TOC (WP10) every 10 cm (*Table 6.8*). On most SAS Ice Stations individual ice cores for DIC + TA (WP10) , NO_2 + NH_4 (WP12+13), and meiofauna (WP1, only ice bottom 10 cm) were taken as well (*Table 6.8*). At all 16 SAS Ice Stations another 12-16 replicate cores were taken and combined into bulk samples for biological SAS Core Parameters (*Table 6.8*). The SAS ice cores were subdivided into four parts: the top ice and bottom ice bulk samples consisted of 12-16 sections of 10-cm length, and the centre-top ice (upper half core minus 10 cm top ice) and centre-bottom ice (lower half core minus 10 cm bottom ice) consisted of 3-8 sections depending of the total length of the ice cores. For buffering microbial cells during the melt process 0.2 μm filtered seawater (FSW; 50 mL per

10-cm ice core length) was added to the ice samples[25], but not to the snow samples. Because of the addition of FSW the measured values of the core parameters in the ice samples need to be multiplied with a dilution factor to obtain the true concentrations in the original sample (*Tables 6.9 and 6.10*).

Snow was sampled close to the ice coring plot by shovelling the rather loose snow layer into six 20-L plastic buckets with a clean metal shovel. Care was taken to not compact the snow in the buckets. Ice-habitat water was sampled with a hand-operated membrane pump after the ice coring work was completed (*Figure 6.13 E*). Melt pond water was sampled ca. 10 cm below the melt pond surface. For sampling brackish brine water, a new borehole was drilled at least 10 m away from the ice coring plot, not all the way through the ice but leaving ca. 0.5 m of ice remaining at the bottom of the borehole. This hole immediately filled up with interstitial brackish brine water. To confirm that the sampled brine water was not contaminated by the seawater below, the salinity was constantly monitored during ice-habitat water sampling with an YSI conductivity meter with a salinity resolution of 0.1 ppt. Water from the ice-seawater interface was sampled from a borehole through the ice, ca. 10 cm below the lower ice margin to avoid contamination by brackish brine from the borehole. The carboys with the water samples were transported back to the ship as soon as possible to avoid freezing of the samples.

The snow, centre-top ice, and centre-bottom ice were on board combined into three bulk samples in three 115-L tanks with a tap and left to melt (*Figure 6.12*), respectively. The 10-cm sections of the top and bottom ice were melted in their 5-L sampling containers and after melting combined into 20-L blue carboys with a tap. The two 20-L carboys (used for sampling on the ice) for each of the ice-habitat water types (melt pond, brackish brine, ice-seawater interface) were combined into 50-L tanks with a tap. The latter samples were processed immediately after sampling while the bulk samples that needed to melt first were processed after they were melted (30-40 hours after sampling). After combining/melting the total water volume in the tanks were read on scales on the tanks and noted. The bulk water samples were then subsampled for the biological SAS Core Parameters. No primary production incubations were performed, and no RNA samples were taken, from the melted habitats because community composition and gene expression will have changed during the melting process.

The main challenge to perform successful ice stations during the SAS-Oden 2021 expedition was to guarantee safe working situations in unpredictable weather conditions. Fog occurred on almost all days during the expedition. Even when an ice station started in clear weather, upcoming fog often forced us to interrupt or abandon the station temporarily. At the last ice station (Station 58), the work was interrupted over-night due to a polar bear inspecting our sampling sites on the ice (*Figure 6.15*). The reason why we were able to complete all 16 SAS Ice Stations at a high success rate was that we gradually developed a highly flexible schedule, in which scientist, SPRS bear guards, ship crew, and helicopter crew were ready to perform sea ice work whenever possible, and interrupt whenever needed.

Altogether, 316 ice cores were sampled for the SAS Core Parameters. The dominating ice type sampled during the SAS-Oden 2021 expedition was second-year ice (formed winter 2019/2020). However, it must be noted that the preconditions for selecting ship-based ice stations caused a bias towards thicker ice, which is why first-year ice (formed winter 2020/2021) was likely under-represented by our sampling. Overall, the measured average ice thickness measured at the 16 SAS coring sites ranged between 112 to 262 cm, and the average core length measured at the 16 SAS coring sites ranged between 106 and 263 cm (*Figure 6.16 A*). The longest core taken during the expedition was 305 cm long (*Figure 6.14 E*). Station 42 had the thickest ice due to a high proportion of multi-year ice (*Figure 6.16 A*). The thinnest ice was present at Stations 6 and 56, both situated close to the marginal ice zone.

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[25] Chaimberlain EJ, et al. (2022) Impacts of sea ice melt procedure on measurements of microbial community structure. Elementa: Science of the Anthropocene (in press).

Average snow depth ranged between 2 and 13 cm (*Figure 6.16 B*), which is typical for the late summer season in the CAO. The average thickness of sea ice protruding above the water level (freeboard) varied between 9 and 30 cm.

Figure 6.13: SAS Ice Stations during the SAS-Oden 2021 expedition. (A) Scientists and polar bear guards on their way to an ice coring plot close to the ship. (B) A crane-operated personnel transfer basket for bringing people from the ship to the ice and back. (C) Overview of an ice station very close to the ship. (D) Ice coring. (E) Hand pump used for sampling water from ice habitats, i.e., water from melt ponds, brackish brine, and the ice-seawater interface. (A) ©Hans-Jørgen Hansen, (B) ©Julia Muchowski, (C) ©Johan Wikner, (D) ©Hauke Flores, (E) ©Flor Vermassen

Figure 6.14: Ice coring during the SAS-Oden 2021 expedition. (A) A 5 ×5 m ice coring plot has been selected and ice coring has started. (B) Measuring ice thickness through a coring hole. (C) Ice core in cradle with notebook, ice saw and drill. (D) Measuring a temperature profile in an ice core in the field. (E) The longest ice core taken (305 cm) during the SAS-Oden 2021 expedition at Station 42, (F) Ice coring plot after the coring had been completed. (A) ©Johan Wikner, (B,C,D,E) ©SAS-Oden sea-ice team, (F) ©Pauline Snoeijs-Leijonmalm
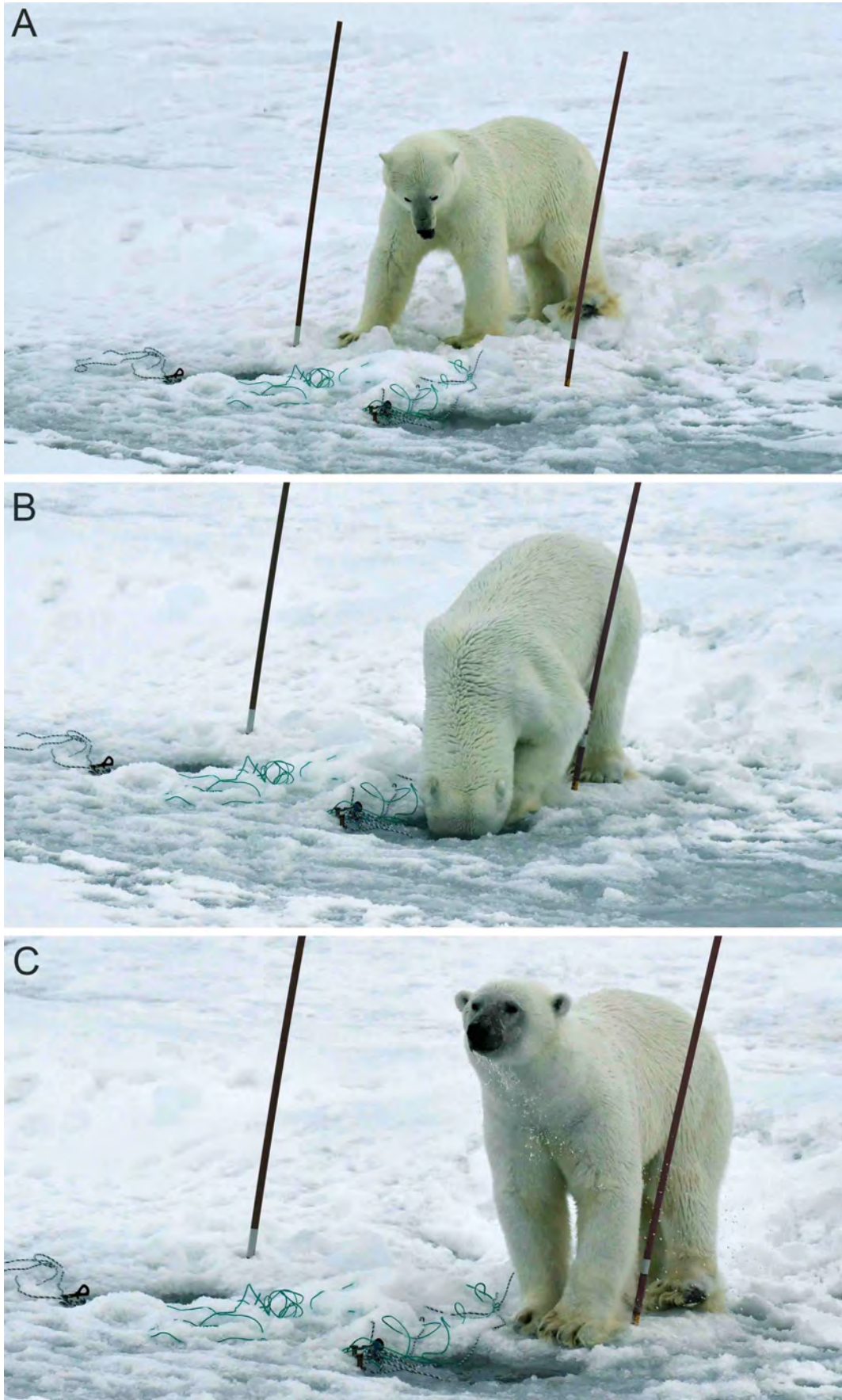
Figure 6.15: A polar bear inspecting Station 58 of the SAS-Oden 2021 expedition. ©Hans-Jørgen Hansen

*Table 6.8: Overview of ice-habitat measurements and sampling at the 16 SAS Ice Stations during the SAS-Oden 2021 expedition. * = the bulk samples were used for later land-based analyses within the SO21 omics collaboration (WP1-9), prokaryote cell density (WP2), viral cell density (WP4), picophytoplankton density and chlorophyll-a (2-200 µm) (WP6), $^{13}C/^{15}N$, POC, PON, POP, primary production, chlorophyll-a (2-200 µm), HPLC pigments, total primary producer cell density, and phytoplankton community composition by microscopy (WP7).*

Measurements or samples from ice cores	Description	Total number of measurements/ samples
Temperature	Immediately after sampling, the temperature in the centre of one ice core was measured every 10 cm in 3 mm wide and 4.5 cm deep holes drilled in the ice core with a VWR TD 320 precision thermometer, resolution 0.1°C, by WP2+7	16 stations 282 measurements
Salinity	The ice core used for the temperature measurements in the field was subdivided into 10-cm sections, melted in the laboratory and salinity in each section was measured with a VWR CO310M conductivity meter, salinity resolution 0.1 ppt, by WP2+7	16 stations 282 measurements
Inorganic nutrients and TOC	The ice core was subdivided into 10-cm sections, melted in the laboratory and inorganic nutrient concentrations in each section were measured on board + the four other ice habitats, as described by WP10 (*Chapter 17*).	16 stations 338 measurements
DIC and TA	The ice core was sectioned and the sections were melted in gas-tight bags. DIC concentrations were measured on board as described by WP10 (*Chapter 17*)	9 stations 134 measurements
NO$_2$ and NH$_4$ WP12 (TRACE) and WP 13 (TGB)	The ice core was sectioned and the sections were melted in gas-tight bags. NO$_2$ and NH$_4$ concentrations were measured on board as described by WP12+13 (*Chapter 19*)	10 stations 151 measurements
Meiofauna WP1 (EFICA)	The lower 10 cm of the ice core was melted in the laboratory and the meiofauna was sieved (10 µm mesh) and preserved in 4% formaldehyde buffered with hexamethylenetetramine for later land-based analysis by WP1+8 (*Chapter 8*)	15 stations 28 samples
Ice habitat bulk sample	**Description**	**Total number of bulk samples**
Top ice	Upper 10 cm of 12-16 ice cores combined into one bulk sample - Salinity was measured after melting	16 stations 16 bulk samples *
Centre-top ice	Upper half ice core minus 10 cm top ice of 3-8 ice cores combined into one bulk sample in a 115-L tank - Salinity was measured after melting	16 stations 16 bulk samples *
Centre-bottom ice	Lower half ice core minus 10 cm bottom ice of 3-8 ice cores combined into one bulk sample in a 115-L tank - Salinity was measured after melting	16 stations 16 bulk samples *
Bottom ice	Lower 10 cm of 12-16 ice cores combined into one bulk sample - Salinity was measured after melting	16 stations 16 bulk samples *
Snow	120 L of snow taken with a clean shovel into six 20-L buckets with lids and combined into one bulk sample in a 115-L tank	11 stations 11 bulk samples *
Melt pond	Hand-pumped 40 L of melt pond water into two 20-L carboys, combined in 50-L tank onboard - Temperature and salinity were measured in the field or in the lab	16 stations 16 bulk samples *
Brackish brine	Hand-pumped 40 L of brackish brine water into two 20-L carboys, combined in 50-L tank onboard - Temperature and salinity were measured in the field or in the lab	16 stations 16 bulk samples *
Ice-seawater interface	Hand-pumped 40 L of ice-seawater interface water into two 20-L carboys, combined in 50-L tank onboard - Temperature and salinity were measured in the field or in the lab	16 stations 16 bulk samples *

Table 6.9: Total upper half ice core length melted from each SAS ice station (top 10 cm and centre-top part separately), the volume of 0.2-µm filtered seawater (FSW) added to achieve a buffered melting process, and the dilution factors with which the measured values of the core parameters in the ice samples need to be multiplied to obtain the true concentrations in the original samples.

Top 10 cm of the sampled ice cores								
Device Operation	Station	Total core length (cm)	FSW added (L)	FSW added (mL/cm)	Melted total volume (L)	Melted ice volume (L)	Melted ice (mL/cm)	Dilution factor
SO21_06-01	6	163.0	8.0	49.1	15.0	7.0	42.9	2.14
SO21_08-10	8	163.0	3.0	18.4	10.0	7.0	42.9	1.43
SO21_13-06	13	149.0	7.5	50.3	15.5	8.0	53.7	1.94
SO21_18-06	18	122.0	6.0	49.2	11.5	5.5	45.1	2.09
SO21_22-13	22	145.0	7.0	48.3	12.0	5.0	34.5	2.40
SO21_26-16	26	117.0	6.0	51.3	12.0	6.0	51.3	2.00
SO21_30-18	30	162.0	8.0	49.4	15.0	7.0	43.2	2.14
SO21_34-01	34	161.0	8.0	49.7	14.0	6.0	37.3	2.33
SO21_35-16	35	161.5	8.0	49.5	15.0	7.0	43.3	2.14
SO21_38-18	38	170.0	8.0	47.1	12.5	4.5	26.5	2.78
SO21_42-18	42	160.0	8.0	50.0	15.0	7.0	43.8	2.14
SO21_49-01	49	160.0	8.0	50.0	14.5	6.5	40.6	2.23
SO21_50-17	50	160.0	8.0	50.0	15.0	7.0	43.8	2.14
SO21_53-16	53	160.0	8.0	50.0	14.0	6.0	37.5	2.33
SO21_56-09	56	163.0	8.0	49.1	15.5	7.5	46.0	2.07
SO21_58-19	58	158.0	8.0	50.6	16.5	8.5	53.8	1.94
Average		154.7	7.3	47.6	13.9	6.6	42.9	2.14
STDEV		14.9	1.4	7.9	1.8	1.1	7.0	0.28

Centre-top part of the sampled ice cores (upper half of the core minus the top 10 cm)								
Device Operation	Station	Total core length (cm)	FSW added (L)	FSW added (mL/cm)	Total melted volume (L)	Melted ice volume (L)	Melted ice (mL/cm)	Dilution factor
SO21_06-01	6	392.0	18.0	45.9	37.1	19.1	48.6	1.94
SO21_08-10	8	631.0	9.6	15.2	40.3	30.7	48.6	1.31
SO21_13-06	13	520.0	28.0	53.8	54.0	26.0	50.0	2.08
SO21_18-06	18	417.0	16.0	38.4	36.0	20.0	48.0	1.80
SO21_22-13	22	481.5	25.0	51.9	45.0	20.0	41.5	2.25
SO21_26-16	26	614.0	32.0	52.1	64.5	32.5	52.9	1.98
SO21_30-18	30	625.0	26.0	41.6	57.0	31.0	49.6	1.84
SO21_34-01	34	694.0	32.0	46.1	65.0	33.0	47.6	1.97
SO21_35-16	35	769.0	34.0	44.2	65.0	31.0	40.3	2.10
SO21_38-18	38	384.5	20.0	52.0	40.0	20.0	52.0	2.00
SO21_42-18	42	515.0	26.0	50.5	54.0	28.0	54.4	1.93
SO21_49-01	49	546.0	27.0	49.5	50.5	23.5	43.0	2.15
SO21_50-17	50	594.0	28.0	47.1	60.0	32.0	53.9	1.88
SO21_53-16	53	427.0	34.0	79.6	58.0	24.0	56.2	2.42
SO21_56-09	56	351.5	18.0	51.2	35.0	17.0	48.4	2.06
SO21_58-19	58	556.0	26.0	46.8	50.0	24.0	43.2	2.08
Average		532.3	25.0	47.9	50.7	25.7	48.6	1.99
STDEV		119.3	7.0	12.5	10.7	5.5	4.7	0.24

Table 6.10: Total lower half ice core length melted from each SAS ice station (centre-bottom part and bottom 10 cm separately), the volume of 0.2-µm filtered seawater (FSW) added to achieve a buffered melting process, and the dilution factors with which the measured values of the core parameters in the ice samples need to be multiplied to obtain the true concentrations in the original samples.

Centre-bottom part of the sampled ice cores (lower half of the core minus the bottom 10 cm)								
Device Operation	Station	Total core length (cm)	FSW added (L)	FSW added (mL/cm)	Melted total volume (L)	Melted ice volume (L)	Melted ice (mL/cm)	Dilution factor
SO21_06-01	6	335.5	18.0	53.7	34.6	16.6	49.5	2.08
SO21_08-10	8	639.0	9.6	15.0	41.2	31.6	49.5	1.30
SO21_13-06	13	410.0	24.0	58.5	46.0	22.0	53.7	2.09
SO21_18-06	18	467.0	16.0	34.3	42.0	26.0	55.7	1.62
SO21_22-13	22	471.5	25.0	53.0	45.5	20.5	43.5	2.22
SO21_26-16	26	705.0	32.0	45.4	68.0	36.0	51.1	1.89
SO21_30-18	30	688.0	26.0	37.8	60.0	34.0	49.4	1.76
SO21_34-01	34	603.0	32.0	53.1	62.0	30.0	49.8	2.07
SO21_35-16	35	880.0	34.0	38.6	75.0	41.0	46.6	1.83
SO21_38-18	38	398.0	20.0	50.3	42.0	22.0	55.3	1.91
SO21_42-18	42	401.0	18.0	44.9	42.0	24.0	59.9	1.75
SO21_49-01	49	566.0	27.0	47.7	56.0	29.0	51.2	1.93
SO21_50-17	50	629.0	28.0	44.5	63.0	35.0	55.6	1.80
SO21_53-16	53	410.0	34.0	82.9	52.0	18.0	43.9	2.89
SO21_56-09	56	350.5	18.0	51.4	26.0	8.0	22.8	3.25
SO21_58-19	58	590.0	25.1	42.5	57.0	31.9	54.1	1.79
Average		534.0	24.2	47.1	50.8	26.6	49.5	2.01
STDEV		152.8	7.1	14.0	13.1	8.6	8.4	0.47

Bottom 10 cm of the sampled ice cores								
Device Operation	Station	Total core length (cm)	FSW added (L)	FSW added (mL/cm)	Total melted volume (L)	Melted ice volume (L)	Melted ice (mL/cm)	Dilution factor
SO21_06-01	6	160.0	8.0	50.0	17.8	9.8	61.6	1.81
SO21_08-10	8	160.0	3.0	18.8	12.8	9.8	61.6	1.30
SO21_13-06	13	152.0	7.5	49.3	16.5	9.0	59.2	1.83
SO21_18-06	18	118.0	6.0	50.8	13.5	7.5	63.6	1.80
SO21_22-13	22	131.0	7.0	53.4	16.0	9.0	68.7	1.78
SO21_26-16	26	176.0	8.0	45.5	19.5	11.5	65.3	1.70
SO21_30-18	30	165.0	8.0	48.5	17.0	9.0	54.5	1.89
SO21_34-01	34	166.5	8.0	48.0	18.0	10.0	60.1	1.80
SO21_35-16	35	163.0	8.0	49.1	18.0	10.0	61.3	1.80
SO21_38-18	38	161.5	8.0	49.5	17.5	9.5	58.8	1.84
SO21_42-18	42	120.0	8.0	66.7	16.0	8.0	66.7	2.00
SO21_49-01	49	160.0	8.0	50.0	18.0	10.0	62.5	1.80
SO21_50-17	50	159.0	8.0	50.3	18.5	10.5	66.0	1.76
SO21_53-16	53	160.0	8.0	50.0	18.0	10.0	62.5	1.80
SO21_56-09	56	160.0	8.0	50.0	18.0	10.0	62.5	1.80
SO21_58-19	58	160.0	8.0	50.0	16.0	8.0	50.0	2.00
Average		154.5	7.5	48.7	16.9	9.5	61.6	1.80
STDEV		16.6	1.3	9.2	1.8	1.0	4.6	0.15
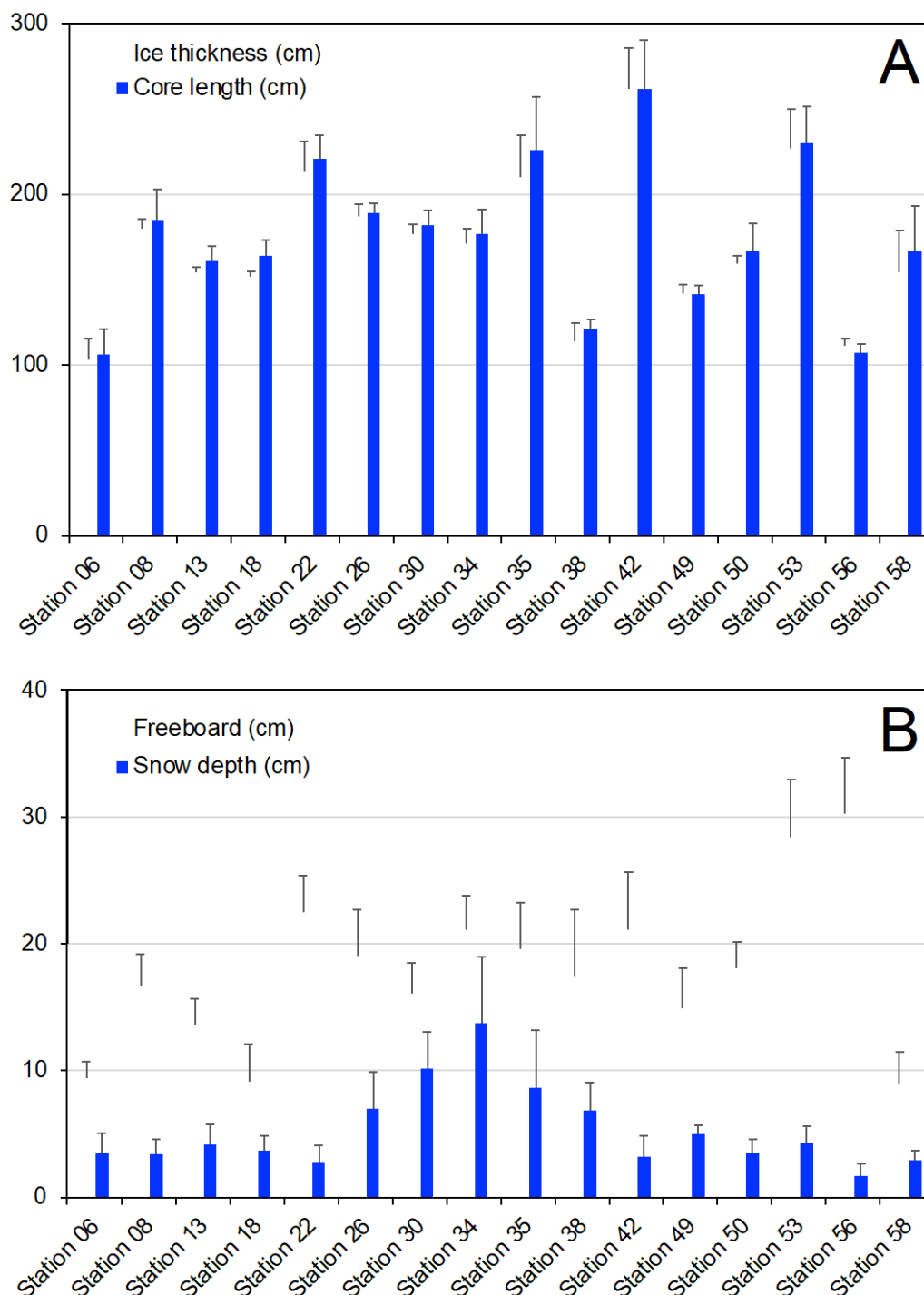
Figure 6.16: Summary of sea ice conditions at the 16 SAS Ice Stations of the SAS-Oden 2021 expedition, N = 18-20 ice cores per station. (A) Average ice thickness measured with an ice-thickness gauge after sampling of the ice cores and average core length of the sampled ice cores. (B) Average freeboard and average snow depth. ©Pauline Snoeijs-Leijonmalm

Air temperature at the 16 SAS Ice Stations during sampling varied between -7.6 and 0.6°C and decreased towards the surface water depending on the air temperature (*Table 6.11*). The temperature and salinity measurements of the ice cores showed different profiles (*Figure 6.17*). Temperature measured in the field varied between -3.5 and -6°C and salinity measured after melting of the ice sections in the laboratory varied between 0.0 and 4.4 ppt.

Table 6.11: Overview of temperature measurements made in the field while sampling at the 16 SAS Ice Stations of the SAS-Oden 2021 expedition. * = temperature not recorded during the field work on the ice but can be derived from the CTD data.

Device Operation	Air temperature (ºC)	Snow surface temperature (ºC)	Snow/ice interface temperature (ºC)	Surface water temperature (ºC)
SO21_06-01	0.5	-0.3	-0.5	-1.6
SO21_08-10	0.6	-0.3	-0.6	-1.2
SO21_13-06	-0.1	-0.5	-0.6	-0.7
SO21_18-06	0.2	0.0	-0.7	-0.7
SO21_22-13	-2.3	-2.0	-1.0	*
SO21_26-16	-0.2	-0.7	-0.7	-1.3
SO21_30-18	-2.2	-1.8	-0.9	-1.6
SO21_34-01	-3.3	-2.9	-1.8	-1.6
SO21_35-16	-7.6	-7.3	-1.5	-1.7
SO21_38-18	-5.1	-3.2	-1.8	-1.7
SO21_42-18	-3.3	-3.8	-1.8	-1.7
SO21_49-01	-0.8	-1.5	-1.6	-2.3
SO21_50-17	-2.6	-2.1	-1.3	-1.5
SO21_53-16	-2.9	-2.7	-1.6	*
SO21_56-09	-4.6	-4.2	-3.2	-1.9
DO21_58-19	-1.6	-1.4	-1.3	-1.7
Minimum	**-7.6**	**-7.3**	**-3.2**	**-2.3**
Maximum	**0.6**	**0.0**	**-0.5**	**-0.7**
Average	**-2.2**	**-2.2**	**-1.3**	**-1.5**
STD	**2.3**	**1.9**	**0.7**	**0.4**
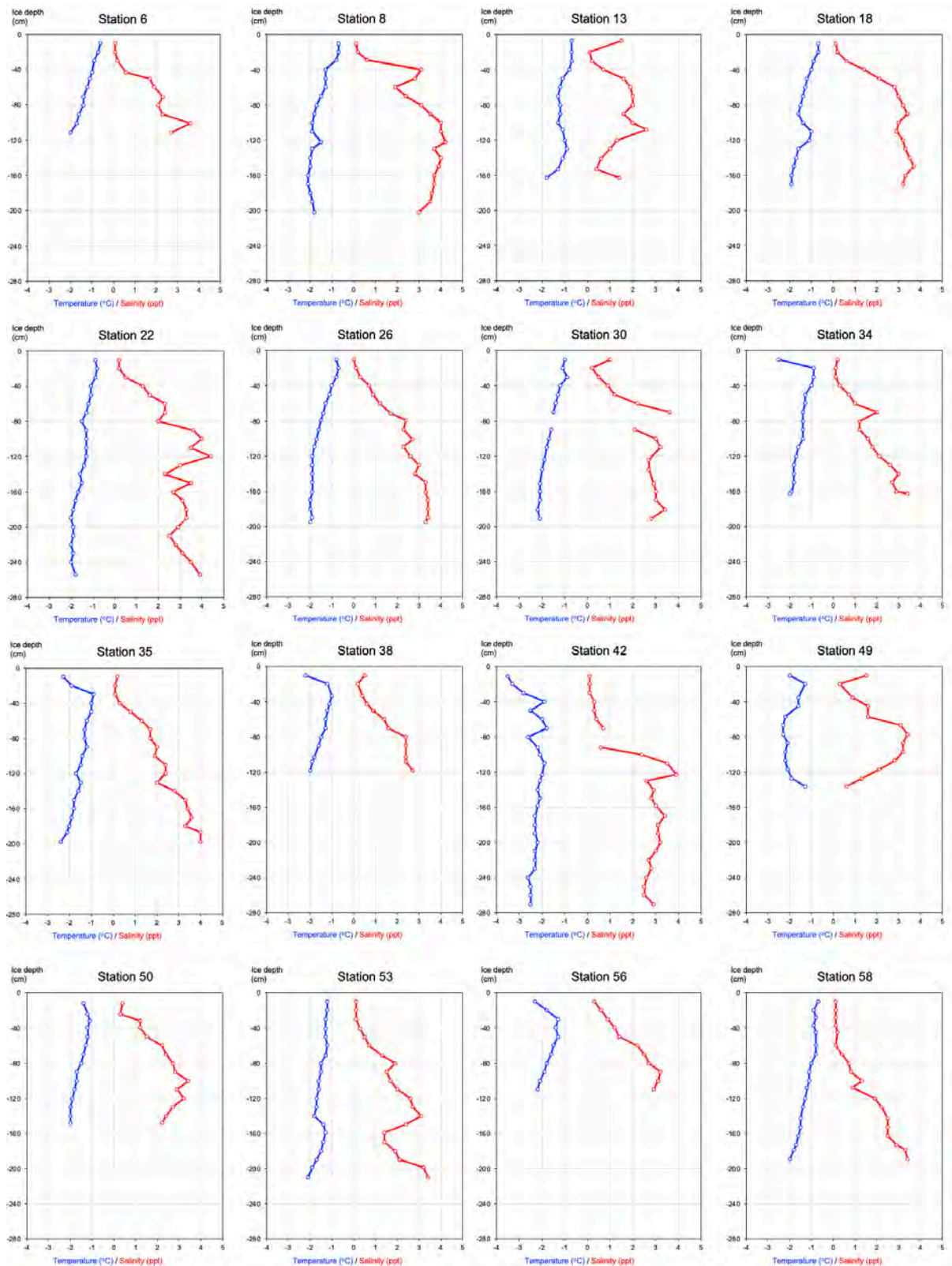
Figure 6.17: Sea ice temperature and salinity at the 16 SAS Ice Stations of the SAS-Oden 2021 expedition, measured from one ice core per station. Missing data (at Stations 30 and 42) are due to lost samples. ©Pauline Snoeijs-Leijonmalm

7 Summary of research data collected

7.1 The SAS-Oden 2021 Research Data Management Policy

All research projects participating in the SAS-Oden 2021 expedition had adopted the "SAS-Oden 2021 Research Data Management Policy" of the SPRS (*Appendix A*) prior to the expedition. This policy is also valid for opportunistic sampling during the expedition (*Table 4.3, Chapter 23*) as well as for use of expedition data by third parties.

Research data is defined as all scientific data collected during the expedition or analysed from samples collected during the expedition. The essence of the policy is that data originating from research projects on board *IB Oden* during the SAS-Oden 2021 expedition are to be published with full open access, with proper attribution to the data owner and field collectors, and acknowledging the SPRS for the infrastructure provided, and with minimum delay, unless otherwise agreed upon by the SPRS. Acknowledgements in any type of publication using data from the SAS-Oden 2021 expedition should read as follows:

"The Swedish Polar Research Secretariat (SPRS; https://polar.se) organized and supported the SAS-Oden 2021 expedition with *IB Oden* in the Central Arctic Ocean. This expedition was the Swedish contribution to the International "Synoptic Arctic Survey" (SAS; https://synopticarcticsurvey.w.uib.no/). We thank the Master and crew of *IB Oden* for expertly undertaking the SAS-Oden 2021 expedition."

The basis of the SAS-Oden 2021 Research Data Management Policy is that the SPRS recognises that high quality research data are valuable products of field work. Data sharing, with fair attribution, is a corner stone for scientific collaboration, not least for the evolvement of interdisciplinary science. Data preservation with open access is fundamental for the legacy of any research activity. Facilitating re-use and re-purposing of research data adds long-term value of the data to scientific research, industry, and society at large. Building on the data statements of the International Council for Science (ICSU) and the International Arctic Scientific Committee (IASC)[26], SPRS works towards ethical open data publication pertaining supported projects, programmes and research infrastructure. SPRS seeks to promote high standards and best practice for management of research data.

Research data includes any information necessary to validate and reproduce the results of scientific research. This could be, e.g., field notes, primary data files, images, or audio-visual materials. Metadata refers to structured information about the data such as information on each measurement and sample taken (immediately after the expedition and submitted with this report) or elaborated data files on which publication is based (immediately after publication of a scientific paper or technical report). Necessary information provided should also include technical information (such as file formats) as well as descriptions of provenance and context (purpose of study, timeframe, sampling locations, equipment used etc.). The data and metadata should be descriptive and detailed enough to enable independent interpretation and re-use of the data. Published research data and metadata should be accurate, complete, identifiable, and openly accessible, and strive to meet the FAIR principles[27]. The datasets together with accompanying metadata should be archived securely and safely in appropriate formats to ensure long-term usability. The data publication should also be given a

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[26] https://iasc.info/images/data/IASC_data_statement.pdf
[27] https://www.force11.org/fairprinciples

unique persistent identifier enabling citation, versioning, and proper attribution. The SAS-Oden 2021 Research Data Management Policy stipulates the following principles for research data and metadata:

(1) The SPRS is the data owner of all data originating from installations on *IB Oden* funded and/or operated by SPRS. Metadata and data are to be made available with free, unrestricted, complete access, without charge and with minimum delay in the SND data repository[28] after completion of the cruise. This includes the basic ship data (date, time, position, depth, meteorology, etc.), the acoustic data, and the CTD data including the data from all instruments attached to the CTD rosette provided by the SPRS but excluding the data collected by extra instruments attached to the CTD rosette by specific research projects. This does not include the use of devices owned by the SPRS for sampling water (e.g., CTD rosette) or ice station sampling equipment (e.g., ice corers and drills).

(2) Data collected during the expedition within the scope of a separately funded research project that requires specific installations or activities outside the premises of (1). This also applies to opportunistic sampling listed in *Table 4.3*. Either the organisation of the principal investigator (PI) or the PI is owner of data, depending on applicable regulations or agreements. Metadata and data are to be made available with free, unrestricted, complete access, and without charge within the framework of current legislation. Metadata are to be submitted to the SND data repository or other suitable data repository with minimum delay after completion of the cruise. Access to data can be restricted by the PI for a maximum of two years (period of moratorium). If the funder of the research project has stipulated a shorter period of moratorium this has precedence.

For clarity, the data ownership and collector details of all parameters measured during the SAS-Oden 2021 expedition are summarized in *Table 7.1*. For all use of the collected data in scientific publications or other publication means by others than the 16 SAS-Oden 2021 projects, written permission from the data owner must be obtained. All use of the data owned by the SPRS, as described under (1) above, is allowed for use in scientific publications or other publication means by the 16 SAS-Oden 2021 projects without written permission, but only as long as ethical limits between projects are not violated. For example, each SAS-Oden 2021 project that wishes to relate its own measurements in the water column to collectively measured environmental conditions such as depth, temperature, salinity, oxygen concentration, chlorophyll fluorescence, CDOM fluorescence, etc., is free to do so if this is relevant for the project's research questions stated in this report. However, this should not compete with the research questions of other SAS-Oden 2021 projects as described in *Chapters 8-22*, e.g., WP14 and WP15 focus on physical oceanography (temperature, salinity), WP2 on microbial C and N metabolism, notably biological N_2-fixation and ammonium oxidation, WP7 on primary production (including chlorophyll fluorescence), WP10 on the carbon cycle (including CDOM fluorescence). Projects are encouraged to collaborate and co-publish as soon as research questions overlap.

Similarly, each SAS-Oden 2021 project that wishes to relate its own measurements in the ice habitats to depth, temperature, salinity, and other environmental conditions measured collectively at the ice stations is free to do so if this is relevant for the project's research questions stated in this report. However, specific measurements performed on water from the ice habitats by, e.g., WP7 and WP10 with focus on nutrients and ecosystem productivity are of course published by the appropriate project as described in *Chapters 8-22*. Projects are encouraged to collaborate and co-publish as soon as research questions overlap.

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[28] https://snd.gu.se/en

7.2 Ownership of the SAS-Oden 2021 data

All data collected during the SAS-Oden 2021 expedition measured with instruments owned by the SPRS are available in the SND data repository. All scientific metadata from the expedition (*Table 7.1*), including those from all 16 SO21 research projects and all opportunistic sampling, are available in the SND at the time of publication of this report. All full project data from the expedition should be available in the SND latest by 1 October 2023 according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). If there are any problems uploading the final project data, please contact the SPRS. It is also recommended to publish (elaborated) project data to journal web sites with the publication of scientific papers.

With respect to the purpose of the expedition, i.e., the international SAS collaboration, the SAS Core Parameters were incorporated in the 16 research SAS-Oden 2021 research projects. Data ownership of all data collected during the expedition is defined in *Table 7.1*, and anyone who wishes to use these data for use in a publication should contact the data owner for consultation and collaboration. The data and samples for most of the SAS Core Parameters have been collected within the research projects and the data owner is the PI of the project. This means that if you would like to use the SO21 chlorophyll *a* data in a publication, you need to make an agreement with the data owner of the SO21 chlorophyll *a* data in *Table 7.1* about this – even after the moratorium.

Table 7.1: Data ownership of the data and samples collected during the SAS-Oden 2021 expedition, for project-specific experimental incubations, etc., see the respective WP chapters (8-22).

SAS Core Parameter	Measurements/ samples collected	Main SO21 data users or purpose	Main onboard data/sample collectors	Data/sample owner (organisation/ project PI)
Water-column data: measured with instruments				
Pressure (depth)	112 CTD casts	Most WPs, SO21 omics	WP14 + WP15	SPRS (instrument owner)
Temperature	112 CTD casts	Most WPs, SO21 omics	WP14 + WP15	SPRS (instrument owner)
Salinity	112 CTD casts	Most WPs, SO21 omics	WP14 + WP15	SPRS (instrument owner)
Dissolved oxygen	112 CTD casts	Most WPs, SO21 omics	WP14 + WP15	SPRS (instrument owner)
Chlorophyll fluorescence	50 CTD casts	WP6, WP7, WP10, SO21 omics	WP14 + WP15	SPRS (instrument owner)
CDOM fluorescence	62 CTD casts	WP7, WP10, SO21 omics	WP14 + WP15	SPRS (instrument owner)
PAR	62 CTD casts	WP6, WP7, WP10, SO21 omics	WP14 + WP15	SPRS (instrument owner)
Turbidity	62 CTD casts	WP1, WP7, WP10, WP14, SO21 omics	WP14 + WP15	SPRS (instrument owner)
LADCP	53 CTD casts	WP14	WP14 + WP15	SPRS (instrument loan)
Light sensor (TDR-Mk9-404A tag)	50 CTD casts	WP1	WP1	Pauline Snoeijs-Leijonmalm (SU)
Secchi depth	21 stations	WP10	WP10	Marcus Sundbom (SU)
EK80 hydroacoustics (raw data)	Continuous along ship route	WP1 + WP14	WP1 + WP14	SPRS (instrument owner/ loan)
WBAT hydroacoustics	49 CTD casts	WP1	WP1 + WP14	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)

UVP optics	38 CTD casts	WP1	WP1 + WP14	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)
LOKI optics	11 stations	WP1	WP1	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)
Mini FishCam	15 stations	WP1	WP1	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)
Water-column data: measured from water samples taken with the CTD rosettes				
Salinity	10 stations 60 samples	For calibration of CTD salinity sensors	WP10	Adam Ulfsbo (GU)
CDOM	7 stations 72 samples	For calibration of CTD CDOM sensor	WP10	Marcus Sundbom (SU)
Dissolved oxygen (DO)	32 CTD full depth 673 samples	WP10	WP10	Adam Ulfsbo (GU)
Dissolved inorganic carbon (DIC)	32 CTD full depth 673 samples	WP10	WP10	Adam Ulfsbo (GU)
Total alkalinity (TA)	32 CTD full depth 673 samples	WP10	WP10	Adam Ulfsbo (GU)
pH	32 CTD full depth 673 samples	WP10	WP10	Adam Ulfsbo (GU)
CFC-12 and SF_6	32 CTD full depth 709 samples	WP11	WP11	Toste Tanhua (GEOMAR)
Nitrous oxide concentration	32 CTD full depth 605 water samples	WP12	WP12 + WP13	Damian L. Arévalo-Martínez (GEOMAR)
Methane concentration	32 CTD full depth 605 water samples	WP13	WP12 + WP13	Brett Thornton (SU)
Inorganic nutrients (N, P, Si)	32 CTD full depth 683 samples	WP7, WP10, SO21 omics	WP10	Marcus Sundbom (SU)
$\delta^{18}O$ of H_2O	32 CTD full depth 421 samples	WP10	WP10	Marcus Sundbom (SU)
DOC Dissolved organic carbon	32 CTD full depth 691 samples	WP10	WP10	Marcus Sundbom (SU)
Particulate organic carbon (POC), including ^{13}C	20 CTD >500 m 180 samples	WP7 + WP10	WP10	Marcus Sundbom (SU)
Particulate organic carbon (POC), including ^{13}C	18 CTD 0-500 m 216 samples	WP7 + WP10	WP7	Pauline Snoeijs-Leijonmalm (SU)
Primary production (^{13}C incubations)	18 CTD 0-500 m 432 samples (light+dark)	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Particulate organic nitrogen (PON), incl. ^{15}N	18 CTD 0-500 m 216 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Particulate organic phosphorus (POP)	18 CTD 0-500 m 216 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
HPLC pigments (chlorophylls and carotenoids)	18 CTD 0-500 m 216 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Chlorophyll concentration (0.3-2 μm)	18 CTD 0-500 m 216×2 samples	WP6	WP6	Hanna Farnelid (LNU)
Chlorophyll concentration (2-200 μm)	18 CTD 0-500 m 216×2 samples	WP7	WP7	Hanna Farnelid (LNU)
Virus density (flow cytometry)	18 CTD full depth 317 samples	WP4, SO21 omics	WP7	Karin Holmfeldt (LNU)
Prokaryote density (flow cytometry)	18 CTD full depth 317 samples	WP7, SO21 omics	WP7	Pauline Snoeijs-Leijonmalm (SU)
Prokaryotic abundance (microscopy)	13 stations 182 samples	WP5	WP5	Johan Wikner (UMU)
Prokaryotic biomass growth	13 stations 749 measurements	WP5	WP5	Johan Wikner (UMU)

Plankton respiration	13 stations 96 measurements	WP5	WP5	Johan Wikner (UMU)
Phytoplankton density (flow cytometry)	18 CTD 0-500 m 216×3 samples	WP6, WP7, SO21 omics	WP7	Hanna Farnelid (LNU)
Phytoplankton composition (Lugol)	18 CTD ChlMax 18 samples	WP7, SO21 omics	WP7	Pauline Snoeijs-Leijonmalm (SU)
DNA	18 CTD profiles 408 samples	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
RNA	18 CTD profiles 406 samples	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
DNA + RNA virus	9 CTD stations 18 samples	SO21 omics (WP2+4)	WP4	Karin Holmfeldt (LNU)
Water-column data: net samples				
Microzooplankton biomass and composition	11 stations 11 community samples	WP1 + WP8	WP1 + WP8	Pauline Snoeijs-Leijonmalm (SU), and Samuel Hylander (LNU)
Mesozooplankton biomass and composition	16 stations 84 community samples	WP1 + WP8	WP1 + WP8	Pauline Snoeijs-Leijonmalm (SU), and Samuel Hylander (LNU)
Macrozooplankton biomass and composition	9 EFICA Master Stations 50 community samples	WP1 + WP8	WP1 + WP8	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)
Zooplankton and fish ^{13}C, ^{15}N, fatty acids	Different nets 567 zooplankton samples	WP1	WP1	EU Commission (EFICA) contact: Pauline Snoeijs-Leijonmalm (SU)
Surface-water data (underway ship seawater intake)				
pH	Continuous	WP10	WP10	Adam Ulfsbo (GU)
Total alkalinity (TA)	Continuous	WP10	WP10	Adam Ulfsbo (GU)
Underway biological O_2 saturation (O_2/Ar ratios)	Continuous	WP10	WP10	Adam Ulfsbo (GU)
Nitrous oxide concentration	120 samples	WP12	WP12 + WP13	Damian L. Arévalo-Martínez (GEOMAR)
Methane concentration	120 samples	WP13	WP12 + WP13	Brett Thornton (SU)
Bacterial and phytoplankton community composition and nutrients	37 stations	WP6	WP6	Hanna Farnelid (LNU)
Ice station data (ice cores, snow, melt ponds, brackish brine, ice-seawater interface)				
Salinity	All eight ice habitats	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
Temperature	All eight ice habitats	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
Inorganic nutrients (N, P, Si) + TOC	10 stations (ice + water) 345 samples	WP10, SO21 omics	WP10	Marcus Sundbom (WP10)
DIC + TA	9 ice stations (ice cores) 134 samples	WP10, SO21 omics	WP10	Adam Ulfsbo (GU)
Nitrous oxide concentration	10 stations (ice cores) 151 samples	WP12	WP12 + WP13	Damian L. Arévalo-Martínez (GEOMAR)
Methane concentration	10 stations (ice cores) 151 samples	WP13	WP12 + WP13	Brett Thornton (SU)
Organic nutrients (totN, totP)	16 stations (water) 48 samples	SO21 omics (WP1-WP9)	WP7	Pauline Snoeijs-Leijonmalm (SU)
Particulate organic carbon (POC), including ^{13}C	16 ice stations 116 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Particulate organic nitrogen (PON), incl. ^{15}N	16 ice stations 116 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)

Particulate organic phosphorus (POP)	16 ice stations 116 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Primary production (¹³C incubations)	16 ice stations 232 samples (light + dark)	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Chlorophyll concentration (0.3-2 μm)	16 ice stations 116×3 samples	WP6	WP6	Hanna Farnelid (LNU)
Chlorophyll concentration (2-200 μm)	16 ice stations 116 samples	WP7	WP7	Hanna Farnelid (LNU)
HPLC pigments (chlorophylls and carotenoids)	16 ice stations 116 samples	WP7	WP7	Pauline Snoeijs-Leijonmalm (SU)
Virus density (flow cytometry)	16 ice stations 116 samples	WP4, SO21 omics	WP7	Karin Holmfeldt (LNU)
Prokaryote density (flow cytometry)	16 ice stations 116 samples	WP7, SO21 omics	WP7	Pauline Snoeijs-Leijonmalm (SU)
Phytoplankton density (flow cytometry)	16 ice stations 116 samples	WP6, WP7, SO21 omics	WP7	Hanna Farnelid (SU)
Phytoplankton composition (Lugol)	16 ice stations 48 samples	WP7, SO21 omics	WP7	Pauline Snoeijs-Leijonmalm (SU)
DNA	16 ice stations 445 samples	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
RNA	16 ice stations 192 samples	SO21 omics (WP1-WP9)	WP1-WP9	Pauline Snoeijs-Leijonmalm (SU)
DNA + RNA virus	10 ice stations 34 samples	WP4, SO21 omics	WP7	Karin Holmfeldt (LNU)
DNA aerosols	9 EFICA Master Stations 25 samples	WP2, SO21 omics	WP2	Caroline Leck (SU)

7.3 Ship data

The data collected by instruments belonging to *IB Oden* ("Ship data") were submitted to the SND data repository by the SPRS. This includes high-resolution expedition route data (date, time, geographical position) and meteorological data. An excel file with average data per 30 minutes covering the time 2021-07-27 until 2021-09-18 was made by the Chief Scientist since this is detailed enough for most of the SO21 projects. This file, called "SO21_Route_30_minutes", is available in the SND and its contents are summarized in *Table 7.2*.

Table 7.2: Organisation of the excel file "SO21_Route_30_minutes" of the SAS-Oden 2021 expedition.

Parameter	Explanation
DateTime	Date and UTC (Coordinated Universal Time)
Oden.Ship.LatitudeDegrees	Decimal degrees (DD) express latitude and longitude geographic coordinates as decimal fractions of a degree
Oden.Ship.LongitudeDegrees	Decimal degrees (DD) express latitude and longitude geographic coordinates as decimal fractions of a degree
Oden.Ship.HDT	Ship heading (degrees)
Oden.Ship.COG	Ship course over ground (degrees)
Oden.Ship.SOG	Ship speed over ground (knots)
Oden.Met.WindSpeedRel	Ship-relative wind speed, from the two wing-mounted ship anemometers (m/s)
Oden.Met.WindDirectionRel	Ship-relative wind direction, from the two wing-mounted ship anemometers (direction FROM, wind on bow is 0)
Oden.Met.WindSpeedTrue	True wind speed, from the two wing-mounted ship anemometers (m/s)
Oden.Met.WindDirectionTrue	True wind direction, from the two wing-mounted ship anemometers (direction FROM, northerly wind is 0)

7.4 The SO21 omics collaboration

The WP1 (EFICA) and WP2 (MIME) project descriptions include a full metagenomics (taxonomic) and metatranscriptomics (gene expression) biodiversity record of water-column, sea ice, and sediment habitats along the SAS-Oden 2021 expedition route. For EFICA the goal is to assess the eDNA distribution of nekton (fish and squid), their zooplankton prey, and their bird and mammal predators in the CAO. For MIME the goal is to assess microbial (prokaryote and eukaryote) community composition and functional diversity with emphasis on carbon and nitrogen metabolism with the specific hypotheses (a) that diazotrophy (biological N_2-fixation) is a significant source of new nitrogen to the oligotrophic CAO marine ecosystem, and (b) that urea is an alternative substrate for ammonium oxidation and chemoautotrophic CO_2 fixation in the CAO at low ammonium availability.

However, during the preparations for the expedition it became clear that all SO21 biological projects (WPs 1-9) had an interest in taking DNA and RNA field samples for various purposes with overlapping sampling, thereby strongly violating the water budget. To avoid double work and enhance collaboration between the nine biological SO21 projects, it was decided that the sampling of DNA and RNA field samples (metagenomics, metatranscriptomics, 16S, 18S) would be performed in a large combined sampling effort by WPs 1-9 called the "SO21 omics collaboration". Thus, all DNA/RNA field samples taken during the SAS-Oden 2021 expedition for assessing species composition (DNA) and functional diversity (RNA) along the SAS-Oden 2021 expedition route are included in the SO21 omics collaboration based on collaborative sampling. The metadata for this collaboration are provided in the excel file "SO21_Metadata_Omics" in the SND data repository and summarized in *Tables 7.4-7.7*. Note that this includes only field samples and excludes samples taken for DNA/RNA analyses related to the incubation experiments carried out by WP2 (MIME), WP3 (ProMis), WP4 (VIRUS), WP5 (ASAP), and WP6 (PICO).

The collective sampling by WPs 1-9 during the SAS-Oden 2021 expedition made it possible to collect a significantly larger biodiversity dataset from field samples along the SAS-Oden 2021 expedition route (more stations, more habitats) than would have been possible to achieve by WP1 and WP2, or any of the other WPs, alone. During the expedition, the SO21 omics sampling programme was coordinated by WP2. All field and laboratory equipment and consumables were brought by WP1 and WP2, and the extractions, sequencing and bioinformatics will be performed by WP1 and WP2 after the expedition. Pauline Snoeijs-Leijonmalm, as PI of WP1 and WP2, is the data owner of the SO21 omics dataset, and the following agreements were made with all collaborators before the expedition:

(1) All collaborators will together publish the full SO21 omics dataset (*Tables 7.4-7.7*), which will be a unique and comprehensive survey of taxonomic biodiversity and gene expression along the SAS-Oden 2021 expedition route, including the water column, ice habitats, deep-sea sediments and aerosols. This publication will be coordinated by Pauline Snoeijs-Leijonmalm and co-authored by all who had a substantial role in designing the study, sampling and filtering the SO21 omics samples onboard *IB Oden*, extracting the DNA/RNA, and contributing with bioinformatics analyses for the publication. This includes metagenomics, metatranscriptomics, 16S, and 18S data.

(2) After the full SO21 omics dataset has been published in an international journal (foreseen in spring 2023), all sequencing data will be on-line with full open access at the European infrastructure for life science/sequence data (EBI[29]). Anybody can then dig deeper into the dataset with bioinformatic methods, especially the onboard SO21 projects because they have other measured data to back up their specific hypotheses, e.g., WP1 for eDNA, WP2 for microbial metabolisms, WP3 for fungi, WP4 for viruses, etc.

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[29] https://www.ebi.ac.uk

(3) After the full SO21 omics dataset has been published, left-over extracted DNA and RNA aliquots, as well as replicate filters (although one filter will always be kept as a reference library filter) can be requested from Pauline Snoeijs-Leijonmalm by onboard SO21 projects, as well as by third parties for testing specific scientific hypotheses not competing with the onboard SO21 projects. Examples of onboard projects using these aliquots are, e.g., barcoding of the CO1 and 12S amplicons (WP1), barcoding of the *nif*H amplicon (WP2), etc. Since metagenomics and metatranscriptomics exists for exactly the same samples it is then possible to cross-link bioinformatics analyses between the different data types.

The SO21 omics samples were obtained from seawater from the bow CTD (*Chapter 6.2*), seawater from the stern CTD (*Chapter 6.3*), sediment cores from the box core (*Chapter 6.6*), ice habitats from the SAS Ice Stations (*Chapter 6.7*), and the aerosol pump, fog and surface water sampling (*Chapter 23.1*). From the melted ice and snow only DNA samples (no RNA samples) were taken because RNA (gene expression) changes very fast when environmental conditions change and 3-40 hours of melting generates an enormous change of environmental conditions, so that analysing RNA would be unrealistic.

The SO21 water-column samples were taken at seven standard depths (*Figure 7.1; Table 7.3*) or – at stations shallower than 3000 m – the possible standard depths and the bottom. From the stern CTD (down to 1000 m), six Niskin bottles (72 L) per depth were reserved for the SO21 omics collaboration as this was necessary because of leakage of the Niskin bottles on this CTD rosette, and from the bow CTD (below 1000 m) two Niskin bottles (24 L) per depth.

Figure 7.1: Retrieval of the CTD omics SAS at the stern of IB Oden, *illustrating the collaboration at the CTD container. In the back from left to right: SPRS technicians Joachim Gyllestad (only hands and feet) and Niklas Vestin, and CTD team members Alexandra Padilla and Salar Karam (his back). In the front from left to right: Javier Vargas Calle (WP2), Prune Leroy (WP2), Claudia Morys (WP2), and Clara Pérez Martínez (WP7) ready to sample the SO21 omics water.* ©Yannis Arck

The SO21 omics water samples, as well as the bulk samples from melted ice and snow, were filtered on 0.2 μm Sterivex™ filter units (four replicates as a standard) with a pore size of 0.2 μm, flash-frozen in liquid nitrogen, and stored at -80°C according to "SO21 SOP: omics" (*Chapter 24*). Immediately after sampling, the water-column water (from the CTD) and the ice-habitat water (ice-seawater interface,

brackish brine, melt pond) was, were brought to the unheated laboratory containers onboard as fast as possible (*Figure 7.2 B-F*). These labs were unheated and illuminated with red light to not contaminate photosensitive cells by white light. In the labs, other scientists than those sampling from the CTD or on the ice had already prepared the peristaltic pumps with tubing and 0.2 µm Sterivex™ filter units. This fast procedure was necessary for two reasons: (1) RNA degrades very fast and gene expression changes very fast when environmental conditions change, and (2) the water in the carboys partly freezes when they are left outside for more than 10 minutes, which may destroy cells. The ice-seawater interface, brackish brine, and melt pond water were combined into bulk samples in 50-L containers. The RNA samples were filtered first for max. 30 minutes, all four replicates simultaneously with max. 45 minutes between sampling, filtered and flash-frozen in liquid nitrogen to avoid changes in gene expression as much as possible. The DNA samples were filtered after the RNA samples for max. 60 minutes and flash-frozen. The viral DNA samples were, after iron chloride treatment to form aggregates, filtered from the filtrate of the RNA and DNA filters (still containing viruses) on a 142 mm diameter Omnipore PTFE membrane filter with pore size 1.0 µm filter on a membrane filter with pore size 0.8 µm as a support filter, and stored at -80°C according to "SO21 SOP: viromics" (*Chapter 24*).

During the SAS-Oden 2021 expedition, SO21 omics DNA samples were taken from 18 CTD stations and 16 SAS ice stations. CTD stations and ice stations were located at the same geographical position (ice stations next to the ship) or within one nautical mile from the ship (ice stations by helicopter), except for in the very beginning of the sampling programme when there were problems with the CTD.

Altogether, 896 SO21 omics DNA filters (*Table 7.4*) and 596 SAS-Omics RNA filters (*Table 7.5*) were made during the expedition. From filtrates of the SO21 omics DNA and RNA 0.2 µm Sterivex™ filter units, 52 virus "Viromics" filters were made (*Chapter 11*, *Table 7.6*). These "Viromics" filters were treated in a different way to bind smaller particles (viruses and possibly also eDNA) with iron chloride.

The SO21 omics sediment samples (6 stations, 10 replicate sediment cores per station) were taken with polycarbonate cores of 4 cm in diameter (*Figure 7.3*) according to "SO21 SOP: box core" (*Chapter 24*). Each sediment core was sealed and immediately frozen at -80°C. DNA extraction and metagenomic analyses will be performed on 1-cm sediment slices.

The SO21 omics aerosol samples fall within the category "opportunistic sampling" (*Chapter 23*) for Caroline Leck (SU, Sweden), and are summarized in *Table 7.7*. These samples consist of particles in air on filters using an air pump on the roof of the "Triple lab", bottles with fog samples (stored at -80°C), and bottles with surface-water samples (stored at -20°C).

Table 7.3: Standard depths of the SO21 omics water-column samples taken during the SAS-Oden 2021 expedition, the CTD used for water sampling, and the number of Niskin bottles reserved for the SO21 omics collaboration.

Standard SO21 omics depth	CTD	Number of Niskin bottles
ChlMax (variable depth, 12-45 m)	Stern	6
100 m	Stern	6
TempMax (variable depth, 200-411 m)	Stern	6
1000 m	Stern	6
2000 m	Bow	2
3000 m	Bow	2
Bottom (variable depth, deepest 4335 m)	Bow	2

Figure 7.2: Filtrations for the SO21 omics collaboration in the red-light laboratory containers. (A) The 115-L containers for melting bulk samples of ice and snow. The snow was sampled in the white buckets and the top and bottom ice core sections in the containers with red lids. Filtered seawater was available in the blue carboys. (B) The 50-L containers with the ice-seawater interface, brackish brine, and melt pond bulk samples. (C) collecting water from the bulk samples into 10-L bottles with scales to read the volume, working in the laboratory from back to front: Claudia Morys (WP2), Janina Rahlff (WP4), and Claire Bird (WP9). (D) The peristaltic pumps used, working in the laboratory: Prune Leroy (WP2). (E) Filtration on Sterivex™ filter units. (F) After the filtrations the peristaltic-pump tubing was rinsed with MilliQ water. (A,B,C,D, F) ©Pauline Snoeijs-Leijonmalm, (E) ©SPRS

Table 7.4: Overview of the SO21 omics water samples for DNA metagenomic analyses, including the bulk samples from melted ice and snow, filtered on 0.2 µm Sterivex™ filter units, flash-frozen in liquid nitrogen and stored at -80°C, during the SAS-Oden 2021 expedition. The average volume of water filtered was 3.2 L (range 0.5 – 10.0 L), the maximum filtration time per filter unit was ca. 60 minutes. The filtration procedure is described in "SO21 SOP: omics" (Chapter 24).

Ship station	SAS Ice Station	ChlMax	100 m	TempMax	1000 m	2000 m	3000 m	4000 m or bottom	Ice-seawater interface	Brackish brine	Melt pond	Top ice (10 cm)	Ice Centre-top	Ice Centre-bottom	Bottom ice (10 cm)	Snow	Total
3		4	4	4	4												16
	6								4	4	4	4	4	4	4		28
7		4	4	4	4	4	4	4									28
8	8	4	4	4	4	4		4	4	4	4	4	4	4	4	4	56
13	13	4	4	4	4	4	4	4	4	4	4	4	4	4	4		56
16		4	4	4	4	4	4	4									28
18	18	4	4	4	4	4	4	4	4	4	4	4	4	4	4		56
22	22	4	4	4	4	4	4	4	4	4	4	4	2	3	4	4	57
26	26	4	4	4	4			4	4	4	4	4	4	4	4	4	52
30	30	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	60
33	34	4	4	4	4	4		4	4	4	4	4	4	4	4	4	56
35	35	4	4	4				4	4	4	4	4	4	4	4	4	52
38	38	4	4	4	4			4	4	4	4	4	4	4	4	4	52
42	42	4	4	4				4	4	4	4	4	4	4	4	4	48
48	49	4	4	4	4			4	4	4	4	4	4	4	4		48
50	50	4	4	4				4	4	4	4	4	4	4	4	3	47
53	53	4	4	4	4	4	4	4	4	4	4	4	4	4	4		56
56	56	4	4	4	4				4	4	4	4	4	4	4	4	48
58	58	4	4	4	4			4	4	4	4	4	4	4	4	4	52
Total		**72**	**72**	**72**	**64**	**36**	**28**	**64**	**64**	**64**	**64**	**64**	**62**	**63**	**64**	**43**	**896**

Table 7.5: Overview of the SO21 omics water samples for RNA metatranscriptomic analyses filtered on 0.2 µm Sterivex™ filter units, flash-frozen in liquid nitrogen and stored at -80°C, during the SAS-Oden 2021 expedition. RNA was not sampled from the bulk samples with melted ice and snow because the RNA was modified during the melting process. The average water volume filtered was 2.5 L (range 0.5 – 4.8 L), the maximum filtration time was ca. 30 minutes. The filtration procedure is described in "SO21 SOP: omics" (Chapter 24).

Ship station	SAS Ice Station	ChlMax	100 m	TempMax	1000 m	2000 m	3000 m	4000 m or bottom	Ice-seawater interface	Brackish brine	Melt pond	Total
3		4	4	4	4							16
	6								4	4	4	12
7		4	4	4	4	4	4	4				28
8	8	4	4	4	4	4		4	4	4	4	36
13	13	4	4	4	4	4	4	4	4	4	4	40
16		4	4	4	4	4	4	4				28
18	18	4	4	4	4	4	4	4	4	4	4	40
22	22	4	4	4	4	4	4	4	4	4	4	40
26	26	4	4	4	4			4	4	4	4	32
30	30	4	4	4	4	4	4	4	4	4	4	40
33	34	4	4	4	4	4		4	4	4	4	36
35	35	4	4	4	4			4	4	4	4	32
38	38	4	4	4	4			4	4	4	4	32
42	42	4	4	4				4	4	4	4	28
48	49	4	4	4	4			4	4	4	4	32
50	50	4	4	4				4	4	4	4	28
53	53	4	4	4	4			4	4	4	4	32
56	56	4	4	4	4	4		2	2	4	4	34
58	58	4	4	4	4			4	4	4	4	32
Total		**72**	**72**	**72**	**64**	**36**	**24**	**66**	**64**	**64**	**64**	**596**

Table 7.6: Overview of the SO21 omics water samples for small-particle analyses (viruses) filtered by WP4 from the filtrate of 0.2 μm Sterivex™ filter units in Tables 7.4 and 7.5 , flash-frozen in liquid nitrogen and stored at -80°C, during the SAS-Oden 2021 expedition. For the filtration procedure, see"SO21 SOP: viromics" (Chapter 24).

Ship station	SAS Ice Station	ChlMax	TempMax	Ice-seawater interface	Brackish brine	Melt pond	Snow	Total
3		1	1					2
	6			1	1	1		3
7								0
8	8	1	1	1	1	1		5
13	13	1	1					2
16								0
18	18	1	1					2
22	22			1	1	1		3
26	26			1	1	1		3
30	30	1	1	1	1	1	1	6
33	34	1	1					2
35	35			1	1	1		3
38	38			1	1	1		3
42	42	1	1					2
48	49	1	1					2
50	50			1	1	1		3
53	53			1	2	2		5
56	56	1	1					2
58	58			2	2	2		4
Total		**9**	**9**	**11**	**11**	**11**	**1**	**52**

Table 7.7: Overview of the SO21 omics aerosol samples: (1) air particles on filters from an air pump on the roof of the "Triple lab", collected by Pauline Snoeijs-Leijonmalm at the nine EFICA Master Stations, flash-frozen in liquid nitrogen and stored at -80°C, (2) fog samples taken at 12 stations by John Prytherch and Sonja Murto stored at -80°C, and (3) water samples taken at four stations by Frank Menger (melt pond), John Prytherch / Sonja Murto (leads), and Maria Samuelsson (surface water CTD) during the SAS-Oden 2021 expedition, stored at -20°C. The SO21 omics aerosol samples fall within the category "opportunistic sampling" for Caroline Leck (SU, Sweden), and the sampling procedures are described in Chapter 23.1. The numbers within brackets are blank samples (empty filters or empty bottles).

Ship station	Sampling date	Air-pump filters	Fog samples	Surface water melt pond	Surface water lead	Surface water CTD	Total
7	2021-08-05		2				2
8	2021-08-07/08	1 (5)	2	15 (1)			18
13	2021-08-10		2				2
14	2021-08-11		2				2
16	2021-08-12		2 (1)				2
22	2021-08-15/16	1 (5)					1
26	2021-08-19/20	1 (5)	2		15 (1)		18
28	2021-08-21		2				2
30	2021-08-22/23	1 (5)					1
35	2021-08-26/27	1 (5)	2		15 (1)		18
38	2021-08-28/29	1 (5)	2 (1)				3
42	2021-08-30/31	1 (5)					1
46	2021-09-02		2 (1)				2
48	2021-09-03		2				2
50	2021-09-05/06	1 (5)					1
53	2021-09-06		2 (1)				2
58	2021-09-10/11	1 (5)				15 (1)	16
Total		**9**	**24**	**15**	**30**	**15**	**93**
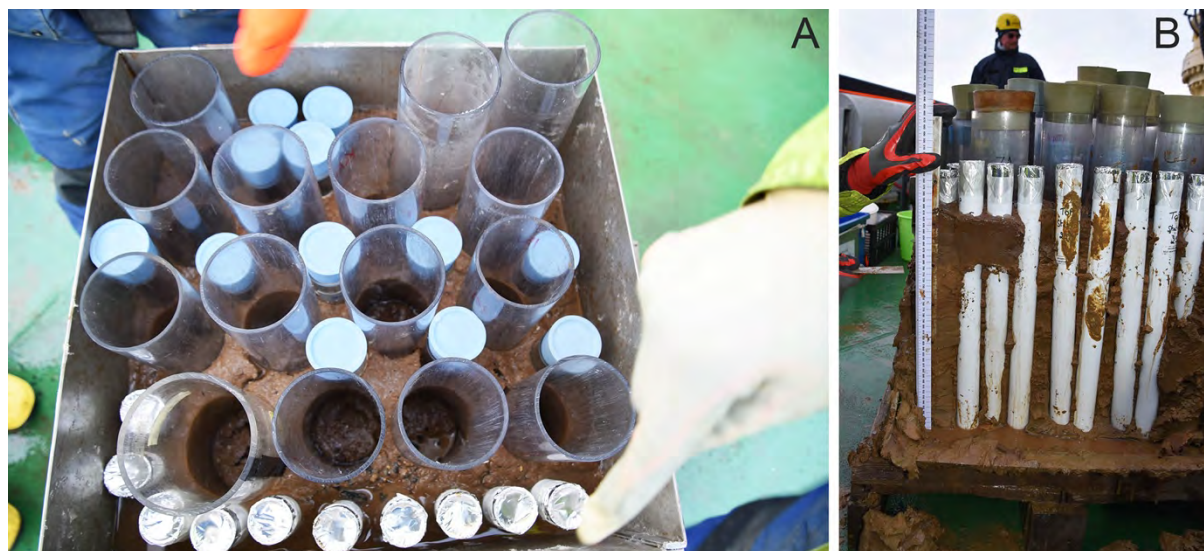
Figure 7.3: Subsampling from a box core sample for SO21 omics sediment cores during the SAS-Oden 2021 expedition. (A) Nine SO21 omics cores were taken from each of six box core samples from six stations (Figure 1.1). (B) The SO21 omics samples covered both the darker and lighter-coloured sediment layers (presumably the Holocene and interglacial layers). ©Pauline Snoeijs-Leijonmalm

8 WP1 (EFICA)

Pauline Snoeijs-Leijonmalm, Julek Chawarski, Serdar Sakinan, Baldvin Thorvaldsson, Frank Menger, Hauke Flores, Nicole Hildebrandt, Julia Muchowski, Prune Leroy, Claudia Morys, Javier Vargas Calle, Clara Pérez Martínez

Project title: Ecosystem mapping in the Central Arctic Ocean during the SAS-Oden expedition (EFICA)

8.1 Resources

External project funding: European Commission (CINEA/DG MARE): Framework Contract EASME/EMFF/2018/003, Specific Contract 06 (SC06) EASME/EMFF/2019/1.3.2.1/03/SI2.840508: "Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition" and Specific Contract 07 (SC07) CINEA/EMFF/2020/3.2.1/01/SC07/SI2.860330: "Sample elaborations, data analyses, results and conclusions from the sea-going MOSAiC and SAS expeditions in the Central Arctic Ocean" to the EFICA Consortium coordinated by Pauline Snoeijs-Leijonmalm (SU), and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for seven berths on the SAS-Oden 2021 expedition to Pauline Snoeijs-Leijonmalm. All project participants are listed in *Table 8.1*.

Table 8.1: Overview of all onboard and onshore participants of the WP1 (EFICA) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (berth)	Pauline Snoeijs-Leijonmalm	pauline.snoeijs-leijonmalm@su.se	PI, WP Leader, FishCam	SU, Sweden
Yes (berth)	Julek Chawarski	julian.chawarski@gmail.com	UVP, WBAT, light sensor, otoliths	SU, Sweden
Yes (berth)	Serdar Sakinan	serdar.sakinan@wur.nl	EK80, WBAT	WMR, Netherlands
Yes (berth)	Baldvin Thorvaldsson	baldvin.thorvaldsson@slu.se	Beam net, MIK net, Longlines, traps	SLU, Sweden
Yes (berth)	Frank Menger	frank.menger@slu.se	Longlines, traps	SLU, Sweden
Yes (berth)	Hauke Flores	hauke.flores@awi.de	Ice fauna, zooplankton	AWI, Germany
Yes (berth)	Nicole Hildebrandt	nicole.hildebrandt@awi.de	Multinet, bongo net, LOKI, zooplankton	AWI, Germany
Yes (WP2)	Prune Leroy *	prune.leroy@slu.se	eDNA sampling	SU, Sweden
Yes (WP2)	Claudia Morys	claudia.morys@gmail.com	eDNA sampling, box corer	SU, Sweden
Yes (WP2)	Javier Vargas Calle	javier.vargas@slu.se	eDNA sampling	SLU, Sweden
Yes (WP7)	Clara Pérez Martínez *	clara.perezmartinez@lnu.se	eDNA sampling	SU, Sweden
Yes (WP14)	Julia Muchowski **	julia.muchowski@geo.su.se	EK80, WBAT	SU, Sweden
No	Christian Stranne	christian.stranne@geo.su.se	Physical oceanography	SU, Sweden
No	Hans Nilsson	hans.nilsson@slu.se	Fishery biology	SLU, Sweden
No	Jonas Hentati-Sundberg	jonas.sundberg@slu.se	Fishery biology	SLU, Sweden
No	Joakim Hjelm	joakim.hjelm@slu.se	Fishery biology	SLU, Sweden
No	Filip Volckaert	filip.volckaert@kuleuven.be	Fish population genetics	KUL, Belgium
No	Stefan Bertilsson	stefan.bertilsson@slu.se	eDNA, bioinformatics	SLU, Sweden
No	Moritz Buck	moritz.buck@slu.se	eDNA, bioinformatics	SLU, Sweden
No	Marine Vandewalle	marine.vandewalle@slu.se	DNA extractions	SLU, Sweden
No	Kim Vane	kim.vane@awi.de	Otolith analyses	AWI, Germany
No	Barbara Niehoff	barbara.niehoff@awi.se	UVP, LOKI analyses	AWI, Germany

* Employed at SU for the expedition, the regular affiliation of Prune Leroy is SLU (Sweden), and that of Clara Pérez Martínez is LNU (Sweden), ** Employment during the expedition funded by EFICA

Figure 8.1: The EFICA Team of the SAS-Oden 2021 expedition gathered at the North Pole. Back row from left to right: Nicole Hildebrandt (AWI), Clara Pérez Martínez (SU), Hauke Flores (AWI), Pauline Snoeijs-Leijonmalm (SU), Frank Menger (SLU), Serdar Sakinan (WMR), Baldvin Thorvaldsson (SLU), Julek Chawarski (SU), Front row from left to right: Prune Leroy (SU), Javier Vargas Calle (SLU), Julia Muchowski (SU), Claudia Morys (SU). ©Pauline Snoeijs-Leijonmalm

8.2 Scientific motivation and specific research questions

There is almost no knowledge about the presence and distribution of the pelagic fish stocks in the rapidly changing CAO ecosystem. Critical gap analyses performed by the EFICA Consortium within a previous Specific Contract (SC02)[30] highlighted that the knowledge gaps for the CAO ecosystem are enormous and obstruct any quantitative analyses of its fish stocks. This is the second expedition to the CAO of the EFICA Consortium – the first one was the international MOSAiC expedition with the German icebreaker *RV Polarstern* in 2019-2020[31]. The measurements and samples collected by WP1 (EFICA) will be elaborated together with the samples collected by EFICA during the MOSAiC expedition 2019-2020.

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[30] Snoeijs-Leijonmalm P, et al. (2020) Review of the research knowledge and gaps on fish populations, fisheries and linked ecosystems in the Central Arctic Ocean (CAO). Publications Office of the European Union [https://data.europa.eu/doi/10.2826/387890]

[31] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the MOSAiC expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2926/714618]

Using acoustic data from the 2016 *IB Oden* expedition, we previously discovered a mesopelagic deep scattering layer (DSL) – suggesting the presence of zooplankton and small fish at 300-600 m of depth[32]. This central Arctic DSL is located in the "warm" (0.5-1.5°C) Atlantic water layer of the ice-covered CAO. The small Arctic endemic gadoid *Boreogadus saida* (polar cod) could be a candidate for occurrence in the central Arctic DSL given its association with the CAO pack ice as juveniles and the high abundances and its critical role in the pelagic food webs of the Arctic shelf seas. Later on, the DSL was again observed in an eight-months study along a 3170 km long transect of the MOSAiC drift expedition that crossed the Amundsen and Nansen Basins in 2019-2020[33]. In the latter study it was shown that the DSL contained low abundances of *Gadus morhua* (Atlantic cod) and *Arctogadus glacialis* (ice cod), along with lanternfish and *Gonatus fabricii* (armhook squid). However, no *Boreogadus saida* was caught from the DSL, and its occurrence in the DSL remained unproven.

During the SAS-Oden 2021 expedition, the EFICA project aims to sample nekton (actively swimming organisms, i.e., fish and squid) and environmental DNA (eDNA) from the mesopelagic zone, and to use the newest multi-frequency broad-band acoustics technology (WBAT) and optics (UVP, LOKI, FishCam) to observe zooplankton and fish. This acoustic and optic equipment will enable us to quantify mesopelagic fish and zooplankton. Chemical and isotopic otolith analyses could enable the reconstruction of migration pathways, age and food sources. Bioinformatic analyses of eDNA are expected to enable estimates of the occurrence and abundance of fish, squid, fish prey (zooplankton), and fish predator (bird, mammal) species.

The specific research questions of the WP1 (EFICA) project are:

(1) Which species of nekton (fish, squid), mammal predators and zooplankton prey occur in the High Seas and the Atlantic inflow? (eDNA, FishCam, fish samples)
(2) What is the distribution and biomass of nekton and zooplankton prey in the High Seas and the Atlantic inflow? (hydroacoustics, eDNA, FishCam)
(3) What is the ecology of the pelagic fish in the High Seas and the Atlantic inflow? - food web, migration, etc. (fish samples, otoliths)
(4) What is the carrying capacity of the High Seas with respect to primary production, zooplankton and nekton?

8.3 Summary of field work performed

EFICA Master Stations: Nine EFICA Master Stations were performed during the SAS-Oden 2021 expedition (ship stations 8, 22, 26, 30, 35, 38, 42, 50, 58) of on average 33 hours during which the ship was lying still with engines turned off for collecting good hydroacoustic data, project-specific EFICA Device Operations (Beam net, CTD wbat, etc.), 24-hour long-line and trap deployments by helicopter, and other fishing efforts from the ice and from the ship. During the EFICA Master Stations all SO21 projects had the opportunity to access the ice for sampling (*Figure 8.2*).

Note: All Device Operations from the ship are described in *Chapter 6*. The eDNA sampling is described under the SO21 omics collaboration (*Chapter 7.4*). Part of the field work performed by WP1 (Serdar Sakinan and Julek Chawarski as members as the EK80 team) is reported in *Chapter 20*.

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[32] Snoeijs-Leijonmalm P, et al. (2021) A deep scattering layer under the North Pole pack ice. Progress in Oceanography 194:102560 [https://doi.org/10.1016/j.pocean.2021.102560]

[33] Snoeijs-Leijonmalm P, et al. (2022) Unexpected fish and squid in the centra Arctic deep scattering layer. Science Advances 8:eabj7536 [https://www.science.org/doi/10.1126/sciadv.abj7536]

SAS Core Parameters: WP1 (EFICA) contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*). WP1 was also responsible for running the ship's EK80 echosounder together with WP14 (MWA).

Figure 8.2: During the EFICA Master Stations IB Oden was lying still and all SO21 projects had the opportunity to access the ice for sampling. (A) The multinet being winched down and WP16 sampling from the lead that was cut through the ice by the ship, (B) Beam net deployment: the beam net connected to the towing wire. (A) ©SPRS, (B) ©Kimberley Bird

EK80 equipment

The EK80 echosounder was operated from the bridge (*Figure 8.3*). The ship-mounted echosounder system on *IB Oden* originally consists of a Simrad EK60 that runs a split-beam Simrad ES18-11 transducer designed to be operated for narrow-band applications. During the SAS-Oden 2021 expedition, this transducer was coupled to an EK80 transceiver and used in wideband mode to generate frequency modulated signals. The full bandwidth that can be achieved with this transducer extends from 15 to 28 kHz. However, previous experiences with this special configuration have shown that the signal quality deteriorates towards the higher end of the full bandwidth.

The EK80 / ES18-11 transducer were calibrated on 1 August by WP1 and WP14 with the help of many other people on board (see *Chapter 20*). EK80 data were collected during the whole expedition: from leaving the Norwegian EEZ (29 July at ca. 18:30) until arrival in Helsingborg after the expedition (19 September). For biological observation of individual targets at low density and high range (250-600 m), higher echosounder sensitivity is desirable. However, this comes with a trade-off regarding noise sensitivity. To achieve the best signal-to-noise ratio for measurement of living targets, the bandwidth was reduced to 16-19 kHz, thereby preventing the dilution of transmitted energy that would accompany higher bandwidth data requiring noise filtering during post-processing of the data.

Due to different requirements in measuring biological backscatter for WP1 (EFICA) and physical phenomena for WP14 (MWA, geophysical mapping), the SAS-Oden 2021 expedition used two alternating EK80 settings. The multibeam and the sub-bottom profiler were turned off when the ship was drifting with the ice at sampling stations and the EK80 was in standalone mode with the WP1 settings. This allowed for an increased ping rate, thus improving the temporal resolution of the dataset. During selected operations aiming at quantifying fish, the recording range of the EK80 was reduced to 1200 m to further increase the ping rate of the system. The WP14 settings were typically in place during transit, and then the EK80 was synchronized with the multibeam and the sub-bottom profiler. During selected operations aiming at quantifying fish, the recording range of the EK80 was reduced to 1200 m to further increase the ping rate of the system.

Figure 8.3: Serdar Sakinan (WP1) working with the EK80 on the bridge. ©Pauline Snoeijs-Leijonmalm

A series of tests was performed during the transit before arriving at the calibration station close to the marginal ice zone. The tests consisted of systematically changing the settings and assessing the noise levels. The test results showed that the cleanest data for observation of biological targets would be achieved by using a relatively narrow bandwidth and the following pulse parameters: FrequencyStart = 16 kHz; FrequencyEnd = 19 kHz ; PulseDuration = 4.096 milliseconds; SampleInterval = 0.256 milliseconds; TransmitPower = 1600 Watt.

Since the density of biological targets along the SAS-Oden 2021 expedition route was very low, we focussed on detecting the targets individually, which would eventually allow a detailed understanding of behaviour and size distribution. This requires receival of multiple hits from the same target which then can allow for isolation and tracking of the target. Collecting observations of this quality required a high frequency with which an ultrasonic pulse is sent into the water column by an echo sounder through the transducer (a high ping rate). Multiple factors hindered this objective, such as synchronization of the EK80 pings with the multibeam and the sub-bottom profiler in a sequential mode. Since the synchronization substantially reduced the pinging rate for the EK80, it was decided to switch the multibeam and sub-bottom profiler off during all sampling stations.

EK80 ping rate

A second issue with respect to ping rate was the depth of the central Arctic basins where seafloor depth extended below 3000-4000 m. A ping adjustment that enabled coverage of this full range required intervals between 6 to 10 seconds. This substantially reduced the pinging rate. Therefore, it was decided to reduce the observation range to 1200 m of depth and to adjust the ping rate accordingly while keeping the aliasing signals from the bottom returns (false bottom) out of the observed range. This adjustment generally allowed for a ping rate of 3 seconds. However, during deep CTD deployments bottom detection was needed and required either expansion of the observation range or switching on the multibeam. Furthermore, during the box core stations a detailed characterization of the seabed was needed that required the multibeam and the EK80 to run in parallel. However, these activities were limited in time and caused only minor loss of temporal resolution or interference issues.

EK80 disturbances

During the SAS-Oden 2021 expedition it was difficult to obtain high-quality acoustic EK80 recordings due to disturbances by noise (*Figure 8.4*). This observation was similar to experiences with the same EK80 equipment on previous expeditions (e.g., the Ryder expedition with *IB Oden* in 2019) when the shipborne noise substantially impacted the quality of the data.

From a general overview of the data from the entire SAS-Oden 2021 expedition, it was estimated that the usable portion of the dataset is ca. 42%. Icebreaking was the major disturbance and generated almost no usable data. When the engines were turned off and the ship was drifting with the ice there were other sources of noise. A regular noise that significantly disturbed data collection was that from a steam hammer in the ship's fuel heating system. This noise was in the form of irregular stripes becoming stronger with depth that were especially amplified by the time-varied gain. There was also a reoccurring temporary elevated broadband background noise that was potentially caused by power usage. Although continuous communication was sustained with the ship's crew, and generally ad-hoc solutions were found for temporary improvements, noise remained to be a perturbation of the data quality.

In addition to acoustic noise there was a failure in the EK80 software that caused data loss a few times. Our understanding of the origin of this problem was that a bug in the software caused recording failure in a random fashion when user settings had been switched. Normally, there were two main settings

that were being alternated (EFICA and Geophysical Mapping). In order to prevent operator-related errors due to alternation of settings (e.g., mistyping parameters, forgotten changes), pre-defined user setting files were generated and used to for importing exact settings. This is an EK80 functionality used commonly for this purpose. However, in a few instances this caused the recording to stop without notice. This was a deceptive problem as the recording alert falsely indicates that recording was ongoing. The data loss caused by this error mostly coincided with transit periods. Furthermore, thanks to generated screen shots of these periods, a detailed representation for the lost section is stored as jpeg images. These cases occurred partially on 14 August, 16-19 August and 28 August. Post-inspection of the images indicated that these data losses would not result in noticeable gaps for the biological observations from the EFICA perspective.

Higher acoustic target density was expected between SO21 stations 50 and 59 when the SAS-Oden 2021 expedition crossed the Atlantic water inflow region to the CAO. This expectation was based on the observation of high fish density near the Yermak Plateau during the MOSAiC expedition[34]. A special setting was used to maximize the ping rate during this part of the expedition route by decreasing the recording range to 800 m (instead of 1200 m). This track partially coincided with open water conditions, which enabled collection of usable data. The ship was instructed to stop for 10 minutes every six hours between SO21 stations 50 and 53, every three hours between SO21 stations 53 and 58, and every hour along the slope of the Yermak Plateau (*Figure 8.5*). During these EFICA stops it was possible to collect relatively clean data to assess the density of the DSL.
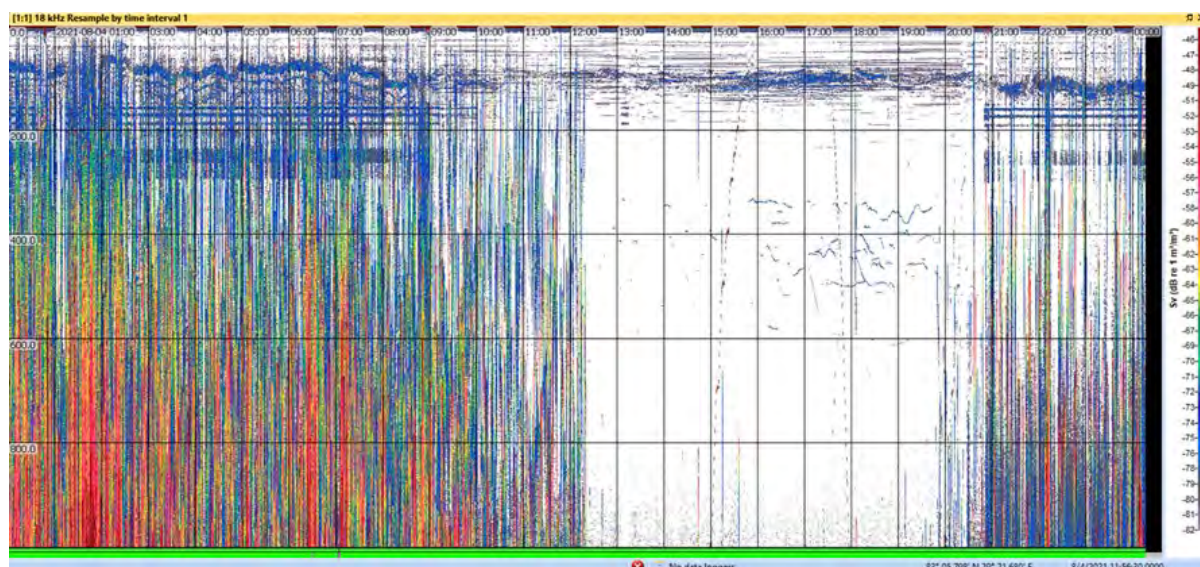
Figure 8.4: Echogram showing that it was possible to obtain high-quality recordings during the SAS-Oden 2021 expedition. Gaps in the data occur due to different sources of noise as shown in this 24-hour echogram from 4 August 2021 when ca. 35% of the data is of usable quality. ©European Commission[35]

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[34] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the MOSAiC expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2926/714618]

[35] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

EK80 living targets actively avoided sampling devices

Throughout the SAS-Oden 2021 expedition we regularly observed that targets resembling fish actively avoided the sampling devices that were lowered into the water column with winches (*Figure 8.6*). These observations suggest that the targets were actively swimming organisms that were able to change direction very fast, i.e., fish and not zooplankton. Squid and zooplankton other than some species of siphonophores are not recorded at 18 kHz[36].
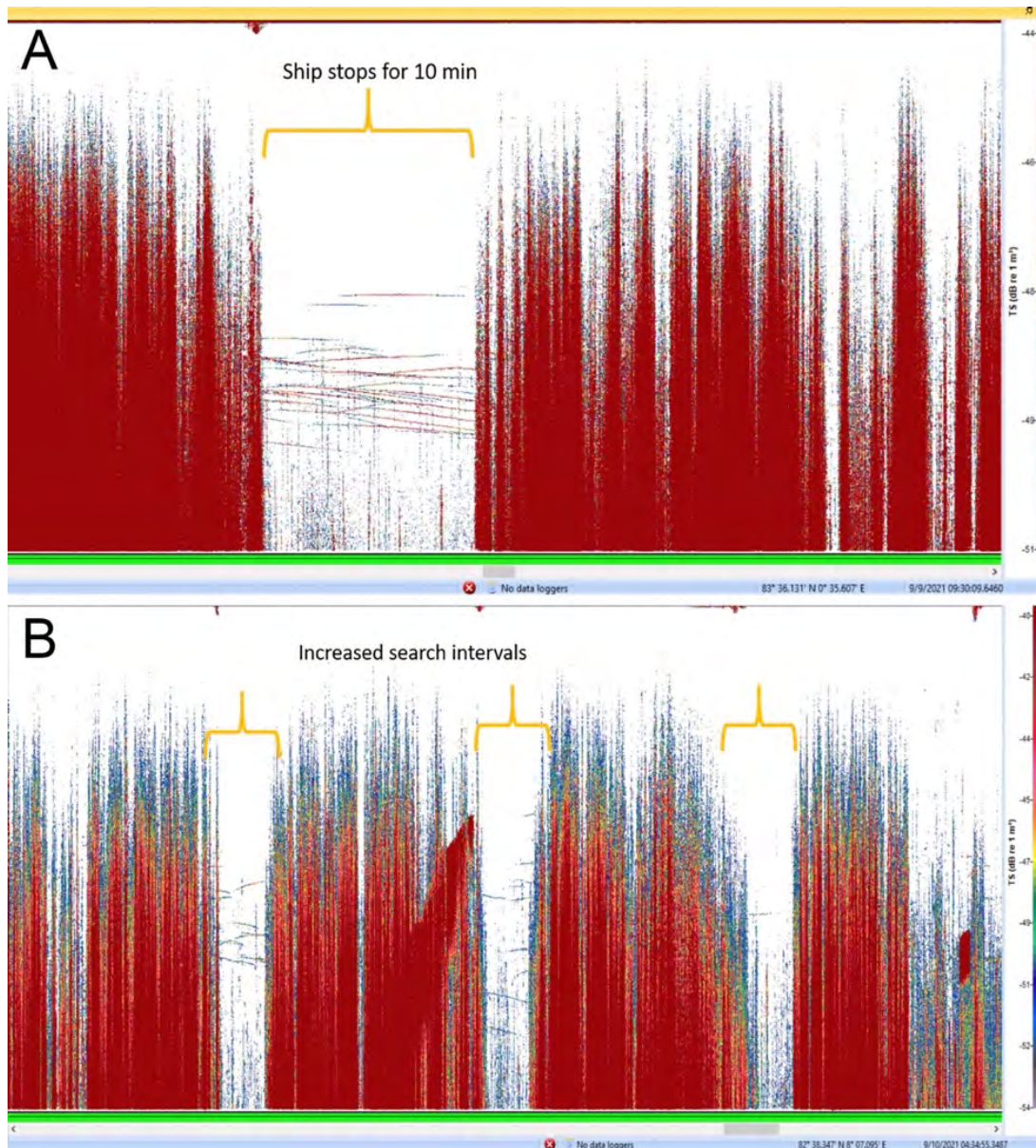
Figure 8.5: Echograms of the ship's EFICA stops between Stations 50 and 59 (A) A 10-minute stop showing single targets receiving multiple hits as (nearly) horizontal lines. (B) Three 10-minute stops along the slope of the Yermak plateau on 10 September 2021 between 3 am and 6 am UTC (lower panel). The red colour shows disturbances from icebreaking. ©European Commission[37]

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[36] Snoeijs-Leijonmalm P, et al. (2022) Unexpected fish and squid in the centra Arctic deep scattering layer. Science Advances 8:eabj7536

[37] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]
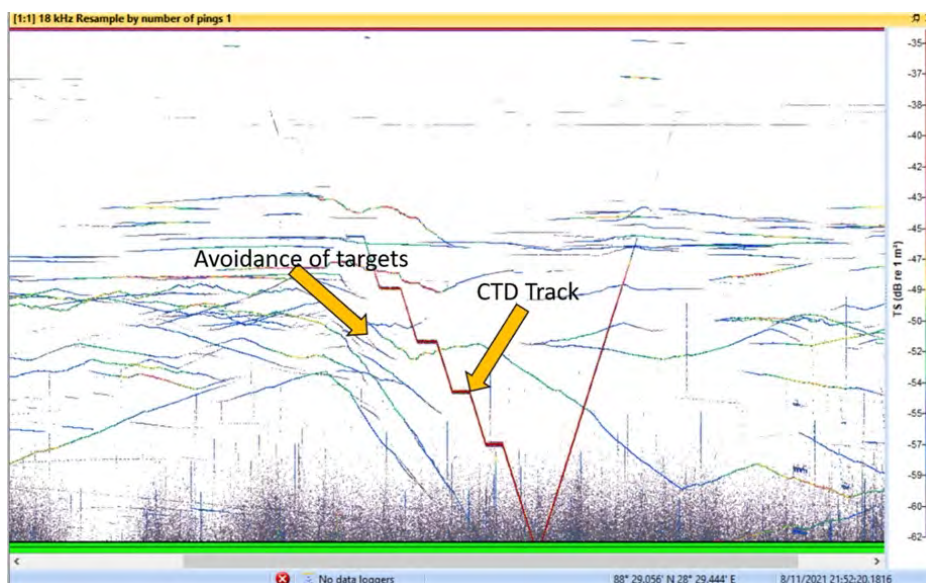
Figure 8.6: Living targets (fish) clearly avoiding the CTD rosette on 11 August 2021 during the SAS-Oden 2021 expedition. ©European Commission[38]

WBAT equipment

Two of the EFICA Consortium partner institutes (SU and SLU) each provided a Simrad™ Wideband Autonomous Transceiver (WBAT) system for the EFICA acoustic measurements during the SAS-Oden 2021 expedition. Each WBAT was equipped with two split-beam transducers (SU 38/333 kHz; SLU 38/200 kHz). The SU WBAT was mounted on the CTD rosette operated from the stern for water-column profiling between 0 and 1000 m of depth and is referred to as the "CTD WBAT". The SLU WBAT was designated to deployments from the ice to collect drifting time series of ca. 24 hours during the nine EFICA Master Stations and is referred to as the "ICE WBAT".

CTD WBAT

The CTD WBAT was equipped with two split-beam transducers with the center frequencies of 38 kHz (ES38-18DK) and 333 kHz (ES333-7CD) (*Figure 8.7*). Two types of CTD cast were used (*Figure 8.8*) down to a maximum depth of 1000 m, typically alternating between each frequency.
First, the downward-facing 38 kHz transducer was configured with an observation range of 200 m. During CTD casts using this transducer, the CTD rosette was stopped at discrete depths to collect approximately 3 minutes of data (180 pings). The 333 kHz transducer was mounted facing sideward and was configured with an observation range of 50 m. These profile types were collected during continuous lowering of the CTD rosette. In both cases, the primary focus of data collection was on the downcast of the CTD rosette. After each sampling station, the WBAT was rinsed with freshwater, carefully dried and all data was copied from the internal memory.

The two frequencies (38 and 333 kHz) were used alternating between the casts with the aim to obtain at least one full profile per frequency per station. Starting with SO21 station 22 (15 August 2021), a video camera with a red light source was coupled with one specific cast at each station (corresponding to 38 kHz). In addition, when sufficient time was available, additional CTD casts were performed opportunistically. The CTD casts with different configurations were identified as follows:

CTD bio SAS: 38 kHz stops every 100 m for 3 min periods during down cast, a video camera attached for continuous recording.

CTD omics SAS: 333 kHz without any stop during down cast and no video camera

CTD wbat EFICA: 38 kHz with stops similar to CTD bio SAS but without video camera
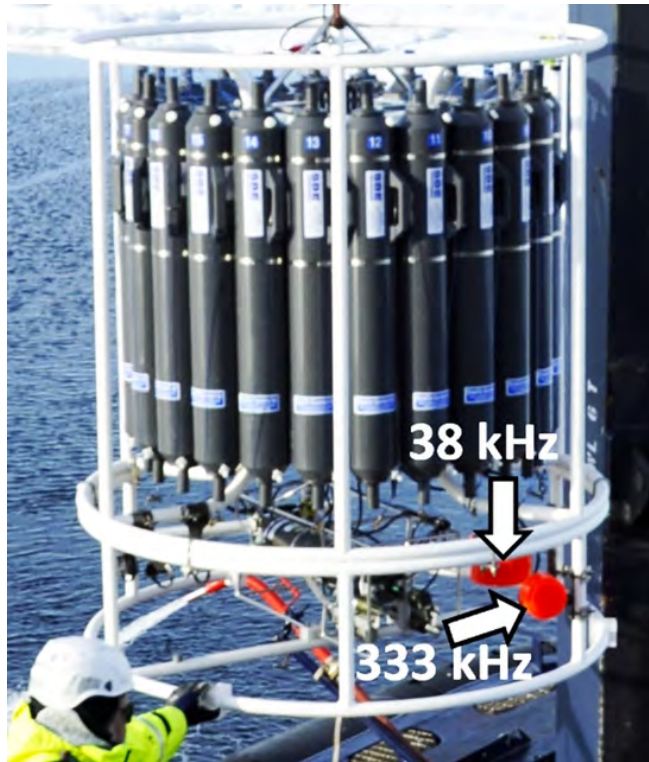
Figure 8.7: Photograph of the CTD WBAT on the stern CTD (maximum depth 1000 m) below the Niskin bottles. The arrows show the 38 KHz and 333 kHz transducers. ©SPRS
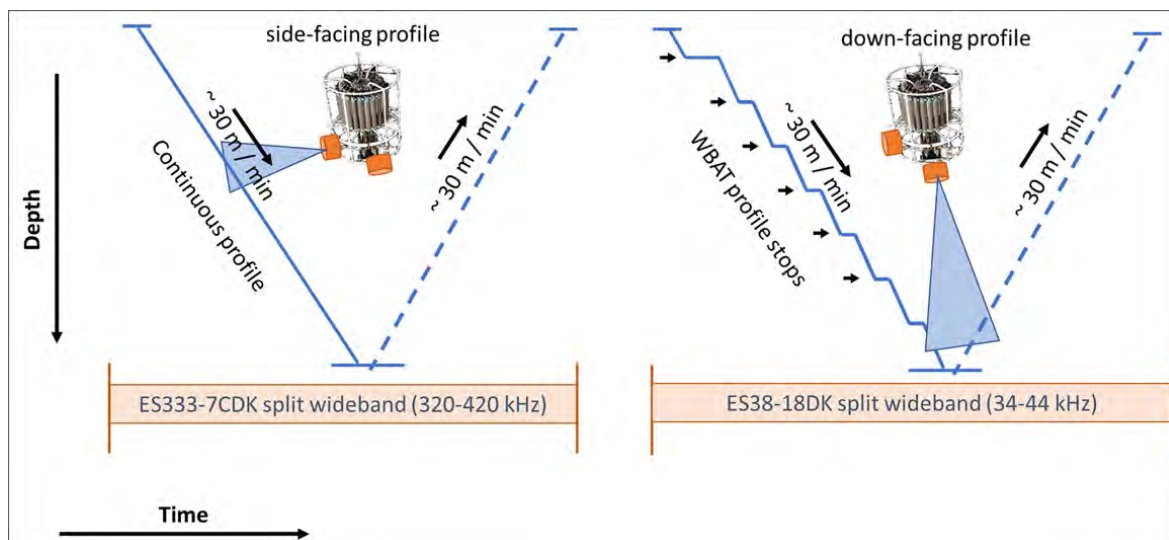
Figure 8.8: The two CTD WBAT deployment configurations (38 and 333 kHz). Data collection was primarily focused on the downcast, as upcasts (heaving) resulted in some bubble artefacts and occasional stops to fire Niskin bottles for water collection. ©European Commission[39]

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[39] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

CTD WBAT calibration

The calibration of the 38 kHz and 333 kHz transducers of the CTD WBAT was performed at SO21 station 58 on 10 September 2021 (9°14.351′E, 82°27.752′N) over ca. 1000 m water depth. Prior to departure from the ship, the WBAT was mounted to a "Simrad stand" (*Figure 8.9*) designed as a stand-alone probe equipped with one WBAT tube and two split-beam transducers. The slim profile of this design was ideal for lowering the echosounder into a narrow 4-bore ice hole. The 38 kHz was calibrated in FM mode with the same acquisition parameters used for the regular deployments using a 38.1 mm tungsten carbide sphere that was hanging ca. 12 m below the transducer. Similarly, the calibration of the 333 kHz was performed with a 22 mm tungsten carbide sphere. In both cases a satisfactory coverage of the center axis and 3 dB beam coverage was achieved. The entire operation took 1 hours and 5 minutes between leaving and returning to the ship.

Figure 8.9: Calibration of the CTD WBAT used during the SAS-Oden 2021 expedition. (A) Preparing the WBAT for calibration on the ice at SO21 station 58. (B) Manoeuvring the calibration spheres. ©Serdar Sakinan

CTD WBAT standard settings

Data acquisition of the WBAT was carried out using pre-programmed commands ("mission plans") that were uploaded to the instrument prior to deployment. A Simrad pressure switch was used to automatically initiate these mission plans soon after the WBAT had been submerged (at ca. 4 m depth), which also stops the data acquisition as soon as the WBAT is close to the surface (back to ca. 4 m). After a few tests at the first two stations, a standard mission plan for each frequency (*Table 8.2*) was used consistently during the rest of the expedition.

Table 8.2: Mission plans for the two transducers of the CTD WBAT during the SAS-Oden 2021 expedition.

Transducer	Ping interval (seconds)	Range (m)	Power	Start frequency (kHz)	End frequency (kHz)	Pulse length (milliseconds)
333 kHz	0.6	50	Max	320	420	2.086
38 kHz	1	200	Max	35	44	2.086

CTD WBAT considerations

A few details are worthwhile mentioning regarding data quality issues and challenges. A challenge was interference by the echoes due to reflections from the sea surface/ice in the 38 kHz data. To our understanding, this issue should be related to the lack of proper dampening of the top part of the 38 kHz transducer which caused sound transmission leaks towards the opposite direction of the main beam. This caused perturbed sections in the data at a range equal to the distance between the transducer and the surface (*Figure 8.10*). The effect of this perturbation was minor in the CTD WBAT and disappeared as soon as the WBAT reached 200 m of depth. However, for similar applications in the future, it is recommended to better insulate the top part of the transducer to prevent such sound leakage and undesirable echoes.

False bottom reflections were noted at a few stations where the seafloor depth was shallower than 2000 m and this affected a small portion of the data. This is an unavoidable problem as there is no possibility to interactively alter the ping rate to prevent false bottom echoes.

Another issue was related to unpredictable movements of the CTD rosette. Since the transducer was attached to the frame of the CTD, it was affected by its motions such as tilt and rotation. This complicates interpretation of individual targets. During the last five casts a magnetometer (IMP) that measures the tilt, acceleration and azimuth was attached to the CTD rosette to understand the CTD motion and to correct single target position data.

Mechanical noise from the ship had some minor effects on the CTD WBAT data when the CTD rosette was close to the surface. This effect was negligible below 200 m of depth. During the deployments, the EK80 was kept pinging with the aim to collect data simultaneously with both instruments. This caused some interference spikes in the WBAT recordings. Some preliminary postprocessing trials shown that these spikes can be cleaned in a relatively straightforward way and the effect on the data quality is very minor.

Some technical issues were experienced in the beginning of the expedition during the four casts that failed to collect WBAT data. The first problem was the consumption of the rechargeable battery much faster than expected. This high battery consumption was probably related to the low water temperature of the Arctic Ocean. To remedy this, the rechargeable battery was replaced with a long-life battery, and the problem never occurred again during the expedition.

The second technical problem that appeared in the beginning of the expedition was related to an extension cable that connects the pressure switch to the WBAT. The pressure switch was used to automatically start and stop a mission plan based on the depth (at ca. 4 m below the surface). For an unexplainable reason, the pressure switch sent stop commands to 38 kHz missions at unpredictable times, which caused loss of data acquisition of two CTD casts. This problem was solved by removing the extension cable and connecting the pressure switch directly to the WBAT.
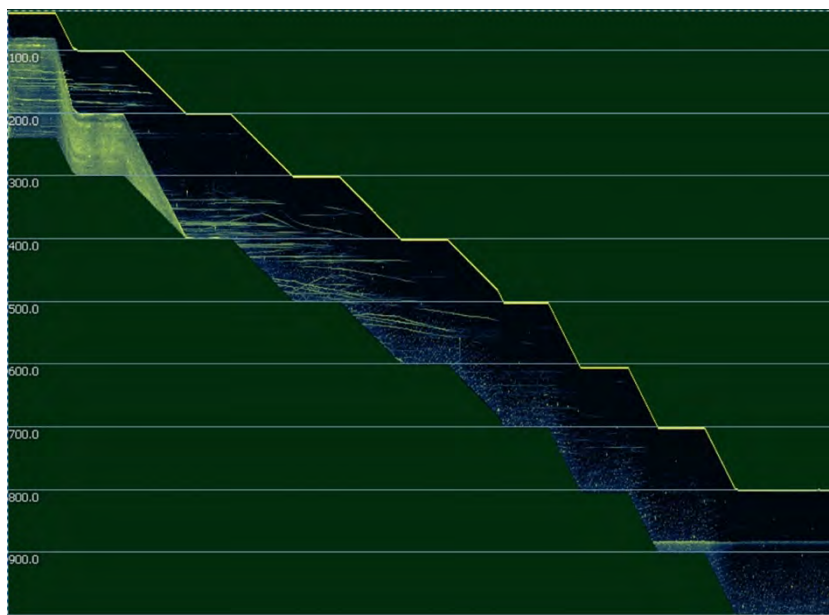
Figure 8.10: A full downcast profile with the CTD WBAT, 38 kHz during the SAS-Oden 2021 expedition. The perturbations due to surface reflection are visible until the WBAT reaches down to 200 m. The zig-zag pattern in the data is due to 3-minute stops every 100 m for pinging. ©European Commission[40]

ICE WBAT

Two ice deployments with the ICE WBAT were performed, a 4-hour test at SO21 Station 4 on 3 August, and a ca. 24-hour deployment as SO21 Station 8 on 7-8 August, followed by an on-ice calibration on 8 August. Unfortunately, the ICE WBAT was severely damaged due to a power surge at the end of the calibration operation and could not be used anymore during the expedition. The failure occurred when the WBAT was recovered from the ice followed by disconnection of transducer cables for helicopter transport. Once on the ship, we had some trouble communicating with the WBAT, but after several trials we once again established a connection and tried a test mission plan. However, this mission plan generated no .raw files. Upon visual inspection of the circuit board, significant electrical damage was observed. For the remainder of the expedition, the SU WBAT became the WBAT for WP1 (EFICA) to achieve its goals.

The last available file was an empty file generated approximately 20 minutes after ice recovery, presumably while the WBAT was still operating a mission. Unable to receive a mission plan to initialize, we decided to have a look at the internals to possibly identify the problem. On the transceiver portion, we found brown material on the motherboard with 2-3 bulged circuits and one that was visibly broken on one end of the solder. This observation was accompanied by a strong smell of a burned circuity. We very gently touched the black piece of the circuit and it crumbled as if burned. After that, we were unable to communicate with the WBAT and transceiver. According to the feedback we received from the technical service of the Simrad, the problem was related to the power transistors with its surrounding components in the transmitter of one of the four channels. We were informed that this was one of the most critical parts of the design and there was no way of repairing this in the field. Therefore, we were advised against further use of this instrument until the damaged board has been replaced.

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[40] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

FishCam equipment

During the MOSAiC expedition, the EFICA Consortium used a custom-built deep-sea camera system (MacArtney Underwater Technology, Germany). This "FishCam" was installed under the ice for many months and connected by electricity and fibre-optical cables to the ship and took several days to deploy and several days to retrieve[41].

It was not possible to use the MOSAiC FishCam for the SAS-Oden 2021 expedition because the ship moved between sampling stations as fast as possible. Therefore, EFICA used three smaller stand-alone camera systems "Mini FishCams" consisting of a custom-programmable small camera (go-flo, GitUp 2 action camera) and a custom-programmable LED light (Nautilux, white light, 1500 lumens), each with one 12 V battery. All equipment was mounted in water-tight housing that could be deployed down to 1750 m of depth. For programming and memory card and battery maintenance the system needed to be opened.

One of the Mini FishCam equipments was sideward-facing and used for shallow deployments (0-5 m) under the ice (*Figure 8.11*). Another one was downward-facing and used for deep deployments (300-400 m) under the ice. The latter system had two battery compartments for the camera and two for the LED light so that 24-hour deployments would be possible. The third Mini FishCam mostly had red light and was mounted on the CTD that stopped every 100 m for 3 minutes when the CTD went down. During continuous CTD deployment (1 m s^{-1}), no living organisms could be distinguished on the video recordings because of the high speed of the winch in relation to the resolution of the camera.

After each deployment, the Mini FishCam was programmed and prepared with newly loaded batteries, a formatted memory card, new tightening rings and silicone grease in a warm room (ca. 20°C), but needed to be opened in the CTD container and on the ice to connect the batteries (*Figure 8.11 C*). The conditions on the ice were ca. -5 to °C.

On 3, 5 and 6 September a ruler was attached to the camera system, which showed that organisms could be seen in red light within ca. 0.8 m from the camera lens, but not beyond this (*Figure 8.12*).

FishCam considerations

Unfortunately, the Mini FishCam that was deployed from the ice in the DSL was destroyed during the first deployment by leakage that was caused by delivery of a too short screw by the manufacturer, which caused loss of data from the DSL that would have complemented the data from the CTD-mounted camera system. The Mini FishCam that was deployed from the ice ca. 2 m under the ice did not work after 19 August because the connection cable between battery and camera was compromised. Using a Mini FishCam immediately under the ice was not planned for the expedition, and the loss of this camera system did not affect the planned EFICA data collection.

The Mini FishCam on the CTD worked well throughout the expedition, but it was not a suitable instrument for practical field use in the Arctic Ocean because it had to be opened under heavy field conditions to connect the batteries. This was a sensitive moment and yielded some failures. There was always the risk of water leakage into the housing. Even if the housing was guaranteed until a depth of 1750 m, it was very difficult (screwing by hand) and time-consuming to prepare the Mini FishCam for being water-tight before deployment. The video recordings from the Mini FishCam were not of the same quality as those from the MOSAiC FishCam, but different groups of taxa could be recognized (*Figure 8.13*)

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[41] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the MOSAiC expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2926/714618]

Figure 8.11: The Mini FishCam used during the SAS-Oden 2021 expedition. (A) Sideward-facing Mini FishCam on its stainless steel frame (built on board by SPRS technician Niklas Vestin) with the two battery tubes under the camera and the LED light above the camera. (B) Downward-facing Mini FishCam: camera and programming unit housing below the battery housing tube. (C) Downward-facing Mini FishCam: connection programming unit to the left and connection battery unit to the right. ©Pauline Snoeijs-Leijonmalm
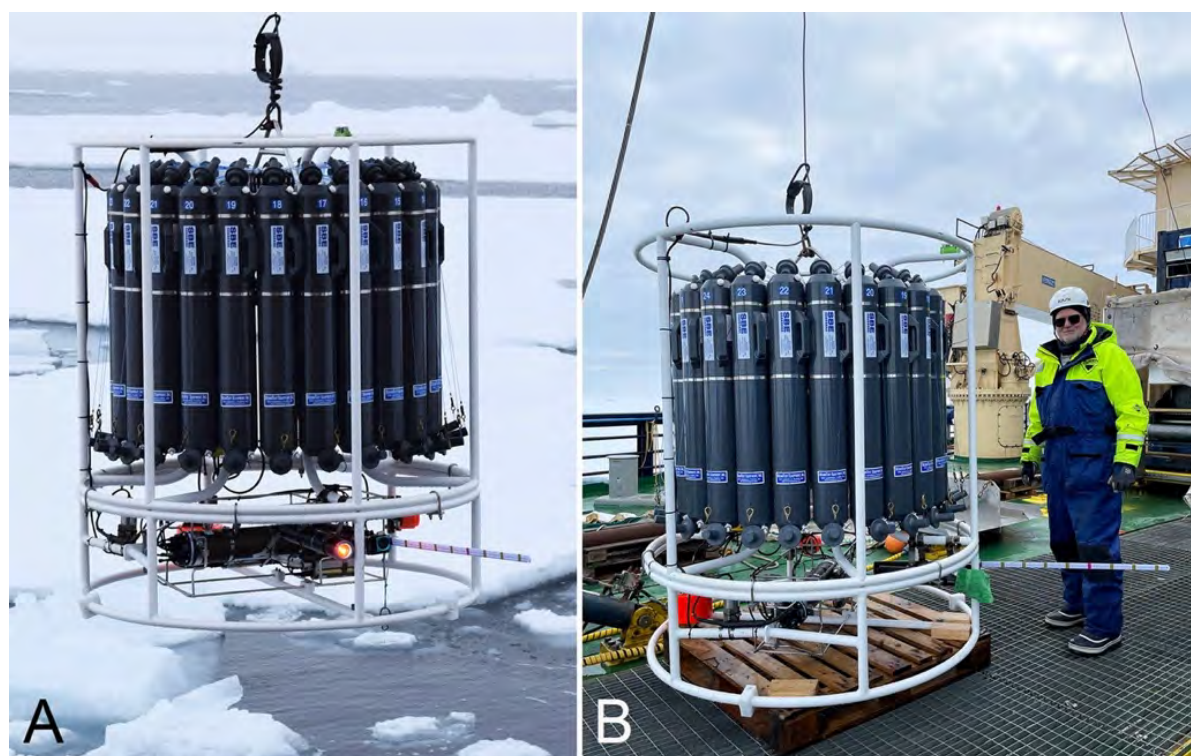
Figure 8.12: CTD FishCam calibration using a ruler of 100 cm length. (A) Mini FishCam with red light on the CTD (together with the CTD WBAT). (B) Calibration of the Mini FishCam sight field on the CTD with a ruler. (A) ©Hans-Jørgen Hansen, (B) ©Pauline Snoeijs-Leijonmalm
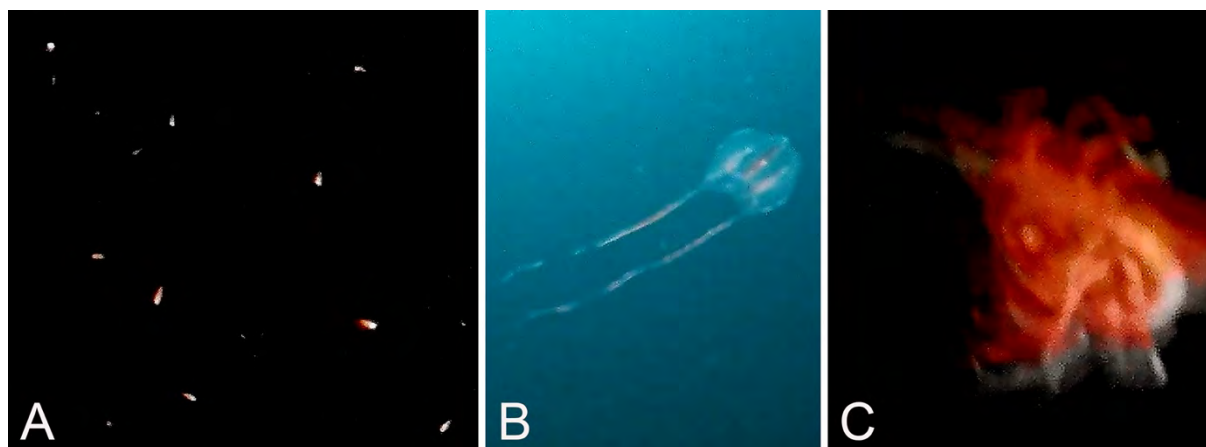
Figure 8.13: Screen captures from video recordings of macrozooplankton made with the Mini FishCam. (A) Amphipods. (B) Mertensia sp. (jellyfish). (C) Jellyfish. ©European Commission[42]

UVP equipment

The Underwater Vision Profiler 6 (HD)[43] brought by EFICA partner SU (Serial number 00126), is an optical instrument used for capturing images of particles ranging from ~50 to 2000 μm. Particles can include phytodetrital aggregates, suspended sediment, and zooplankton. The instrument was delivered as a prototype by Hydroptic. Due to production setbacks, the instrument was not field tested nor ready for deployment until approximately one week into sampling.

The instrument was installed on the rosette frame of the CTD operated from the bow with the fore-deck winch (operated to full depth, i.e., to max. ca. 4350 m during the SAS-Oden 2021 expedition). The UVP was operated autonomously during each of the ship's CTD casts from the bow. To run autonomously, each profile requires a basic "rinsing" sequence, whereby the rosette is lowered to 15 m depth to initiate the instrument and then raised to 2 m depth before conducting a full water column profile. Once the rosette is heaved 30 m, the instrument shuts off to ensure that data is only collected during the down cast. The instrument was maintained alongside other rosette instruments using standard procedures (i.e., rinsing, charging, lubricating connections, etc.).

UVP standard settings

The UVP arrived with an incorrect manual. It did not run unless you exactly follow the UVP6-HF_CTD_user_manual_mini.pdf instructions, which were provided only once we were underway and past our first sampling stations. This means that the UVP is not piloted using the UVPapp, but the OctOS.exe command line software. The UVP was already setup to run in CTD mode and these settings were not changed prior to deployment.

The UVP box did not contain important instructions on how to connect the battery. These instructions arrived only on 7 August 2021: "You need to pull the orange thread in order to remove the titanium cap. Then connect the molex connector…". We pulled the orange thread but we were not able to push it back in all the way after connecting the molex connector below the titanium cap. I considered this to not be a risk to the battery.

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[42] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

[43] Picheral M, et al. (2021) The Underwater Vision Profiler 6: an imaging sensor of particle size spectra and plankton, for autonomous and cabled platforms. Limnology & Oceanography: methods, 15 pp. [http://doi.org/10.1002/lom3.10475]

After getting the UVP powered, there were no problems communicating with the instrument. The static IP address on the laptop was set to the same IP address as seen in the manual: 193.49.112.130. Each time the UVP was plugged into the battery via red cable, it flashed three times indicating that it was in stand-by mode. The CTD operators charged the battery in-between each cast. Due to some long back-to-back casts, we lost some data at the end of casts, when presumably the battery died. The battery life is approximately 10 hours.

Upon installation on the rosette, we discovered that the manufacturer made holes in the frame on the wrong plane of the bracket (x vs y). We fixed this by drilling new holes in the steel arm. It fitted on the rosette without any interference with the ADCP or Niskin bottles.

We followed the instructions to lubricate connections during each connection to the UVP. CTD operators confirmed that they followed these instructions. However, there was some obvious corrosion occurring on the red connector to the UVP. One of the pins was corroding faster than the others. This connector was cleaned as often as possible using Lectra clean solution and the connection was relubricated. Nonetheless, by the end of the expedition, the red connection side of the y-cable appeared heavily corroded. During data processing it was noticed that some of the later casts had power failures with resets in the middle of casts. In order to process these casts, merging of sequences is required to create single samples.

UVP considerations

Do not send the UVP out on expedition until it has been inspected for corrosion-related problems. During the last eight casts of the SAS-Oden 2021 expedition, five had power supply problems on the way down. An inspection is required at the manufacturer of the UVP (Hydroptic), they need to upgrade the firmware and replace the lights. Neither are tested and may not be available until early 2022. Please contact Hydroptic for customs and shipping information. Have Hydroptic send a spare y-cable and a spare charging cable in case one gets damaged. These types of cables are very complicated to assess for damage and likely impossible to repair on the ship. Request the proper documentation from Hydroptic to run the UVP. However, despite that we lost some precious sampling time due to careless packaging and incorrect manuals, the data that we collected during the SAS-Oden 2021 expedition are of very good quality (*Figure 8.14*).

LOKI

The other optical instrument operated by WP1 (EFICA), next to the UVP, the LOKI[44] (*Chapter 6.5*) also yielded excellent depth profiles of zooplankton images (*Figure 8.15*).

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44 Schulz J, et al. (2009) Lightframe On-sight Key species Investigation (LOKI). IEEE OCEANS 2009-EUROPE [http://doi.org/10.1109/OCEANSE.2009.5278252]
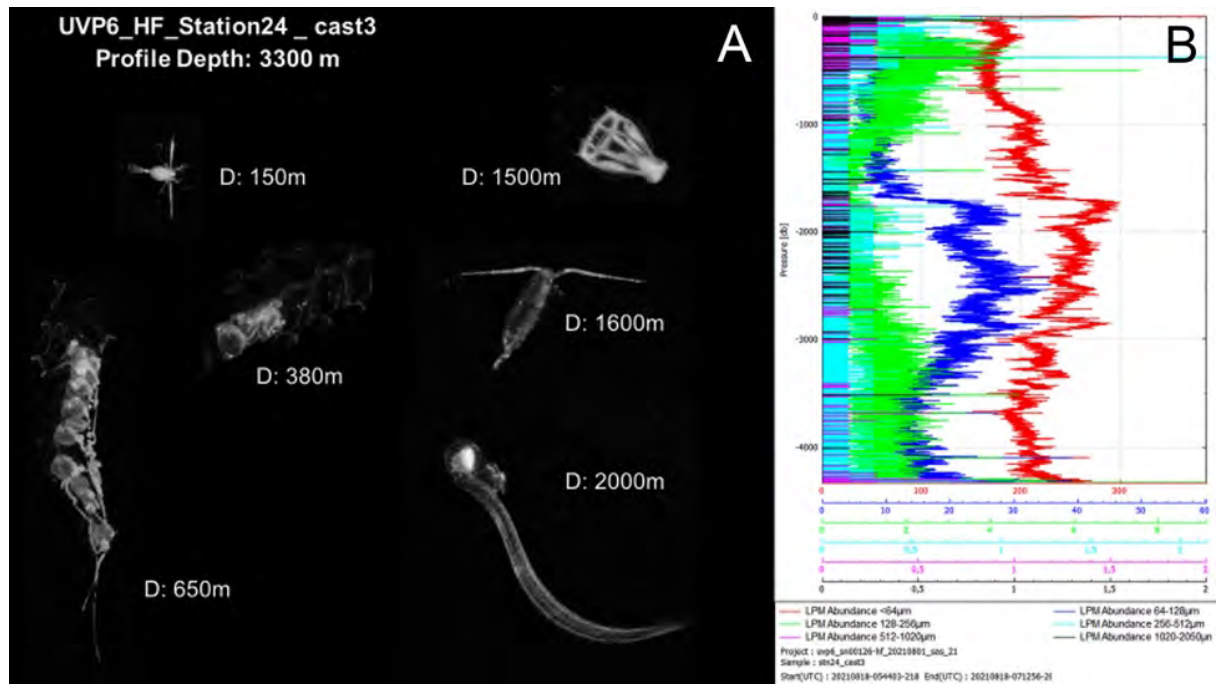
Figure 8.14: Examples of output from the UVP at SO21 station 24. (A) Images of zooplankton. (B) Particle profiles. ©European Commission[45]
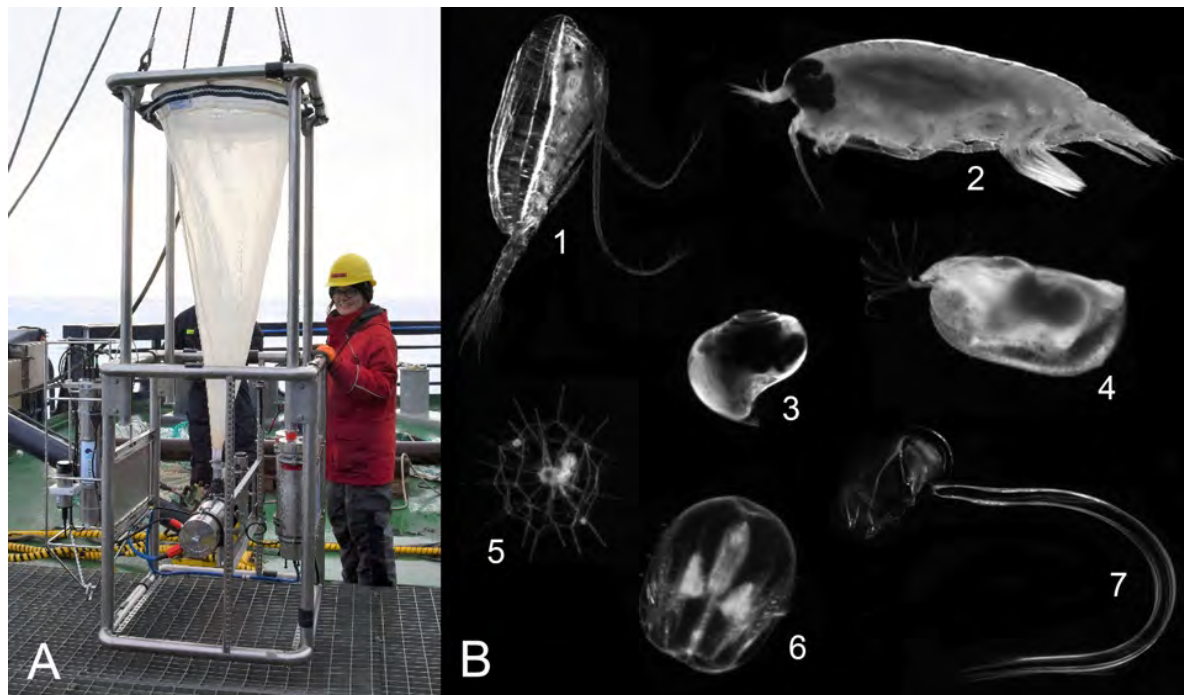
Figure 8.15: The Lightframe On-sight Key species Investigation system (LOKI). (A) After retrieval during the SAS-Oden 2021 expedition. (B) Zooplankton images recorded with the LOKI during previous expeditions. (1) Calanus glacialis (Copepoda), (2) Cyclocaris guilelmi (Amphipoda), (3) Limacina helicina (Gastropoda), (4) Ostracoda, (5) Radiolaria, (6) Mertensia ovum (Ctenophora), (7) Oikopleura sp. (Appendicularia). Images are not drawn to scale. (A) ©Hans-Jørgen Hansen, (B) ©Nicole Hildebrandt

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[45] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

Longlines and traps equipment

Longlines (*Figure 8.16*) and trap lines (*Figure 8.17*) targeting fish in the DSL were deployed at ice stations reached by helicopter. Ice thickness ranged between ca. 1 and 2 m. An auger (diameter 25 cm) was used to drill holes for each longline (one hole, *Figure 8.16 B*) and each deep trap line (four auger holes united, *Figure 8.17*). The holes for the deep trap line were cut to a rectangular opening with an ice saw. The preferred deployment time for both devices was 24 hours, however, shorter deployments were also performed whenever a time in the water of >4 hours could be realized. The time, date and geographical position were recorded at the start and end of each deployment (fully submerged and out of the water, respectively).

Figure 8.16: Deployment of longline. (A) Scientist carrying a 25-cm diameter auger used for making holes in the ice for the deployments during the SAS-Oden 2021 expedition. (B) A long line is hauled in with the help of a battery-driven winch on a tripod. (C) Hooks on a longline prepared for deployment. (D) Different hook sizes with artificial bait used for longline fishing. (E) The EFICA ice stations were reached by helicopter. (F) Line retrieval by hand-hauling. ©Frank Menger

Figure 8.17: A deep trap line with six traps and a rectangular hole (four auger holes united) for deployment made by combining four 25-cm diameter auger holes in the ice. ©Frank Menger

Longlines standard settings

Longlines were deployed between 300 and 700 m depth to sample fish from the DSL. A 5-kg bottom weight was attached to the long line, and true deployment depth was monitored using one or two Star Oddi TD probes (bottom or bottom and top of the longline, depending on Star Oddi probe availability). Each longline had 150 hooks with 2.8 m spacing between hooks, and either squid, shrimp, herring or artificial bait was used to attract fish. Hooks were set up in a sequence of 15 defined hooks and the sequence was repeated ten times (*Figure 8.16 C,D*, *Table 8.3*). Three hooks with squid were followed by three hooks with shrimp and three hooks with herring as bait, followed by one squid hook and three hooks with different artificial bait, e.g., fluorescence or fly hooks, to test if this might attract the fish. The hooks with natural bait consisted of one small, one medium and one large hook (*Table 8.3*). Longlines were retrieved either by line hauler or by hand-hauling (*Figure 8.16 B,E,F*) depending on weather conditions. Hand-hauling was the more controlled and, therefore preferred, method, but also the more physically strenuous retrieval method.

Table 8.3: Standard hook sequence for the longlines. S = small, M = medium, L = large.

	1	2	3	4	5	6	7	8	9	10	11-15
Hook	S	M	L	S	M	L	S	M	L	squid	S or M
Bait	squid	squid	squid	shrimp	shrimp	shrimp	herring	herring	herring	-	artificial

Trap lines standard settings

Four to six tubular traps (*Figures 8.17 and 8.18*) were attached to a line with 30 m spacing in-between them. After an initial trial phase of five deep trap lines to evaluate different setups during 7-19 August, a setup using six traps from 310 to 460 m was defined and deployed as a standard for the eight trap lines deployed after 19 August for the rest of the expedition. The final standard trap line contained three traps with mushed bait (squid, shrimp and herring in equal amounts) and a white light source (Proglow superbright white) were deployed at 460, 400 and 340 m. These were alternated with traps with mushed bait and no light at 430, 370 and 310 m. A 5-kg bottom weight and 2.5-kg weights on each trap were used. A Star Oddi TD probe was attached to the lower end of the trap line to monitor its true deployment depth.
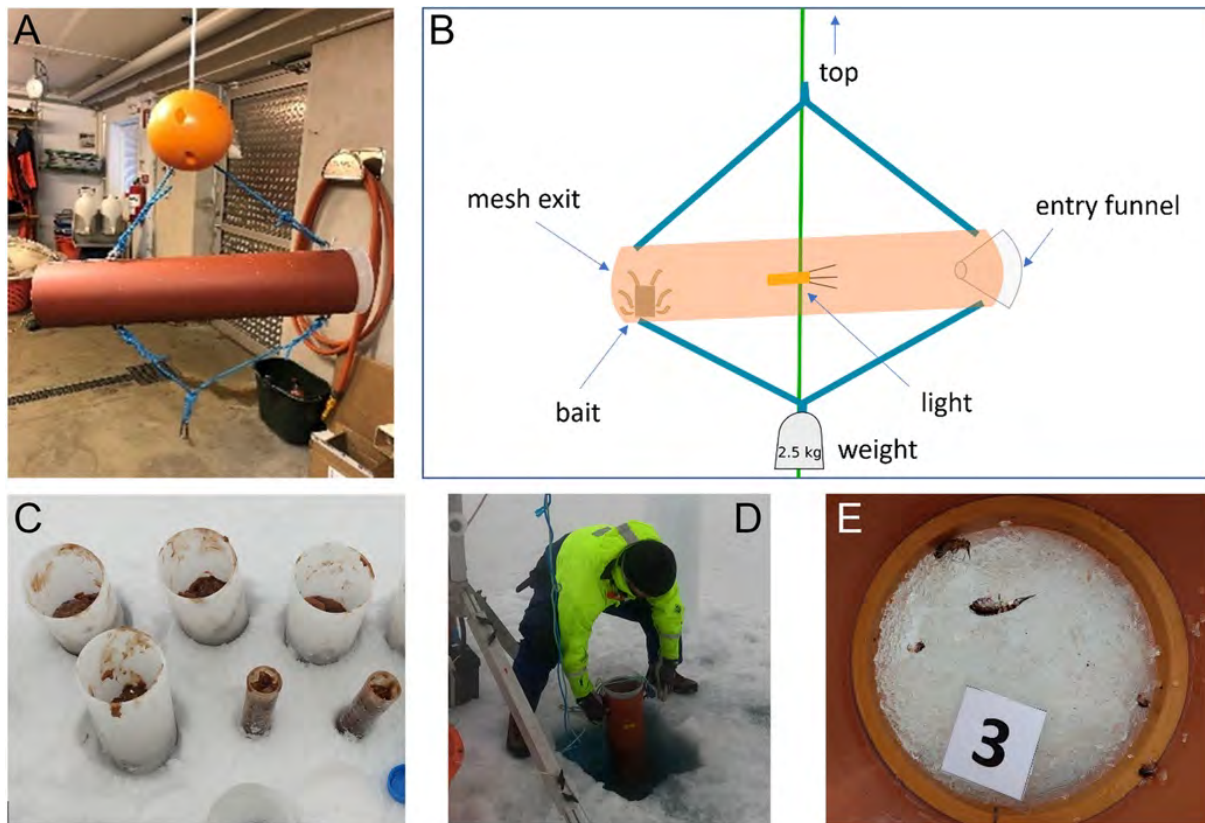
Figure 8.18: Design of the tube traps. (A) A hanging trap. (B) Schematic sketch of a hanging trap. (C) Filled bait containers for the traps. (D) Trap retrieval from the ice. (E) Example of trap catch. (A,B,D) ©Baldvin Thorvaldsson, (C,E) ©Frank Menger

Longlines and traps considerations

The Star Oddi TD probe results showed that deployment depth was strongly affected by ice drift / water currents (*Figure 8.19*). This affected the sampling depth.
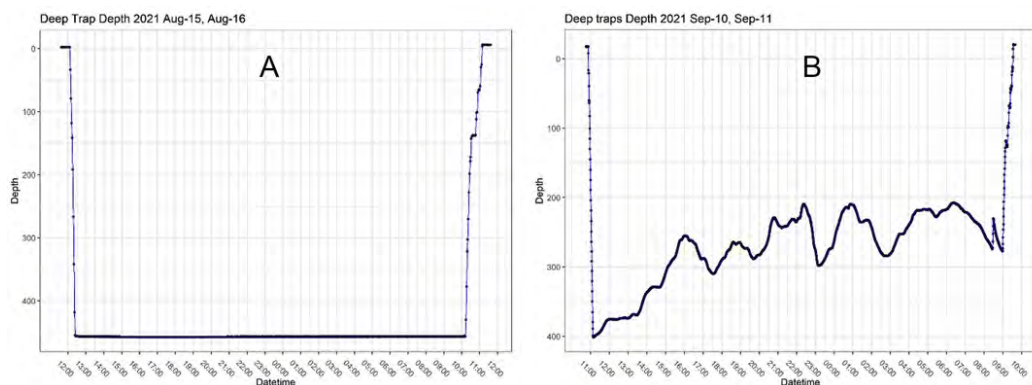
Figure 8.19: Difference in the effect of ice drift / water currents between two trap lines deployed on different dates as shown by Star Oddi TD probe depth measurements. The indicated depth is the depth of the lower end of the trap line. (A) Hardly any effect. (B) Strong effect. ©European Commission[46]

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[46] Snoeijs-Leijonmalm P, et al. (2022) Ecosystem mapping in the Central Arctic Ocean (CAO) during the SAS-Oden expedition. Publications Office of the European Union [https://data.europa.eu/doi/10.2826/958629]

Jigging machines and fishing rods

A jigging machine is automatic fishing gear with hooks that has the capacity to fish very deep and search for fish. Jigging machines are commonly used in fisheries worldwide, mainly for cod, mackerel and squid fishing. A jigging machine consists of a control unit and a winch or drum for the line (*Figure 8.20*). It is electrically-driven by a 12 volt car battery and can fish automatically following a predetermined programme. The two jigging machines brought on the SAS-Oden 2021 expedition by EFICA partner SLU were of the brand Belitronic BJ5000 (manufactured in Sweden).

On the winch/drum of the jigging machines we had 1000 m dyneema line (extra strong rope made of High Modulus Polyethylene fibres), 1.4 mm thick, to be able to fish in the DSL. At the end of the line there were five hooks on a nylon line and a weight of tree kilos. Most often we used the program for fish searching. In this program a starting depth is chosen and how many jigs should be performed before changing to the next water depth. In this way, the machine can search for fish as far up as programmed before it restarts from start depth. We fished with the jigging machine both from the ship and from the ice.
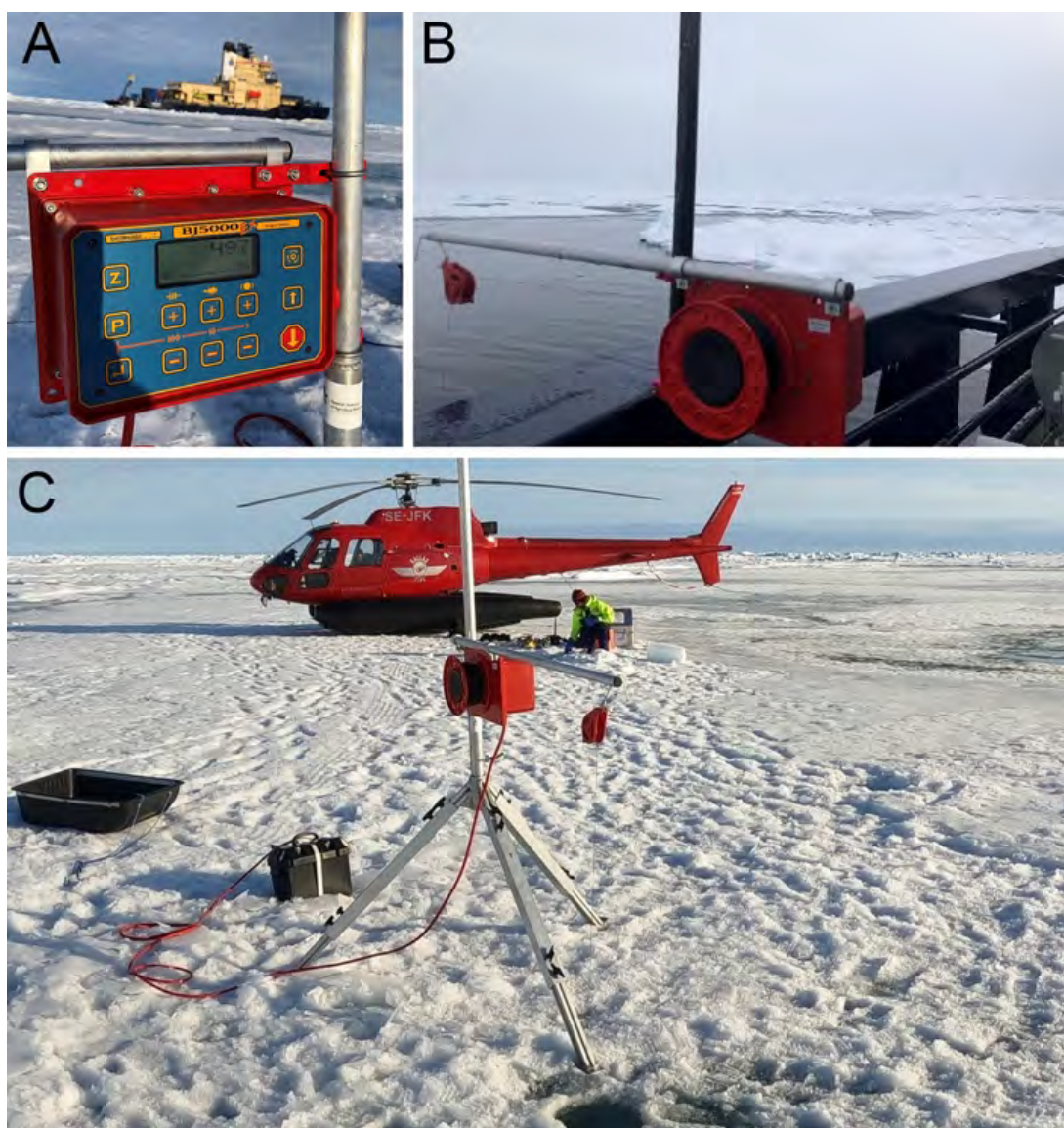
Figure 8.20: BelitonicBJ5000 jigging machine used during the SAS-Oden 2021 expedition. (A) Control unit. (B) Deployment from the ship. (C) Deployment from the ice with a tripod. ©Baldvin Thorvaldsson

We used two types of fishing rods: (1) rods targeting planktivorous fish such as polar cod and myctophids, equipped with small herring hooks and artificial bait resembling zooplankton, partly fluorescent, and (2) rods for large predatory fish equipped with the same hook types as those used with the longlines, with the option to use artificial, fluorescent and/or organic bait (shrimp and herring).

Fishing with rods was performed on an opportunistic basis and would only be recorded when fish was caught. This activity was called "Citizen science" on board (*Figure 8.21*). The equipment for opportunistic fish sampling consisted of rods and reels for angling down to 500 m to be used under cold conditions, such as aluminium reels and special non-freezing rods. Many different artificial baits were brought as well. This equipment could be borrowed by anyone on board in their free time and, in case fish was brought up, the EFICA scientists would get the opportunity to take scientific samples.
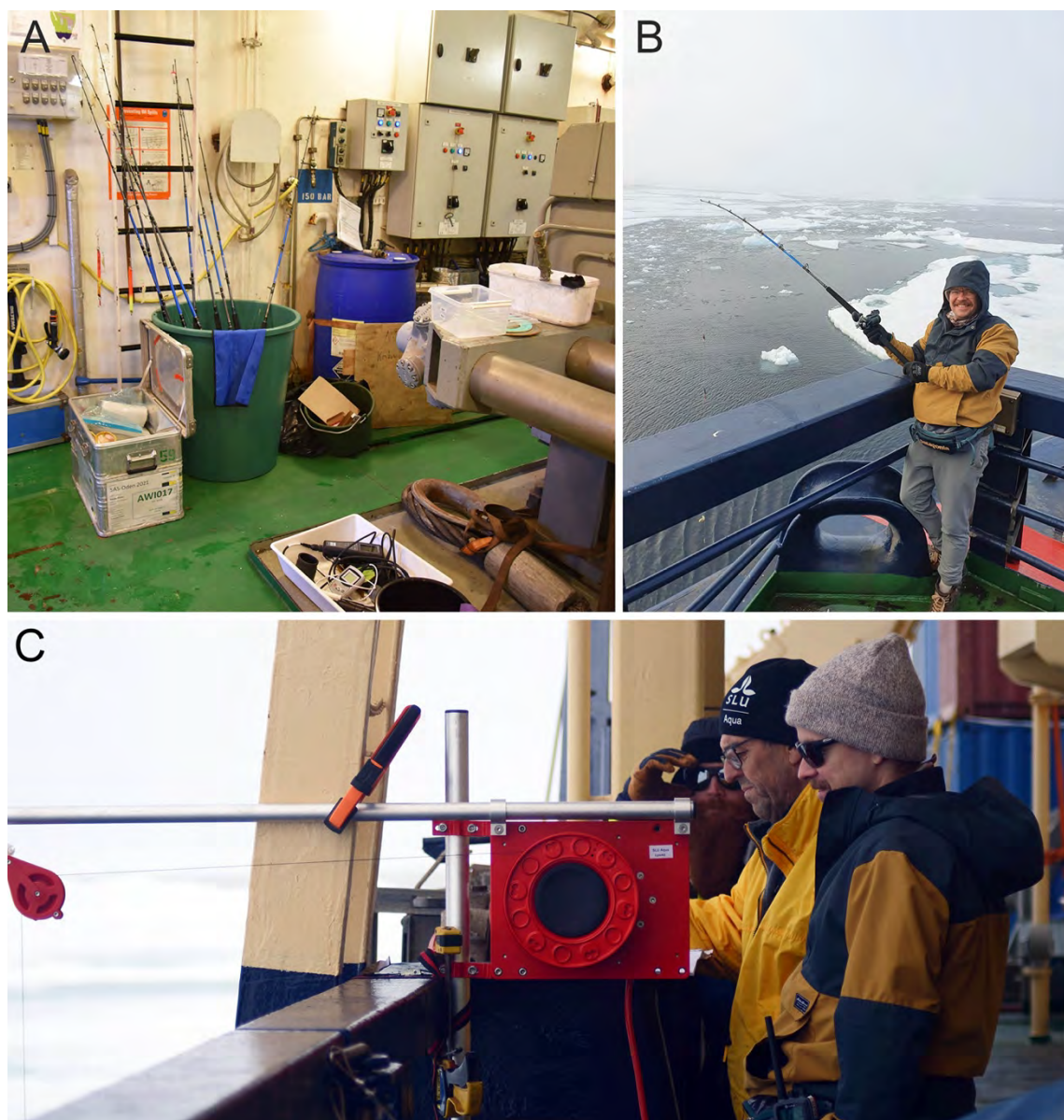
Figure 8.21: "Citizen science" fishing with fishing rods during the SAS-Oden 2021 expedition. (A) The storage site for the fishing rods. (B) Julek Chawarski (WP1) fishing with a fishing rod from IB Oden. *(C) Baldvin Thorvaldsson (WP1), Julek Chawarski (WP1) and Adam Ulfsbo (WP10) using a jigging machine from the ship. (A) ©Pauline Snoeijs-Leijonmalm, (B) ©Anna Lunde Hermansson, (C) ©SPRS*

Under-ice traps

For sampling sympagic (ice-associated) polar cod *Boreogadus saida* and invertebrates, we deployed baited traps in the ice-seawater interface layer. Two types of traps were deployed: umbrella net traps (*Figure 8.22 A*) and tube traps of the same model as used in the mesopelagic sampling (*Figure 8.22 B*).

Umbrella net traps are fish traps with approximately 1 cm mesh which can be deployed through a narrow hole in the ice or from an ice edge. In catching configuration, the net has a chamber of $\sim 50 \times 50 \times 50$ cm size, with a fyke throat as an entrance. To deploy the net, it is compressed to a cylindrical shape in the same way as a folded umbrella. Once the net is under the ice, it can be unfolded with a mechanism triggered with a stick through the ice hole.
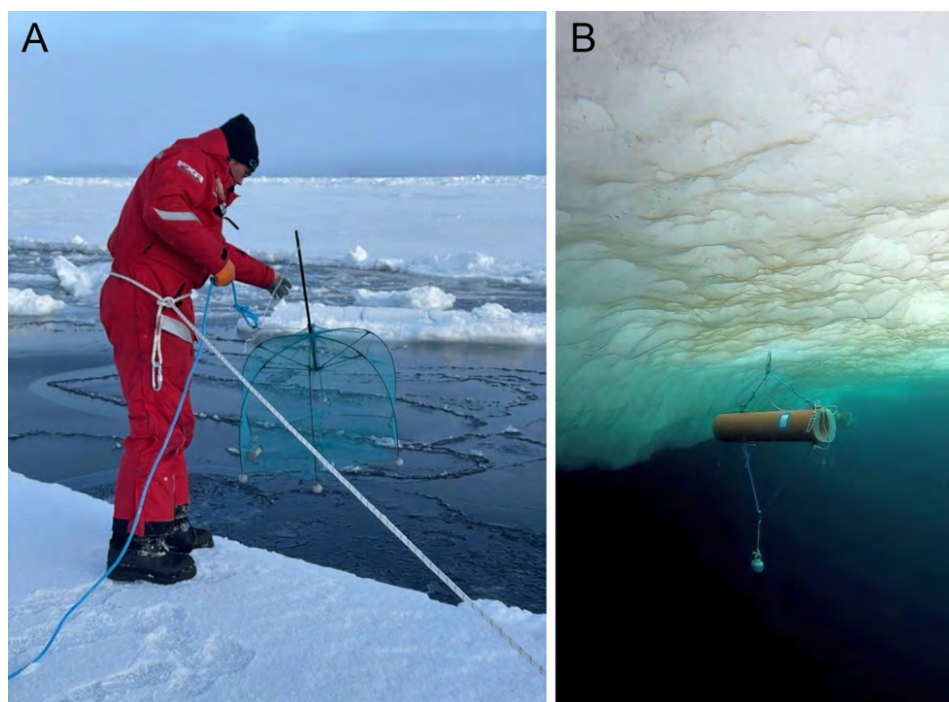
Figure 8.22: The two types of under-ice traps used for catching juvenile polar cod during the SAS-Oden 2021 expedition. (A) Deployment of an umbrella net trap by Hauke Flores (WP1) at an ice edge. (B) A tube trap hanging under the ice. ©Nicole Hildebrandt

Elaboration of zooplankton samples

Mesozooplankton community samples from the multinet (150 μm mesh, *Figure 8.23 A-C*) and microzooplankton community samples from the bongo net (50 μm mesh, *Chapter 6.5*) were conserved in Kautex vials in 4% formaldehyde buffered with hexamethylenetetramine and stored at 4°C according to "SO21 SOP: zooplankton community" (*Chapter 24*). For later analyses of food-web parameters, i.e., C and N content, including δ ^{13}C, δ ^{15}N, and fatty acid analyses of key zooplankton species, individual organisms were sorted from bicycle and bongo net catches. The sorting and identification took place in the unheated "Triple Lab" (*Figure 8.23 D*). The net catches were carefully poured into large photo trays, and zooplankton organisms were picked out and sorted for species and life stage using glass pipettes or tweezers. Target species included copepods (*Calanus* spp., *Paraeuchaeta* spp., *Metridia* sp., *Scaphocalanus* sp., *Spinocalanus* sp., *Gaetanus* spp., *Heterorhabdus* sp., *Microcalanus* spp., *Oithona* spp., *Aetideopsis* spp., *Mormonilla* sp.), amphipods (*Themisto* spp., *Cyclocaris* sp., *Eusirus holmi*), gastropods, chaetognaths, appendicularians, hydrozoans, ostracods, polychaetes and decapods.
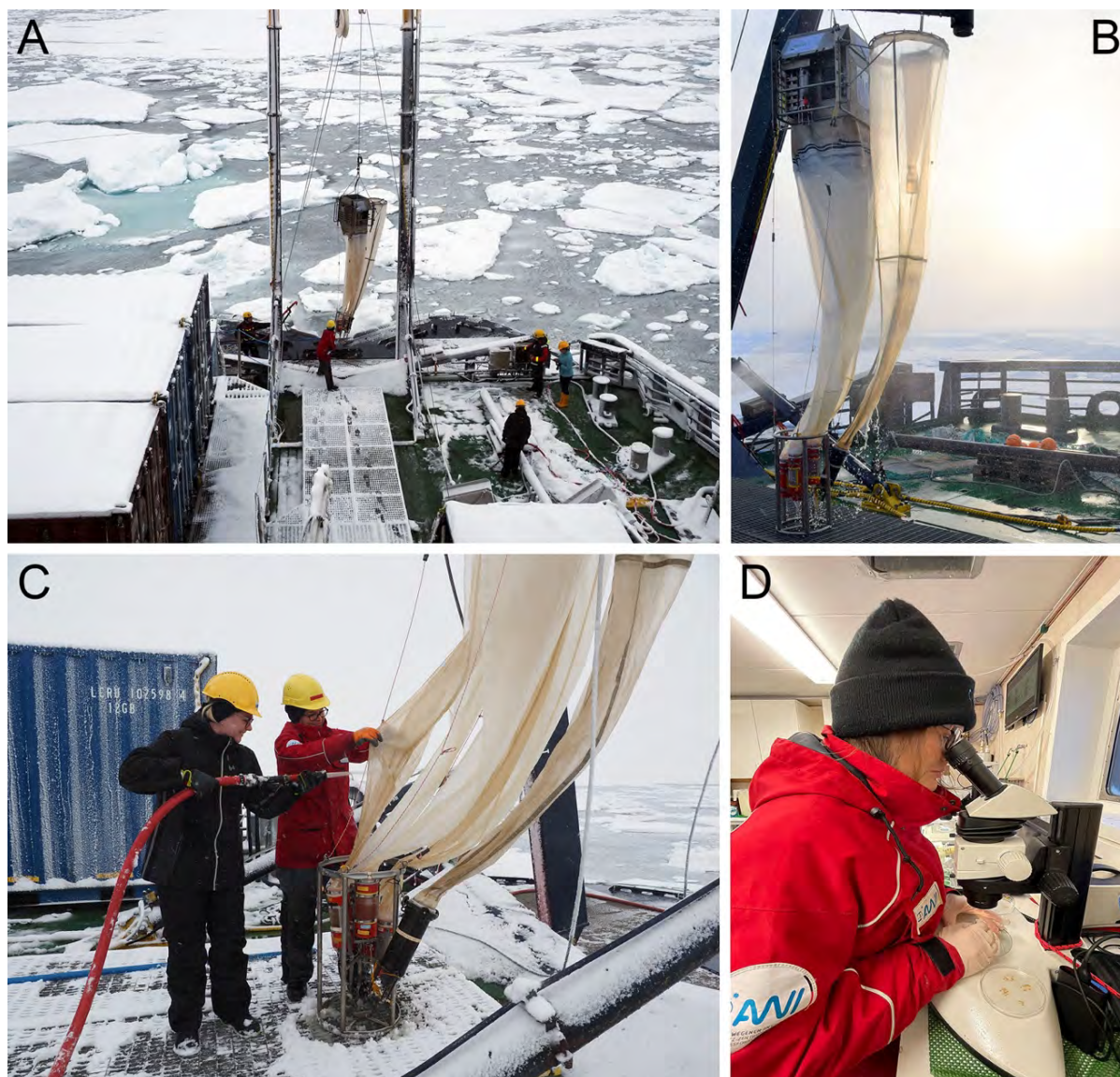
Figure 8.23: Multinet retrieval and species identification during the SAS-Oden 2021 expedition. (A,B,C) Retrieval of the multinet by Nicole Hildebrandt (WP1) and Emma Svahn (WP8), showing the side nets: one "bicycle net" for species sorting by WP1 and WP8, and a smaller "gypsum net" for WP3. (D) Species sorting and identification for the EFICA food-web parameters by Nicole Hildebrandt in the unheated "Triple Lab" on board IB Oden. (A,C) ©SPRS, (B) ©Hans-Jørgen Hansen, (D) ©Pauline Snoeijs-Leijonmalm

All zooplankton individuals sampled for food web parameters were first photographed with a stereo microscope camera in order to perform length measurements later on. Then they were briefly dipped in MilliQ water to remove salt, dabbed dry on a tissue, and placed in pre-weighted tin caps for CN analyses or in pre-combusted, pre-weighted glass vials for fatty acid analyses, either individually (large species) or pooled (smaller species). Individuals that were too big for the smaller containers were placed in plastic bags. For very small copepod species, individuals were pooled on a piece of mesh (50 µm), rinsed with MilliQ water and then placed in a petri dish. The CN samples were stored at -20°C, and the fatty acids samples at -80°C until further processing in the home laboratories. Additionally, two bicycle net samples (SO21 stations 22 and 30) and one bongo net sample (SO21 station 56) were preserved in 95% ethanol for genetic barcoding.

Besides fish, the beam and MIK nets on the SAS-Oden 2021 expedition targeted macrozooplankton to characterize the taxonomic composition of the potential prey field, or competitors (e.g., the comb jelly *Beroe* sp.) of finfish in the CAO. Because of its large opening, the beam net was highly successful in catching larger-sized macrozooplankton (*Figure 8.24*) that are rare in multinet samples. Target groups for the beam and MIK nets were decapods, amphipods, chaetognaths, hydrozoans and ctenophores. Beam and MIK net samples were first photographed as a whole (*Figure 8.24 A*), after which the macrozooplankton individuals were picked out, counted and again photographed (*Figure 8.24 B-D*) for later size distribution measurements. Thereafter, they were either preserved in Kautex bottles with a 4% formaldehyde solution buffered with hexamethylenetetramine or pure (>99%) ethanol and then stored at 4°C, or they were frozen in plastic bags at -20°C (for CN and stable isotope analyses) or in glass vials at -80°C (for fatty acids analyses).
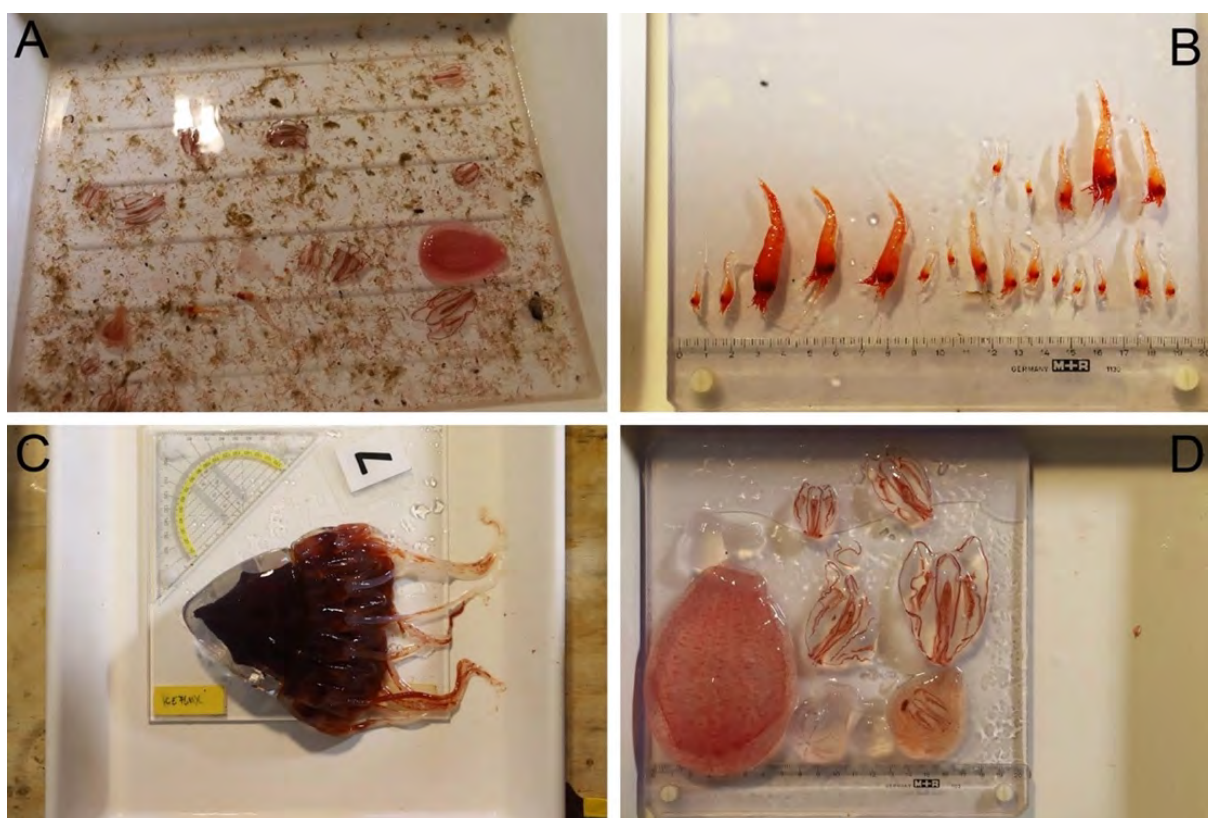
Figure 8.24: Examples of macrozooplankton collected with the beam net and the MIK net. (A) Overview of entire catch, beam net, (B) Size overview of Hymenodora glacialis*, beam net. (C)* Periphylla periphylla*, MIK net, (D) Ctenophores* Beroe *sp. and* Mertensia *sp., MIK net. ©Nicole Hildebrandt*

Elaboration of fish samples

Altogether, three sympagic *Boreogadus saida* (polar cod) were caught with baited under-ice traps and one at the water surface with the beam net. The fish were between 107 and 164 mm long. The largest specimen caught at SO21 station 35 was a gravid female (*Figure 8.25*). The fish caught with under-ice traps were dissected and sampled for otoliths, muscle tissue, guts, gonads, livers and tissue for genetic analysis. The remaining carcasses and the fish caught with the beam net were individually frozen (-20°C) for later analysis in the home laboratory.
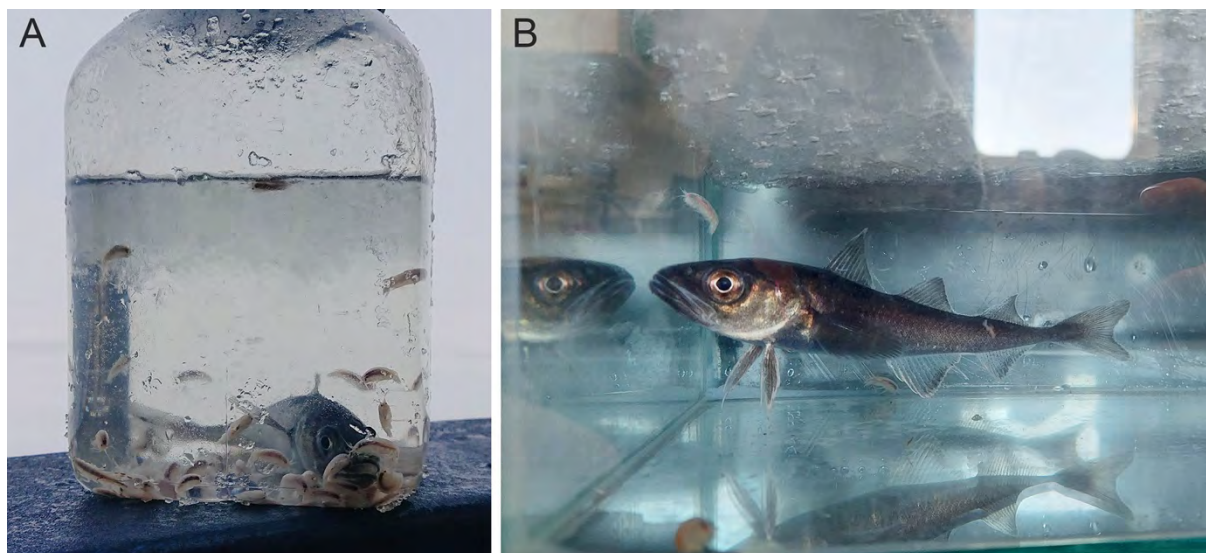
Figure 8.25: Example of catch from ice traps during the SAS-Oden 2021 expedition. (A) Whole catch from one trap consisting of sympagic amphipods and one Boreogadus saida *(polar cod). (B) Gravid female* Boreogadus saida *caught at SO21 station 35. (A) ©John Prytherch, (B) ©Janina Rahlff*

Sediment otoliths

As soon as the first box core sample arrived on deck (*Chapter 6.6*) we discovered that it contained intact fish otoliths (*Figure 8.26 and 8.27*). Otoliths were found at each of the six box core stations of the SAS-Oden 2021 expedition (*Figure 1.1*). Two sample types were taken to analyse otolith sedimentation:

(1) Transparent cores (Plexiglas, diameter 8 cm) were used for benthos subsampling to collect vertically stratified sediment subsamples (see *Chapter 6.6*). Within 24 hours of sampling, 11-12 sub-cores (kept at ambient temperature) were sliced into 1-cm sections and combined into bulk stratified samples (i.e., all 0-1 cm layers of the 12 cores were combined, all 1-2 cm layers of the 12 cores were combined, etc.) for the entire darker, presumably Holocene, layer. After sieving on a 300-µm mesh sieve, the (extremely few) benthic organisms were fixed in 70% ethanol, after which the otoliths and other calcareous structures such as bivalve shells (for sediment dating) were picked out from the benthos samples

(2) After placement of the subsampling cores of all involved WPs according to "SO21 SOP: box core" (*Chapter 24*), the remaining sediment from the darker-coloured (presumably Holocene) upper 10-20 cm layer was carefully removed from around each subsampling core as they were removed from the box core and combined into one bulk sample. At two stations an extra box core was available for EFICA's otolith studies due to obliquely sampled sediment, which was not ideal for the other SAS-Oden WPs. These two extra samples were used for bulk sampling of otoliths as well.

All otoliths and from the stratified samples also calcareous structures such as bivalve shells were stored in jars, photographed on board, and stored in small vials at room temperature. Altogether, WP1 sampled 315 otoliths from a total of 85 sediment samples, including both stratified and bulk samples.

Figure 8.26: The structure of a box core sample taken during the SAS-Oden 2021 expedition – macroscopic animal life was almost absent. (A) The box core sample after removing the bottom water with silicone tubing. (B) The vertical structure of the sediment with the darker upper layer presumably representing the Holocene and lighter-coloured glacial sediment below it. (C) Otoliths were observed on the sediment surface. (D) The pencil is pointing at a relatively large otolith. ©Pauline Snoeijs-Leijonmalm
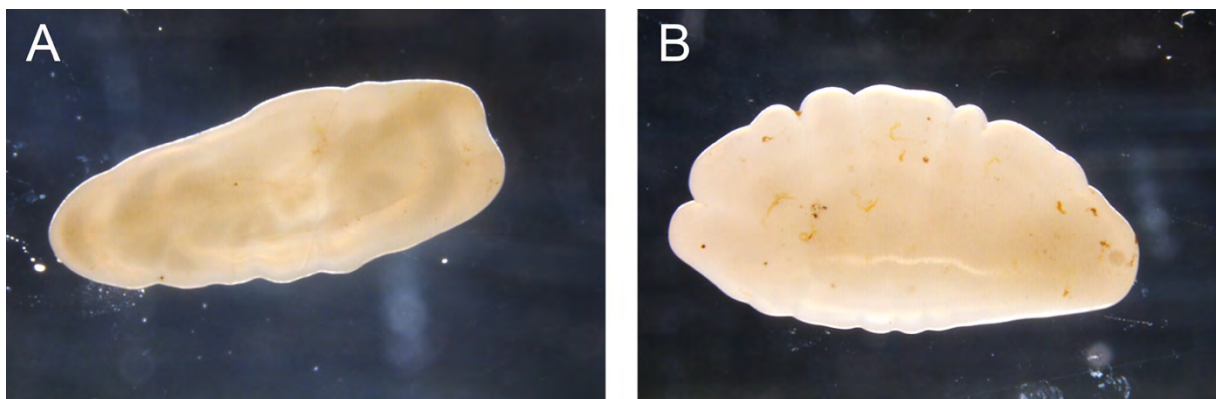
Figure 8.27: Microscopic images of otoliths with two different morphologies found in deep-sea sediments during the SAS-Oden 2021 expedition. (A) A narrow-shaped otolith. (B) An otolith with a broader shape and a more lobed margin. ©Hauke Flores

8.4 Summary of metadata collected

The metadata collected by WP1 (EFICA) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP1_EFICA" in the SND data repository and summarised in *Table 8.4*.

Table 8.4: Overview of all measurements/samples collected by WP1 (EFICA) during the SAS-Oden 2021 expedition.

Device	Depth	Nr of stations	Nr of Device Operations	Number of samples	Data owner
Hydroacoustics					
EK80 echosounder	10-1200 m	All	Continuous	Continuous along route	SPRS
CTD WBAT, 38 kHz	0-1000 m	18	32 CTD casts	Continuous depth profiles	EU Commission
CTD WBAT, 333 kHz	0-1000 m	17	17 CTD casts	Continuous depth profiles	EU Commission
ICE WBAT echosounder	0-500 m	1	1 ice station	Continuous depth profiles	EU Commission
Optics					
CTD Light sensor (TDR-Mk9-404A tag)	0-bottom	32	62 CTD casts	Continuous depth profiles	EU Commission
CTD UVP optics	0-bottom	32	38 CTD casts	Continuous depth profiles	EU Commission
LOKI optics	0-1000 m	11	11 LOKI casts	Continuous depth profiles	EU Commission
CTD deep-sea video camera	0-1000 m	15	15 CTD casts	Continuous depth profiles	EU Commission
ICE deep-sea video camera	2-3 m	3	3 ice stations	Continuous video recordings	EU Commission
Nets					
Beam net	0-800 m	9	45 beam net casts	1 fish caught 45 macrozooplankton community samples	EU Commission
MIK net	0-800 m	2	5 ring net casts	5 macrozooplankton community samples	EU Commission
Multinet (with WP8)	0-2000 m	16	16 multinet casts	84 mesozooplankton community samples	PI WP1 & PI WP8
Bongo net (with WP8)	0-200 m	11	13 bongo net casts	11 microzooplankton community samples	PI WP1 & PI WP8
Different nets	0-bottom	16	Different net casts	567 zooplankton samples for food-web parameters	EU Commission
Fishing lines and traps					
Longline	200-730 m	12	13 longlines, 24 hrs	No fish caught	EU Commission
Trap line	200-460 m	12	12 trap lines, 24 hrs	No fish caught	EU Commission
Trap under ice	2-3 m	11	11 stations, 24 hrs	3 fish caught	EU Commission
Jigging machine	0-500 m	5	5 stations, 1-3 hrs	No fish caught	EU Commission
Fishing rods	0-500 m	ca. 25	Citizen science	No fish caught	EU Commission
Box corer					
Subsampling benthos	900-1500 m	6	6 box core casts	85 benthos samples	PI WP1
Subsampling otoliths	900-1500 m	6	8 box core casts	315 sediment otoliths	EU Commission

8.5 Summary of preliminary results

Routine fisheries assessments are normally based on trawling and hydroacoustics in combination, but logistical difficulties prevent such activities in the CAO. While trawled nets cannot be applied in the CAO today due to its thick ice cover, also acoustic data collection is problematic because hydroacoustic backscatter from organisms is distorted by noise from icebreaking. Similar to the results from the MOSAiC expedition, fish sampling remained a challenge during the SAS-Oden 2021 expedition. Only four sympagic *Boreogadus saida* (polar cod) could be sampled from the ice. No mesopelagic fish were caught during the entire expedition despite numerous efforts with longlines and traps. From the EK80 acoustic measurements it became clear that the acoustic targets that resembled fish actively avoided the beam net, as well as the other sampling gear lowered from the ship, including thin fishing lines.

However, hydroacoustic data of good quality were collected at discrete measuring stations along the expedition route when the ship's engines were turned off and it was drifting with the sea ice. On the EK80 echosounder we observed very low target density in the epi- and mesopelagic portion of the water column (10-600 m). During the periods when the noise level was low, individual targets were observed in desired detail and from these data individuals can be counted, their target strengths can be estimated reliably and their behaviour can be observed. The EK80 sections overlapping with CTD wbat casts are suitable for comparison. From the WBAT hundreds of hits from individual targets were received with the possibility of single-target tracking.

Zooplankton sampling during the SAS-Oden 2021 expedition was very successful. Using both taxonomic composition and the size distribution of each taxon in combination with hydroacoustic data from the WBAT and the ship-mounted 18 KHz EK80, we will be able to quantify the prey biomass distribution along the SAS-Oden 2021 expedition route.

In contrast to the MOSAiC expedition, we did not detect high abundances of Atlantic fish species in the DSL at the inflow of Atlantic water to the CAO near the Yermak Plateau north of Svalbard during the SAS-Oden 2021 expedition. This could suggest seasonal and/or annual variation of Atlantic fish entering the CAO, and needs further study before conclusions can be drawn.

During the SAS-Oden 2021 expedition >300 well-preserved fish otoliths were discovered in the upper sediment layer that presumably represents the whole current geological epoch, the Holocene, that started ca. 11,700 years ago. These were not only otoliths from juvenile fish and might provide us with proof of the occurrence of adults of *Boreogadus saida* (polar cod) and *Arctogadus glacialis* (ice cod) these species in the CAO today (on the sediment surface) and in the past (in the 10-20 cm "Holocene" layer below the sediment surface).

Since no fish samples were collected in the DSL of the CAO, despite numerous attempts with vertical nets, traps and lines, the eDNA samples have become all the more important for the EFICA studies in assessing fish diversity and distribution in the CAO. The SAS-Oden genomic sample collection constitutes of thorough systematic sampling the water-column, eight ice habitats and sediment cores for the purpose of finding evidence of the occurrence of fish, zooplankton, birds and mammals.

8.6 Summary of post-cruise analyses and deliverables

The measurements and samples collected by WP1 (EFICA) will be elaborated together with the samples collected by EFICA during the MOSAiC expedition 2019-2020. Within the EFICA consortium the responsibility of the SAS-Oden 2021 analyses are as follows:

Hydroacoustics

EK80 & WBAT fish & zooplankton acoustics: Serdar Sakinan (WMR, IJmuiden, Netherlands)
EK80 fish acoustics: Hans Nilsson, Jonas Hentati Sundberg (SLU, Lysekil, Sweden)
Fish & zooplankton abundance/oceanography: Christian Stranne (SU, Sweden)

Optics

UVP: Barbara Niehoff (AWI, Germany)
MiniFishCam: Pauline Snoeijs-Leijonmalm (SU, Sweden)

Organisms

Macrozooplankton composition & biomass: Nicole Hildebrandt (AWI, Germany), collaboration WP8
Microzooplankton composition & biomass: Not yet decided, collaboration WP8
Ice invertebrate composition: Hauke Flores (AWI, Germany)
Ice meiofauna composition & biomass: Not yet decided
Boreogadus saida population genetics: Filip Volckaert (KUL, Leuven, Belgium)
$\partial^{13}C$, $\partial^{15}N$ in zooplankton and fish: Hauke Flores (AWI, Germany)
Fatty acids in zooplankton and fish: Pauline Snoeijs-Leijonmalm

Sediment otoliths

Image analysis: Julek Chawarski (SU, Sweden)
$\partial^{13}C$, $\partial^{18}O$, fish aging: Kim Vane & Hauke Flores (AWI, Germany)
^{14}C dating: Flor Vermassen & Pauline Snoeijs-Leijonmalm (SU, Sweden), collaboration WP9
Sediment characteristics: Helen Coxall (SU, Sweden), collaboration WP9

eDNA

Sequencing payment and administration: Pauline Snoeijs-Leijonmalm (SU, Sweden)
DNA & RNA extractions: Stefan Bertilsson, Prune Leroy, Javier Vargas Calle & Marine VandeWalle
Bioinformatics: Stefan Bertilsson & Moritz Buck

Deliverables

The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be delivered to the European Commission in April 2023. After this, the scientific results will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

9 WP2 (MIME)

Pauline Snoeijs-Leijonmalm, Prune Leroy, Claudia Morys, Javier Vargas Calle, Clara Pérez Martínez, Lisa Winberg von Friesen

Project title: Microbial Metabolism - How does meltwater affect marine microbial metabolic diversity, production and nutrient cycling in the Arctic Ocean?

9.1 Resources

External project funding: Swedish Research Council (VR, grant number 2018-04685) and the Swedish Research Council for Sustainable Development (FORMAS, grant number 2018-00509) and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for three berths on the SAS-Oden 2021 expedition to Pauline Snoeijs-Leijonmalm. All project participants are listed in *Table 9.1*.

Table 9.1: Overview of all onboard and onshore participants of the WP2 (MIME) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (WP1)	Pauline Snoeijs-Leijonmalm	pauline.snoeijs-leijonmalm@su.se	PI, WP Leader	SU, Sweden
Yes (berth)	Prune Leroy *	prune.leroy@slu.se	eDNA, CN experiment	SU, Sweden
Yes (berth)	Claudia Morys	claudia.morys@gmail.com	eDNA, box corer	SU, Sweden
Yes (berth)	Javier Vargas Calle	javier.vargas@slu.se	eDNA	SLU, Sweden
Yes (WP7)	Clara Pérez Martínez *	clara.perezmartinez@lnu.se	eDNA	SU, Sweden
Yes (WP7)	Lisa Winberg von Friesen	lisa.vonfriesen@bio.ku.dk	eDNA, CN experiment	UCPH, Denmark
No	Stefan Bertilsson	stefan.bertilsson@slu.se	eDNA, bioinformatics	SLU, Sweden
No	Moritz Buck	moritz.buck@slu.se	eDNA, bioinformatics	SLU, Sweden
No	Marine Vandewalle	marine.vandewalle@slu.se	DNA extractions	SLU, Sweden
No	Lasse Riemann	lriemann@bio.ku.dk	PhD supervisor Lisa	UCPH, Denmark

* Employed at SU for the expedition, the regular affiliation of Prune Leroy is SLU (Sweden), and that of Clara Pérez Martínez is LNU (Sweden)

Figure 9.1: The WP2 team at work during the SAS-Oden 2021 expedition. (A) Prune Leroy, Clara Pérez Martínez and Claudia Morys taking water samples with a hand pump on the ice. (B) Prune, Claudia and Clara in the helicopter on their way to a SAS Ice Station. (C) Javier Vargas Calle taking an ice core. (D) Lisa Winberg von Friesen combining bulk water samples in the "Main Lab" of IB Oden. (A) ©Prune Leroy (selfie), (B) ©Clara Pérez Martínez (selfie), (C) ©Christien Laber, (D) ©SPRS

9.2 Scientific motivation and specific research questions

The purpose of this research project is to complement the existing knowledge on the global marine microbiome (bacteria, archaea, fungi, protists) with data from the area north of Greenland (with the thickest ice cover in the Arctic) and the Central Arctic Ocean (Eurasian Basin, Makarov Basin, Lomonosov Ridge, North pole). This includes not only mapping the full collection of genes in the microbial communities living in different sympagic (ice-associated), pelagic and benthic microhabitats but also to analyse gene expression, both in the field and during incubation experiments, and to relate the results to biogeochemical cycling. This is the second expedition to the CAO for the MIME project – the first one was the international MOSAiC expedition with the German icebreaker *RV Polarstern* in 2019-2020. During the MOSAiC expedition a full seasonal cycle of experiments could not be achieved, partly because of the Covid19 pandemic and partly because of loss of a collaborator, and benthic samples were not taken at all. The SAS-Oden 2021 expedition therefore fills a crucial gap in the final year-round data set, i.e., the summer season. The data collected by WP2 (MIME) during the SAS-Oden 2021 expedition will be elaborated together with those collected by the project during the MOSAiC expedition 2019-2020.

While we got very good winter cover (28 experiments in the polar night, i.e., 24 hours of darkness per day) from MOSAiC spring data are very poor (6 experiments in the polar day, i.e., 24 hours of light per day), and summer experiments were absent. The MOSAiC experiments were carried out with water from the ice-seawater interface, seawater from the chlorophyll maximum (ChlMax), and seawater from the temperature maximum (TempMax) in the Atlantic water layer. The 11 experiments carried out during the SAS-Oden 2021 expedition (polar day) included two more habitats: brackish brine water pumped up from inside the ice and (nearly) fresh melt pond water. Furthermore, one treatment (organic substrate) was added to the experimental set-up used during the SAS-Oden 2021 expedition in collaboration with Lisa Winberg von Friesen and Lasse Riemann (University of Copenhagen, Denmark). Both a strength and a drawback of MOSAiC was that we studied the same ice floe for a whole year so that the variation in ice types is limited. During the SAS-Oden 2021 expedition we encountered first-year ice, multi-year ice, and ice in different melting stages, as well as melt ponds. Melting ice is increasing dramatically in the Arctic Ocean as a result of global warming.

The results of the MIME project will help us identify the microbial processes in the different habitats and add this variability to marine sea-ice models. The methods we will use are: (1) Genomics (16S, 18S, metagenomics, metatranscriptomics) sequencing and bioinformatics of field samples to uncover the taxonomic composition and metabolic functions of microbial community processes in the marine environment (water column) and ice habitats with emphasis on C and N cycling. (2) Community uptake of ^{13}C and ^{15}N in combination with gene expression to model microbial metabolism in general and specifically testing two hypotheses experimentally and from field measurements: (a) that biological N_2-fixation is a significant source of new N to the oligotrophic CAO marine ecosystem, and (b) that urea is an alternative substrate for ammonium oxidation and chemoautotrophic CO_2 fixation in the CAO, especially at low ammonium availability (deep water).

The specific research questions of the WP2 (MIME) project are:

(1) What is the genomic (DNA) and functional (RNA) diversity of the microbial communities in the seawater and sea-ice habitats in the CAO north of Greenland and up to the North Pole along the Lomonosov Ridge?
(2) Will the carbon and nitrogen metabolisms of microbial communities change when the CAO transforms from a permanent ice-covered ecosystem into a seasonal one through increased meltwater influence?

9.3 Summary of field work performed

SAS Core Parameters: WP2 (MIME; *Figure 9.1*) coordinated the SO21 omics collaboration (*Chapter 7.4*). The participants in WP2 performed the majority of the sampling and filtering of DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*). Prokaryote abundance samples (FCM-prok) were taken from the ice habitats, the 12 CTD bio SAS depths, and every 500 m below 500 m of depth (CTD deep SAS) according to "SO21 SOP: flow cytometry" (*Chapter 24*).

The project-specific MIME CN experiment was carried out 11 times by WP2 (MIME). The experimental water was obtained from extra CTD casts (ChlMax, TempMax) or extra ice-habitat water sampling from the ice (melt pond, brackish brine, ice-seawater interface), and once from the seawater tap.

Immediately after water sampling, in different carboys because of the large volume necessary (165 L), and the need to start the experiment with exactly the same water in all 48 experimental bottles, the water from all replicate containers was mixed in a 165-L "mixing tank". With the mixing-tank water, 48 polycarbonate bottles were filled. The experimental design (*Figure 9.2*) consisted of 2.3-L bottles spiked with ^{13}C (bicarbonate), inorganic ^{15}N (nitrate, ammonium), organic ^{15}N (urea, amino acids), as well as a blank treatment called "REF" (no tracers added), and 4.6-L bottles spiked with ^{13}C (bicarbonate) and $^{15}N_2$ gas with and without Organic C[47]. Spiking with $^{15}N2$ gas was performed according to "SO21 SOP: spiking with $^{15}N_2$" (*Chapter 24*). Six 2.3-L bottles were taken from the mixing tank at the beginning of the experiment (t=0 hours), representing the natural water with natural levels of ^{13}C and ^{15}N and with the original microbial community composition. The treatment bottles were incubated for 24 hours (t=24 hours) in onboard fridges with glass doors, one dark fridge (dark treatment), and one with daylight LED-illumination outside the door (*Figure 14.4 A*). The WP2 team member with the main responsibility for the MIME CN experiments during the SAS-Oden 2021 expedition was Prune Leroy.

From each experiment 138 subsamples were taken (*Table 9.2*): water samples (1 mL) for single-cell genomics, water samples (4 mL) for flow cytometry to document community change during the experiment, water samples for inorganic and organic nutrient analyses (45 mL), water samples (10 mL) for MIMS analyses for the $^{15}N_2$ treatments (after addition of $^{15}N_2$ gas), and water samples for nitrification from the ammonium treatment (after incubation). Samples for IRMS (isotope-ratio mass spectrometry) were taken on Advantec® glass fibre filters (diameter 25 mm) and genomic samples for taxonomic composition and gene expression were taken on 0.2 μm Sterivex™ filter units.

WP2 (MIME) also prepared filters for (1) "filter tests" for comparing Advantec® and Whatman® GF/F and glass fibre filters, (2) "spiking tests" to check if the addition of stable isotope tracers added to 0.2 μm-filtered seawater (without prokaryotes and protists) would affect the yield of ^{13}C and/or ^{15}N on the filters, and (3) "kinetics tests" to test how the uptake of the different stable isotope tracers developed over time (after t=0, t=12, t=24, t=48 hours).

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[47] The "Organic C" treatment consisted of a mixture of labile and semi-labile sugars characteristic for Arctic summer waters to a final concentration of 10 μM.
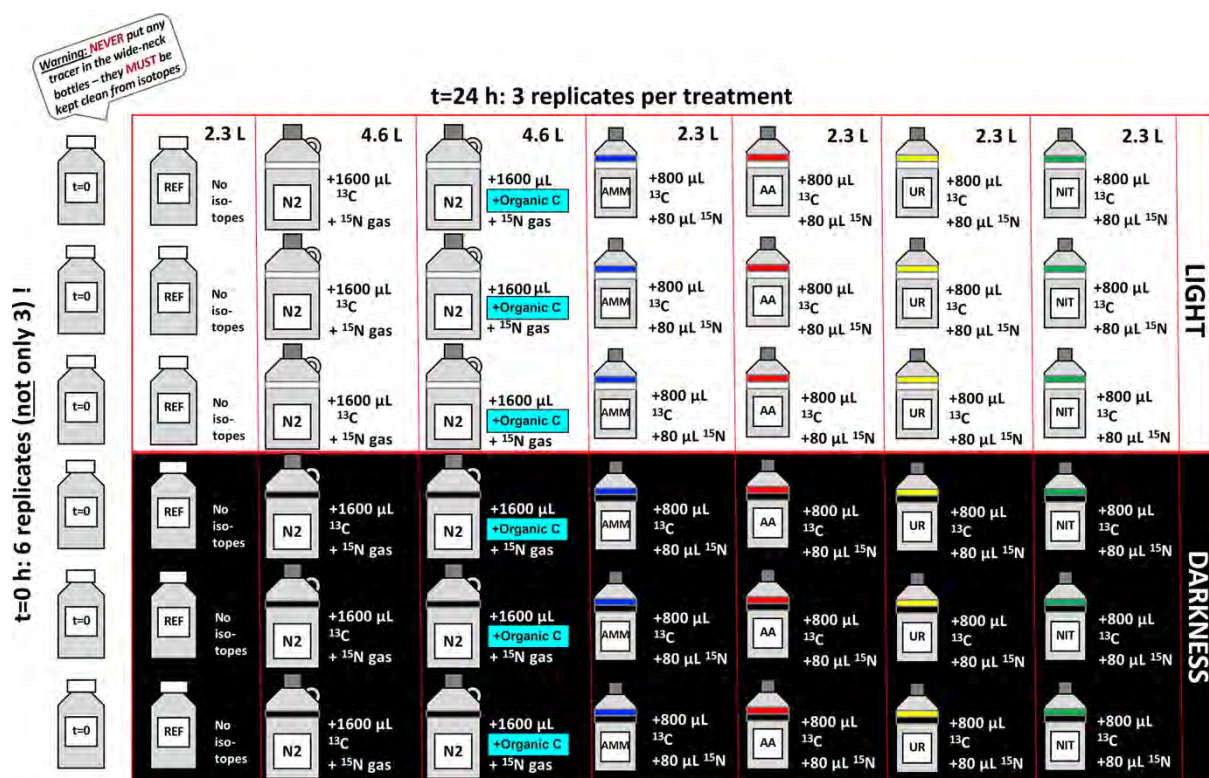
Figure 9.2: Experimental design of the MIME CN experiment carried out during the SAS-Oden 2021 expedition. The same experiment (except the "Organic C" treatment) was carried out during the MOSAiC expedition 2019-2020. ©Pauline Snoeijs-Leijonmalm

Table 9.2: Overview of the subsamples taken during one MIME CN experiment with the experimental design used during the SAS-Oden 2021 expedition (Figure 9.2).

SUB-SAMPLE CHECK-LIST MIME-experiment SAS-Oden 2021

Working order	Sample type	DAY 1	DAY 2							Total
		t = 0	REF	N2	N2-org	AMM	AA	UREA	NIT	
1	Single Cell genomics	3		6	6					15
2	MIMS			6	6					12
3	Nitrification					6				6
Make the Pajamas bottles										
4	Flow cytometry	4	4	4	4	4	4	4	4	32
5	Sterivex filters RNA	2	2	2	2	2	2	2	2	16
Rest of each experimental bottle										
6	Advantec filters	6	6	6	6	6	6	6	6	48
Samples taken from the mixing tank										
7	Inorganic nutrients	3								3
8	Organic nutrients	6								6
9	Sterivex filters DNA	All the rest								0
Samples taken from extra Carboy = has not been in mixing tank										
10	Sterivex filters DNA	All the rest								
Ordered 180 L Tank 165 L	Incubation water	13.8	13.8	27.6	27.6	13.8	13.8	13.8	13.8	138

9.4 Summary of metadata collected

The metadata collected by WP2 (MIME) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP2_MIME" in the SND data repository and summarised in *Tables 9.3 and 9.4*.

Table 9.3: Overview of all measurements/samples collected by WP2 (MIME) during the SAS-Oden 2021 expedition.

Parameter	Nr of samples/ measurements per experiment	Nr of experiments	Total nr of samples/ measurements
MIME CN experiments			
Salinity in mixing tank	1	11	11
Single cell genomics	15	11	165
MIMS (only $^{15}N_2$ treatments)	12	11	132
Nitrification (only AMM treatments)	6	11	66
Flow cytometry (prokaryotes and protists)	32	11	352
Inorganic nutrients	3	11	33
Organic nutrients	6	11	66
Advantec glass fibre filters for IRMS	48	11	528
0.2 µm Sterivex™ filter units for microbial composition (DNA)	3	11	33
0.2 µm Sterivex™ filter units for gene expression (RNA)	16	11	176

Table 9.4: Overview of all samples collected by WP2 (MIME) for the SAS Core Parameter prokaryote density in field samples by flow cytometry (FCM-prok) during the SAS-Oden 2021 expedition.

Parameter	Code	CTD casts	Total nr of samples
Prokaryote density water column 0-500 m	FCM-prok	18 CTD casts	216
Prokaryote density water column >500 m	FCM-prok	18 CTD casts	101
Prokaryote density ice habitats	FCM-prok	16 ice stations	136
Total number of samples			**453**

9.5 Summary of preliminary results

No preliminary results were obtained during the expedition.

9.6 Summary of post-cruise analyses and deliverables

The measurements and samples collected by WP2 (summer field sampling + 11 experiments) will be elaborated together with the samples collected by the MIME team during the MOSAiC expedition 2019-2020 (year-round field sampling + 34 experiments). Within the VR and Formas MIME projects the responsibility of the analyses for both expeditions are as follows:

Post-cruise tasks Stockholm University (SU):

(1) IRMS, MIMS and nitrification analyses at the UC Davis Stable Isotope Facility, University of California (USA) – the POC and PON contents on the filters will also be available from these analyses
(2) Dissolved inorganic and organic nutrient analyses (excluding urea and amino acids) at the accredited nutrient laboratory at the Department of Ecology, Environment and Plant Sciences (SU), including salinity measurements of these samples
(3) Sequencing payment and administration

Post-cruise tasks Swedish Agricultural University (SLU, Uppsala):

(1) Drying and packing of the IRMS samples (not acid-fumed)
(2) Analysis of the FCM samples, both field samples and experiments
(3) Analysis of urea and amino acids in water samples
(4) Extractions of RNA and DNA
(5) Amplicon sequencing of key genes (e.g., *nifH*)
(6) Single-cell genomics

Deliverables:

The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). After the full SO21 omics dataset has been published in an international journal (foreseen in spring 2023), all sequencing data will be on-line with full open access at the European infrastructure for life science/sequence data (EBI[48]). The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[48] https://www.ebi.ac.uk

10 WP3 (ProMis)

Birthe Zäncker, Kimberley Bird

Project title: Production and export of phytoplankton-derived organic matter in the changing Arctic Ocean – Role of parasites, saprotrophs and mineral ballasting (ProMis)

10.1 Resources

External project funding: The Arctic Research Icebreaker Consortium (ARICE, EU[49]), grant number 730965, including two berths on the SAS-Oden 2021 expedition from the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) to Birthe Zäncker. All project participants are listed in *Table 10.1*.

Table 10.1 Overview of all onboard and onshore participants of the WP3 (ProMis) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (berth)	Birthe Zäncker	birzan@mba.ac.uk	Co-PI, WP Leader	MBA, UK
No	Michael Cunliffe	micnli@mba.ac.uk	Co-PI	MBA, UK
Yes (berth)	Kimberley Bird	kimbir@mba.ac.uk	Field sampling and processing	MBA, UK
No	Jutta Wollenburg	jutta.wollenburg@awi.de	Gypsum sample analyses	AWI, Germany
No	Brandon Hassett	brandon.hassett@uit.no	Card-FISH analyses	UiT, Norway
No	Emily Cooper	emicoo@mba.ac.uk	PLFA analyses	MBA, UK

10.2 Scientific motivation and specific research questions

The Arctic summer sea ice extent and abundance of multi-year ice are decreasing[50]. By the middle of this century, the Arctic Ocean will be mostly, if not completely, ice-free during the summer[51]. The loss of sea ice in the Arctic favours "Arctic amplification" – the process of increased heat absorption by the newly exposed Arctic Ocean in a positive feedback loop[52]. These physical changes in the Arctic Ocean are drastically impacting the biology and ecology of the region[53]. However, the exact mechanisms through which the decreasing Arctic sea ice will influence the entire ecosystem are not yet clear, especially at the microbial scale. For example, stronger water-column stratification due to increased introduction of freshwater from the melting sea ice could reduce surface water mixing, making it more difficult for phytoplankton to utilise nutrients from deeper water layers. Higher light intensities due to loss of sea ice at the same time could increase photosynthetic rates and/or cause photoinhibition. This would greatly influence production of phytoplankton-derived organic matter (OM), which underpins the Arctic Ocean food webs and biogeochemical cycles.

Phytoplankton are not only influenced by abiotic factors but also by microbial interactions. Up to 25% of the cells of a single diatom species (*Pleurosigma elongatum*) can be infected by fungi[54], showing not

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[49] https://arice-h2020.eu

[50] Kwok R (2018) Arctic sea ice thickness, volume, and multiyear ice coverage: losses and coupled variability Arctic sea ice thickness, volume, and multiyear ice coverage: losses and coupled variability (1958-2018). Environmental Research Letters 13:105005

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[53] Wassmann P, et al. (2011) Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235–1249

[54] Hassett BT, Gradinger R (2016) Chytrids dominate arctic marine fungal communities. Environmental Microbiology 18:2001–2009

only the importance of parasitic fungi for overall diatom abundance, but also their importance in shaping the phytoplankton community structure via selective parasitism. Studies have shown that fungal infection rates are higher when diatoms are stressed[55]. Therefore, if diatoms are inhibited by light due to melting sea ice, fungal infection rates are likely to increase[56]. However, the specific impacts of Arctic sea ice loss on phytoplankton and their fungal parasites are difficult to predict because little is known about host-parasite interactions and infection mechanisms.

Another factor influencing the export of phytoplankton-derived OM through the food web are saprotrophs that degrade biogenic particles. Part of the dissolved organic matter (DOM) pool excreted by phytoplankton are precursor compounds for the formation of Transparent Exopolymer Particles (TEP)[57], which are polysaccharide-rich biogenic gel-like particles that act as attachment sites and point sources of organic material for saprotrophic bacteria and fungi[58,59]. Due to their stickiness, TEP also combine other particles in the water column and facilitate the formation of larger sinking particles (e.g., marine snow), which maintain the biological carbon pump[60]. At present we have a limited understanding of how TEP production and processing will be impacted by sea ice state.

Mineral ballasting of organic matter also facilitates the biological carbon pump. One of the previously underestimated minerals in this process is cryogenic gypsum[61], which has been observed to ballast a *Phaeocystis* under-ice bloom in the Arctic Ocean, increasing vertical carbon export[62]. Except for this single study, the influence of cryogenic gypsum on ballasting has not been fully evaluated but could potentially impact the carbon export in the Arctic Ocean significantly[63]. Most likely not only the prymnesiophyte *Phaeocystis* and the diatom *Melosira*, which have been previously associated with cryogenic gypsum, but also other microbes are associated with gypsum and are exported from surface waters to the deep ocean. Depending on the magnitude of this process, the export of microbes could alter the OM degradation in surface waters.

The specific hypotheses of the WP3 (ProMis) project are:

(1) Cryogenic gypsum increases TEP-related carbon export in ice-covered sea compared to the open Arctic ocean.
(2) Fungi are abundant and active in the Central Arctic Ocean in the water column and the sea ice.
(3) Fungi control TEP production and concentrations not only by colonizing and consuming carbohydrate-rich particles, but also by parasitizing phytoplankton, the main producers of TEP precursors.

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[55] Hassett BT, Gradinger R (2016) Chytrids dominate arctic marine fungal communities. Environmental Microbiology 18:2001–2009

[56] Hassett BT, et al. (2017) Spatial distribution of aquatic marine fungi across the western Arctic and sub-Arctic. Environmental Microbiology 19:475–484

[57] Passow U, et al. (2001) The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. Continental Shelf Research 21:327–346

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[60] Passow U, et al. (2001) The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. Continental Shelf Research 21:327–346

[61] Wollenburg JE, et al. (2018) Ballasting by cryogenic gypsum enhances carbon export in a Phaeocystis under-ice bloom. Scientific Reports 8:1–9

[62] Wollenburg JE, et al. (2020) New observations of the distribution, morphology and dissolution dynamics of cryogenic gypsum in the Arctic Ocean. The Cryosphere 14:1–14

[63] Wollenburg JE, et al. (2018) Ballasting by cryogenic gypsum enhances carbon export in a *Phaeocystis* under-ice bloom. Scientific Reports 8:1–9

10.3   Summary of field work performed

SAS Core Parameters: WP3 contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*).

Project-specific sampling: Water samples were taken from the bow CTD. 8.5 L was taken from 5-6 depths at the sampled stations. All ice samples were taken at the EFICA Master Stations with a Kovacs ice corer (*Figure 10.1*), and subsequently thawed inside WhirlPak bags in a water bath at 10-15°C for 20-90 min before filtration.
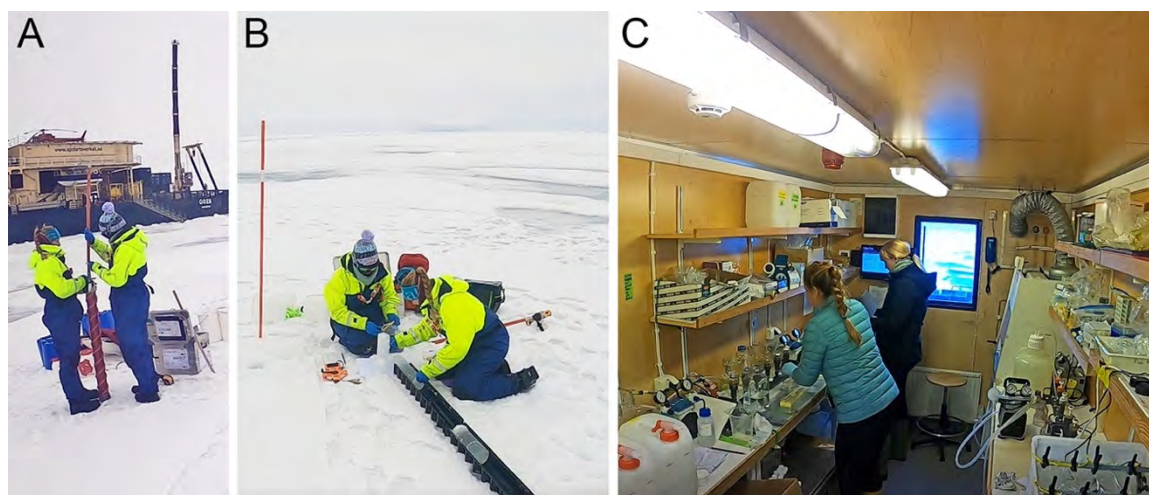
Figure 10.1. Sampling at ice stations for WP3: (A) Taking the ice core, (B) Sectioning the ice core and (C) Filtering the thawed ice in the lab container on board IB Oden. ©*Birthe Zäncker*

DNA/RNA: Water and thawed ice were filtered onto a 47-mm diameter cellulose nitrate membrane filter (0.2 μm pore size). The filters were stored in a 1-mL DNA/RNA shield and frozen at -80°C and will be kept frozen until DNA/RNA co-extraction.

TEP and mesolens[64]: Water and thawed ice were filtered onto a 25-mm diameter Whatman® Nuclepore filter (0.4 μm pore size). The volume filtered ranged from 5 to 250 mL depending on the particle concentration in the water and ice samples. TEP were subsequently stained with 1 mL of freshly 0.2 μm-filtered Alcian Blue for 3 seconds. Excess stain was washed off with MilliQ water and once dry, the filters were stored in petri dishes and frozen at -20°C.

TEP-DNA: Water and thawed ice were filtered onto a 25-mm diameter Whatman® Nuclepore filter (0.4 μm pore size). The water volume was adjusted according to the microscopy samples. One of the two replicates was stained with 1 mL of freshly 0.2 μm-filtered Alcian Blue for 3 seconds. Excess stain was washed off with MilliQ water. The other replicate was left unstained. Both replicates were transferred to a 2-mL Eppendorf tubes with the surface of the filter directed to the middle of the tube, and frozen at -80°C.

Gypsum: Samples for gypsum were taken from a small net attached to the multinet operated by WP1 and WP8 (*Chapter 6.5*), the box corer operated by WP1 and WP9 (*Chapter 6.6*), and from project-specific ice cores taken the nine EFICA Master Stations *Chapter 6.7*). The multinet samples were

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[64] Mesolens = a powerful microscope allowing for the analysis of a wide field of view at high resolution.

directly filtered onto a 30 μm-mesh and preserved in 100% ethanol and stored at 4°C. At each of the ice stations, one core was cut into 10-cm sections. The core sections were placed into 1 L of lukewarm tap water to record the change in volume and then poured onto a 30 μm mesh. The remaining ice on the mesh was subsequently melted under running tap water in 2-3 min. The mesh was then preserved in 100% ethanol and stored at 4°C similar to the multinet samples. For the box corer gypsum samples, 5 mL of the water directly overlaying the sediment was transferred into 40 mL 100% ethanol and stored at 4°C.

Card-FISH: Samples were taken from the bottom 10 cm of one ice core at each ice station. The core section was melted in the water bath and subsequently filtered onto a 47-mm diameter nuclepore track etched membrane (0.2 μm pore size). When only 100 mL were overlaying the filter, 10 mL of 37% formaldehyde were added to preserve the cells. Samples were frozen at -20°C for microscopy.

10.4   Summary of metadata collected

The metadata collected by WP3 (ProMis) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP3_ProMis" in the SND data repository and summarised in *Table 10.2*.

Table 10.2: Overview of all samples collected by WP3 (ProMis) during the SAS-Oden 2021 expedition.

Parameter	Category	CTD casts	Ice stations	Multinet	Box core	Total number of samples
Mesolens	Project-specific	16	10			193
TEP	Project-specific	16	10		3	196
TEP DNA	Project-specific	16	10			193
PLFA	Project-specific	14	9			137
DNA/RNA	Project-specific	16	10			193
FISH	Project-specific		10			10
Gypsum	Project-specific		10	14	4	193

10.5   Summary of preliminary results

No preliminary results were obtained during the expedition.

10.6   Summary of post-cruise analyses and deliverables

Project-specific DNA and RNA samples will be co-extracted in the home laboratory in Plymouth (UK). Samples will be analysed for fungal biomarkers such as the ITS region to identify the predominant fungal community in the water column and sea ice and use it for comparison for the fungal community on TEP. TEP filters will be analysed microscopically for TEP abundance, area and size spectra in the home laboratory at MBA, UK and images will be analysed using "Image J". Mesolens samples will be stained fluorescently to label TEP as well as bacterial and fungal cells attached to TEP, and will then be analysed using the Mesolens. TEP will be washed off from the filters using DNA/RNA shield and single TEP will be sorted using a Zeiss laser capture microdissection microscope (LCM) with subsequent DNA/RNA extractions of the TEP-associated microbial communities. The dataset will be used for comparison of the TEP-associated to the bulk microbial communities using the 16S and 18S data sets. The gypsum samples will be analysed under the microscope at AWI, Germany. Fungal parasites will be

determined at UiT, Norway. The filters will be stained with probes fused to fluorescent dyes and observed under the microscope to count parasitic fungi on sympagic algae.

Deliverables: The data will be made publicly available at the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

11 WP4 (VIRUS)

Janina Rahlff

Project title: Climate change driven effects on the diversity and activity of polar viruses (VIRUS)

11.1 Resources

External project funding: The Linnaeus University Center for Ecology and Evolution in Microbial model Systems (EEMIS), and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for one berth on the SAS-Oden 2021 expedition, to Karin Holmfeldt, and the German Science Foundation, project VIBOCAT, grant number DFG RA3432/1-1, to Janina Rahlff. All project participants are listed in *Table 11.1*.

Table 11.1 Overview of all onboard and onshore participants of the WP4 (VIRUS) project.

On board	First name	E-mail address	Task in project	Affiliation
No	Karin Holmfeldt	karin.holmfeldt@lnu.se	PI	LNU, Sweden
Yes (berth)	Janina Rahlff	janina.rahlff@lnu.se	WP Leader, field sampling & processing	LNU, Sweden

11.2 Scientific motivation and specific research questions

Marine viruses represent the most abundant biological entities in the oceanic water column[65] where they contribute to microbial diversification[66] and carbon cycling by inducing host cell lysis[67]. In the harsh Arctic environment, viruses tend to integrate into the genome of their microbial hosts, particularly during the winter, while replicating lytically during summer months[68,69]. In addition, Arctic bacteriophages can transfer important metabolic genes to their hosts, such as cold shock, resistance[70] or fatty acid desaturase (FAD) genes[71], which aid the host to overcome different kinds of stressors. While previous studies have reported on high viral abundance[72], high viral diversity[73] and high infection rates[74] in polar regions, a comprehensive dataset of viral abundance and diversity for different ice and water habitats derived from the Central Arctic Ocean is still lacking. It has been reported that only 12% of viral populations derived from cryopeg brine, sea-ice brine and melted sea ice could have a taxonomy assigned[75], indicating that many undiscovered viruses (not yet recorded in viral databases) reside in these habitats. Hence, we aim to elucidate the viral diversity of various ice

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[65] Suttle CA (2005) Viruses in the sea. Nature 437:356–361

[66] Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? Environmental Microbiology 6: 1–11

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habitats and the water column of the Central Arctic Ocean and determine to which extend lysogeny is the predominant viral lifestyle.

The surface microlayer (SML) forms a <1000 μm, skin-like layer on top of aquatic ecosystems and has distinct physicochemical characteristics compared to the underlying water[76]. Microbes and viruses with distinctive community structures compared to the underlying water are found in this ecotone between hydrosphere and atmosphere[77,78], where microorganisms contribute to gas and matter exchange processes across the air-sea boundary[79,80]. Furthermore, the SML is a known source of microbes and viruses to be transferred into aerosols[81]. Viral abundance and virus-induced mortality in Arctic SML were shown to be higher compared to the underlying water, and viruses exhibited a stronger degree of lysogeny in the SML[82]. In addition, it has been reported that virus-like particles in SML samples were derived from an open lead during summer in the Central Arctic Ocean[83].

A thorough, qualitative characterization of SML viruses from the field under application of -omics methods is missing to date[84], which is especially true for the polar regions. At the same time viral interactions with hosts as well as the viral role in the Arctic microbial loop remains elusive. When sea ice melts, melt ponds form and cover a considerable surface area of the Arctic (*Figure 11.1*). It has been shown that microbial polymers with a major fraction of proteinaceous compounds accumulate in the SML of melt ponds and that prokaryotes are not enriched in melt pond SML[85]. The role of viruses in shaping melt pond prokaryote communities, and their contribution to carbon-compound release in contrast to Arctic seawater SML, have so far not been investigated. With ongoing climate change, melting of sea ice, especially of thin first year ice, proceeds and entails that the areas covered with melt ponds will grow. This makes it crucial to understand the role of melt ponds as microbial and viral habitat, especially because viral lysis is a strong contributor to the release of organic matter.

The specific hypotheses of the WP4 (VIRUS) project are:

(1) The Central Arctic Ocean has a unique viral community mainly composed of viruses able of lysogenic replication cycles.
(2) Viruses in the surface microlayer have greater resistance to UVA radiation than those in the underlying water.
(3) Melt pond microbial and viral communities of the surface microlayer are stable across different melt ponds and between samples taken in the Arctic summers of 2012 and 2021.
(4) Melt pond microlayer viral communities differ from seawater communities because melt ponds represent closed freshwater systems.

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[77] Hardy JT (1982) The sea surface microlayer: Biology, chemistry and anthropogenic enrichment. Progress in Oceanography 11:307–328

[78] Drucker VV, et al. (2019) Autochthonous bacteriophages in the microbial loop structure of different biotopes of Lake Baikal. Contemporary Problems of Ecology 12:143–154

[79] Rahlff J, et al. (2019) Oxygen profiles across the sea-surface microlayer – Effects of diffusion and biological activity. Frontiers in Marine Science 6:11

[80] Reinthaler T, et al. (2008) Dissolved organic matter and bacterial production and respiration in the sea-surface microlayer of the open Atlantic and the western Mediterranean Sea. Limnology & Oceanography 53:122–136

[81] Aller JY, et al. (2005) The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. Journal of Aerosol Science 36:801–812

[82] Vaqué D, et al. (2021) Enhanced viral activity in the surface microlayer of the Arctic and Antarctic Oceans. Microorganisms 9:317

[83] Leck C, Bigg EK (2017) Biogenic particles in the surface microlayer and overlaying atmosphere in the central Arctic Ocean during summer. Tellus B Chemistry Physics Meteorology 57:305–316

[84] Rahlff J. (2019) The virioneuston: A review on viral–bacterial associations at air–water interfaces. Viruses 11:191

[85] Galgani L, et al. (2016) Biopolymers from a gelatinous microlayer at the air-sea interface when Artic sea ice melts. Scientific Reports 6:29465

Figure 11.1. Melt ponds covering the Arctic ice surface. ©Janina Rahlff

11.3   Summary of field work performed

SAS Core Parameters: WP4 was responsible for the SAS Core Parameters viral abundance and viral metagenomics. WP4 also contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*). Viral abundance samples (FCM-virus) were taken from the ice habitats, the 12 CTD bio SAS depths, and every 500 m below 500 m of depth (CTD deep SAS).

SO21 omics collaboration (*Chapter 7.4*): WP4 was responsible for the viruses "viromics" (code VIROMIC) samples. These were taken from approximately every second CTD omics cast from the chlorophyll maximum (ChlMax) and the temperature maximum (TempMax) (*Figure 11.2 A*), as well as from sea-ice habitats (ice-seawater interface, melt ponds, brackish brine, snow) during most of the nine EFICA Master Stations. The viromics samples were taken from the filtrate of the SO21 omics samples for metagenomic analyses of prokaryotes and protists that was filtered on 0.2 μm Sterivex™ filter units (*Chapter 7.4, Figure 11.2 B*). This will make it possible to couple the metagenomic analyses of the virus fraction directly to those of the prokaryote and protist fraction. Ca. 20 L of the 0.2 μm pre-filtered water were flocculated with iron-III-chloride ($FeCl_3$) with a final concentration of 1 mg $FeCl_3$ per L of sample water[86]. The water was incubated under regular shaking of the carboy for at least 1 hour in the fridge. The flocculates were subsequently filtered onto a 142-mm diameter membrane filter (Merck Millipore) with a pore size of 1 μm using a peristaltic pump at a constant speed of 60-70 (*Figure 11.2 C*). For detailed standard procedures for the viromics filtrations see "SO21 SOP: viromics" (*Chapter 24*).

For seven CTD stations and eight ice stations, additional water samples were taken for virus isolation (code VIRIS, 50 mL of 0.2 μm pre-filtered water into the fridge), which was coordinated with Dennis Amnebrink (WP5), who took samples for bacterial isolation (glycerol stocks) from many of the same but unfiltered samples. For a detailed description of the procedure, see "SO21 SOP: viral isolation" (*Chapter 24*). In addition, virus abundance samples were taken by Hanna Farnelid and Christien Laber (WP6).

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[86] John SG, et al. (2011) A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environmental Microbiology, Reports 3:195202

Figure 11.2: Sampling and filtration of the viromics samples. (A) Water sampling from CDT omics, (B) Sterivex filtration in red light for prokaryote and protist metagenomics, (C) Filtration of viral flocculates under red-light conditions (C). ©Clare Bird

Project-specific water samples (ca. 420-970 mL) were taken from the SML, once from a deep melt pond and five times from seawater using the glass plate method[87] (*Figures 11.3-11.5*; "SO21 SOP: surface microlayer"). This seawater was collected from open leads in front of the ship (*Figure 11.4 A*) or from a platform at the end of *IB Oden*'s gangway lowered starboard (*Figure 11.4 B*). The melt pond SML represents a water sample with salinity close to zero whereas the salinity at the seawater SML samples ranged from 7.2 to 31.5. Water from a ca. 60 cm reference depth was sampled using a weighted hose connected to a 100-mL syringe. Between samplings the glass plate was cleaned with 70% ethanol, and pre-rinsed with sample water. Wind speed at the sampling site was measured a hand-held digital anemometer (Mastech MS6252A) and water temperature and salinity with a thermosalinometer.

Water from the SML and underlying water was size-fractionated by filtration onto 5 µm (eukaryotes, particle-attached microbes, and viruses), 0.2 µm (prokaryotes, host-attached viruses) and 1 µm (virome, after flocculation). Iron flocculation was performed with more $FeCl_3$ than for the SAS viromes: 10 mg L^{-1},according to recent findings[88] for better concentration of viruses from freshwater and seawater samples at higher concentrations of $FeCl_3$. All 40 SML viromics samples were frozen at -80°C for metagenomic analysis (code SMLVO).

From the SML and reference depth, samples for virus isolation (code SMLVI, 13 samples), bacterial isolation (code SMLBI= 13 samples), and virus abundance (code SMLVA, 26 samples) were taken. SMLVI was sampled as mentioned above. For SMLBI 900 µL samples were mixed with 600 µL of 50% glycerol, and for SMLVA 1000 µL samples were mixed with 20 µL of 25% glutardialdehyde (final concentration ~0.5%). SMLVI were stored in the fridge and SMLVA and SMLBI at -80°C.

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[87] Harvey G. W., and Burzell L. A. (1972) A simple microlayer method for small samples Limnology & Oceanography 17: 156-157.

[88] Langenfeld K, et al. (2020) Concentration of ultrafiltration and iron chloride flocculation in the preparation of aquatic viromes from contrasting sample types, Peer Journal 9:e11111

Since we on one occasion observed snowflakes accumulating in the SML and on the glass plate during sampling, we started some opportunistic sampling of freshly fallen snow on the upper deck of *IB Oden*. Snow was collected by using an open container, like an aquarium. Snow was melted in the laboratory container and filtered onto 0.2 µm 47 mm membranes and the viruses were flocculated as described for the SML samples.
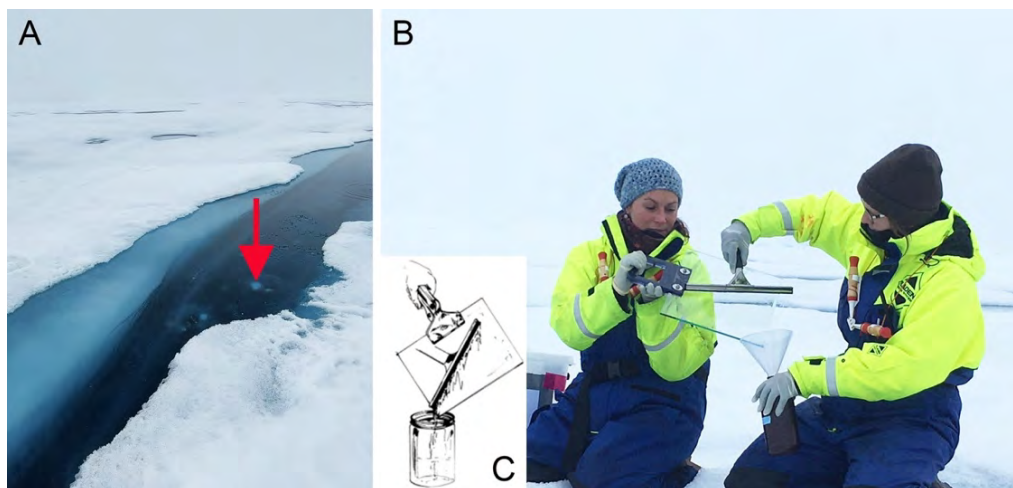
Figure 11.3: Sampling from the melt pond surface microlayer (SML). (A) Deep melt pond with arrow indicating the sampling position, (B) Glass plate sampling procedure. (A) ©Janina Rahlff, (B) ©Prune Leroy

Figure 11.4: Sampling from the ocean surface microlayer (SML). (A) The SML was sampled in an open lead from the ice in front of IB Oden, *sampling position indicated by blue arrow, (B) Sampling from starboard side of the ship from the gangway, (C) Sampling from the edge of an ice floe (C). (A) ©Janina Rahlff, (B) ©SPRS, (C) ©Hans-Jørgen Hansen*

Figure 11.5: Janina Rahlff and Sonja Murto sampling the surface microlayer (SML) during the SAS-Oden 2021 expedition. ©John Prytherch

11.4 Summary of metadata collected

The metadata collected by WP4 (VIRUS) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP4_VIRUS" in the SND data repository and summarised in *Table 11.2*. The sampling stations are shown in *Figure 11.6*.

Table 11.2: Overview of all samples collected by WP4 (VIRUS) during the SAS-Oden 2021 expedition.

Parameter	Code	Category	CTD casts	Ice stations	surface microlayer (SML)	Total nr of samples
Viral density	FCM-virus	SAS Core Parameter	18	16		453
Viromics	VIROMIC	SO21 omics collaboration	9			18
Virus isolation	VIRIS	Project-specific	7			28
Viromics	VIROMIC	SO21 omics collaboration		10		31
Virus isolation	VIRIS	Project-specific		8		32
Viromics	SMLVO	Project-specific			6	40
Virus isolation	SMLVI	Project-specific			6	13
Bacterial isolation	SMLBI	Project-specific			6	13
Virus abundance	SMLVA	Project-specific			6	26

Note: Karin Holmfeldt is the owner of the VIROMIC and VIRIS samples, Janina Rahlff is the owner of the SML-Code samples. For virus abundance samples from CTD casts and ice stations, see WP7.
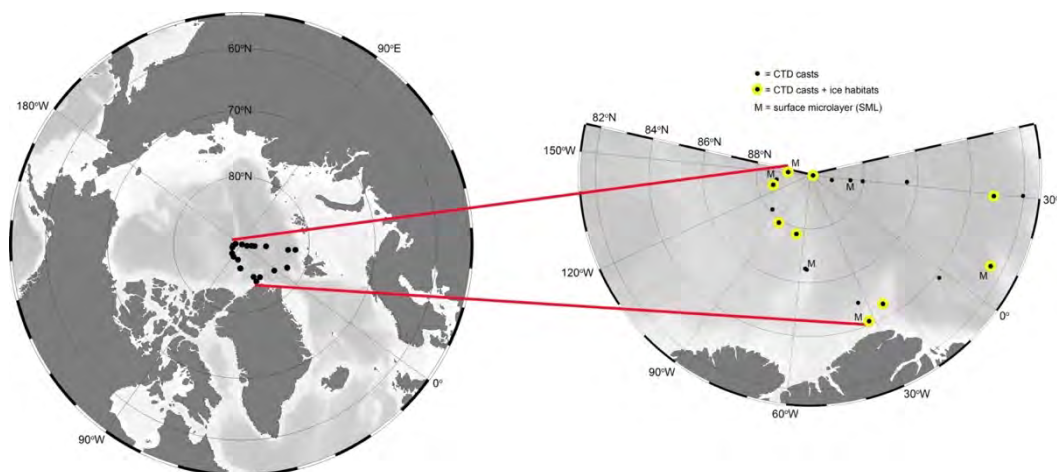
Figure 11.6: Map of the stations sampled by WP4 from CTD casts, ice habitats, and the surface microlayer). Yellow circles represent stations were viromes for ice habitats were samples and "M" represents where the surface microlayer was sampled. Map was generated with Ocean Data View (https://odv.awi.de)

11.5   Summary of preliminary results

Sometimes early leakage of the membrane filters could indicate higher amounts of viral flocculated especially in the ice-seawater interface and melt pond samples compared to the other sample types. At several stations, we observed decaying *Melosira arctica* in copious amounts in sea ice and melt ponds, which may release gels of polysaccharides and organic compounds. Matching previous observations of accumulating microbial polymers in melt pond SML[89], and our observation that the >5 μm fraction of melt pond SML was very slimy and difficult to filter compared to water from the bottom of the melt pond or SML from seawater, we assume that decaying *Melosira* exuding polymeric gels could be a major cause for the observed gelatinous SML. In addition, we found that cellular structures resembling bacteria and protists surrounded decaying *Melosira* cell chains which indicates that the turnover of this ubiquitous diatom is feeding the Arctic microbial loop in sea ice habitats (*Figure 11.7*).
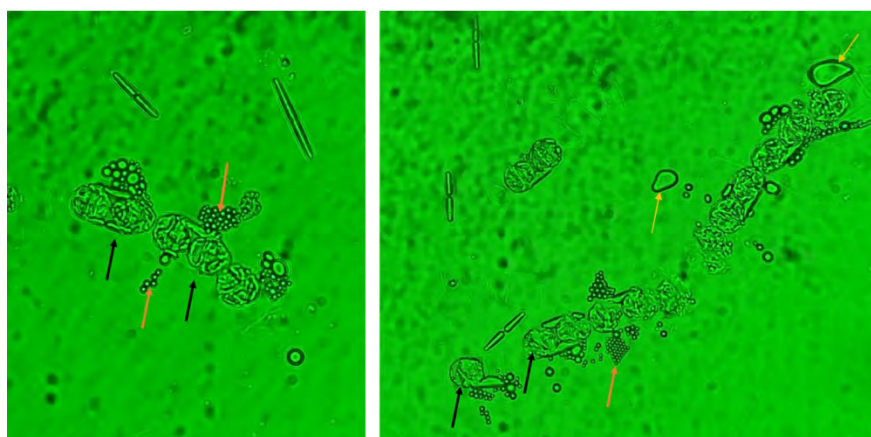
Figure 11.7: Deteriorating Melosira *sp. (black arrows) with surrounding bacterial structures (orange arrows) and likely protist cells (yellow arrows). 100× magnification, sample obtained from an ice hole at Station 18 (sample kindly provided by Hauke Flores). ©Janina Rahlff*

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[89] Galgani L, et al. (2016) Biopolymers from a gelatinous microlayer at the air-sea interface when Artic sea ice melts. Scientific Reports 6: 29465

11.6 Summary of post-cruise analyses and deliverables

Post-cruise analyses

DNA from the CTD and ice station samples will be extracted at SLU (Uppsala) and sequenced and annotated at the Swedish National Genomics Infrastructure (NGI, Uppsala) within the SO21 omics collaboration (*Chapter 7.4*). Project-specific DNA from the SML will be extracted and sequenced at LNU.

Viral density (FCM-virus) will be enumerated either with flow cytometry or epifluorescence microscopy at LNU. Viruses and bacteria will be isolated and purified at LNU. Thereafter the isolated viruses will be sequenced, investigated with transmission electron microscopy (TEM), and virus-host interactions will be studied. This is planned to be a long-term project as we assume these viruses and bacteria are slow-growing and might take weeks to form colonies or plaques.

Regarding the project-specific SML DNA samples we aim for a comparison with unused DNA samples from melt pond microlayer sampled in Arctic summer 2012[90], which would provide insights in how melt pond microbial and viral communities (those associated with hosts) from the SML can differ between years. This would allow us to draw conclusions about the persistence of such communities in the face of climate change-related warming that leads to more ice melting and increased melt pond formation. With mostly seawater SML samples taken during this cruise, we also like to compare which viruses common in seawater SML would be able to establish in melt ponds.

Deliverables

The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[90] Galgani L, et al. (2016) Biopolymers from a gelatinous microlayer at the air-sea interface when Artic sea ice melts. Scientific Reports 6: 29465

12 WP5 (ASAP)

Johan Wikner, Ashish Verma, Dennis Amnebrink

Project title: Adaptive strategies of Arctic prokaryotes at extremely low growth rates – carbon cycling, respiration, biomass, production, gene expression, morphology (ASAP)

12.1   Resources

External project funding: The Kempe foundation (grant number SMK-1854), the Umeå University strategic research programme Ecochange (Dnr 224-919-09), and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for three berths on the SAS-Oden 2021 expedition to Johan Wikner, the Ymer-80 foundation (Decision date 22 February 2021) to Ashish Verma, the Linnaeus University Centre for Ecology and Evolution in Microbial model Systems (EEMiS) to Jarone Pinhassi, and the Swedish Research Council (VR, grant number 2018-02914) to Sun Nyunt Wai. All project participants are listed in *Table 12.1*.

Table 12.1: Overview of all onboard and onshore participants of the WP5 (ASAP) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (berth)	Johan Wikner	johan.wikner@umu.se	PI, WP Leader	UMU, Sweden
Yes (berth)	Ashish Verma	ashish.verma@umu.se	Prokaryotic abundance and morphology	UMU, Sweden
Yes (berth)	Dennis Amnebrink	dennis.amnebrink@lnu.se	Prokaryotic taxonomy and gene expression	LNU, Sweden
No	Jarone Pinhassi	jarone.pinhassi@lnu.se	Prokaryotic ecology	LNU, Sweden
No	Sun Nyunt Wai	sun.nyunt.wai@umu.se	Outer membrane vesicles	UMU, Sweden

12.2   Scientific motivation and specific research questions

Prokaryotes encompass Bacteria and Archaea, two of the three domains in the taxonomic Tree of Life. Prokaryotes comprise the oldest and most numerous organisms on the planet, also having the most rapid growth rates (i.e., short generation times). Their biomass and associated production are therefore considerable despite their small size. Prokaryotes consequently have a profound impact on the cycling and flow of elements in the Ocean ecosystem. WP5 contributes with measurements of the biomass and growth rate of the prokaryotes in the Arctic Ocean. In addition, the plankton respiration will be measured, also to a large extent caused by prokaryotic respiration.

An earlier study of prokaryotic ecology with similar variables have been performed in the Chukchi Sea and Canada Basin[91]. In addition, the SHEBA/JOIS (Surface Heat Budget of the Arctic Ocean/Joint Ocean Ice Study) ice camp experiment also included prokaryotic and respiration measurements, however mainly from one surface depth, drifting through the Canada and Mendeleyev Basins[92]. No studies along the route of the SAS-Oden 2021 expedition in the Amundsen Basin or along the Lomonosov ridge have been reported. Our contribution would complement the earlier studies with measurements in new areas and deeper water layers.

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[91] Kirchman DL, et al. (2009) Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean. Deep Sea Research, Part II 56:1237–1248

[92] Sherr BF, Sherr EB (2003) Community respiration/production and bacterial activity in the upper water column of the central Arctic Ocean. Deep Sea Research, Part I 50:529–542

In a sub-project we will also investigate prokaryotic adaptation to low growth rates and cold temperatures by gene expression and detailed morphological studies by electron microscopy. This aim is to determine the share of maintenance respiration in Arctic prokaryotes, and associated activities. The knowledge provided by WP5 is needed to assess if carbon is mainly oxidized to CO_2 or contributing to the biomass flow in the food web. These alternative processes would result in entirely different effects on the carbon chemistry of the sea water and flow of biomass in the food web.

Plankton respiration and the associated formation of CO_2 have implications for the carbonate system, pH, release of CO_2 to the atmosphere and consumption of oxygen. The extent of prokaryotic carbon demand (production of biomass $C + CO_2$) in the water column will also influence the rate of sedimentation of organic matter to the deep water. Prokaryotic carbon demand is also crucial to understand the fate of terrestrial organic carbon. Our study of maintenance activities will elucidate what adaptive strategies prokaryotes use to maximize ecological fitness in a cold and energy-poor environment. Maintenance activities and growth activities imply different controlling factors for both biomass formation and CO_2 release. From these data prokaryote growth efficiency, per cell respiration and specific growth rate can also be derived.

The specific research questions of the WP5 (ASAP) project are:

(1) How extensive and dependent on ice cover is the spatial variability of plankton respiration, prokaryotic abundance, biodiversity, morphology and activity across the Central Arctic Ocean?
(2) Is temperature sensitivity of plankton respiration in the Arctic Ocean higher than at lower latitudes?
(3) How much of primary produced and terrestrial carbon is metabolized by prokaryotes in the water column?
(4) What is the biomass growth efficiency of Arctic prokaryotes as compared to CO_2 release?
(5) What is the prokaryotic metabolic and morphological adaptation to low growth rate and maintenance activities in the Arctic Ocean?

12.3   Summary of field work performed

SAS Core Parameters: WP5 was responsible for the SAS Core Parameters prokaryotic abundance (by microscopy), biomass growth and plankton respiration. WP5 also contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*), as part of the SO21 omics collaboration (*Chapter 7.4*).

Sampling: Seawater was collected from Niskin bottles on rosette samplers provided by the SPRS (*Figure 12.1 A*) following a CTD cast. Both the stern and bow CTDs were used as appropriate for sampling. Samples were taken from up to nine depths between the ice-seawater interface (ISI, ca. 1.8 m) and 500 m. Water volumes of 3.5 L were consistently booked from the CTD bio from eight depths (10 to 500 m). However, also the CTD omics and the CTD wbat were used several times for better matching collection of the ice-seawater interface sample or providing better working hours after the sampling (6 h of sample processing). Furthermore, seawater was collected by manual deployment of a Ruttner sampler on a rope. An auger (28 cm in diameter) was used to make a hole for the Ruttner sampler used to collect a 3.5 L ice-seawater interface sample (*Figure 12.1 B,C*). A salinometer (brought by WP1) was used to measure temperature and salinity *in situ*. Multiple 12-L Niskin bottles were used for sampling 100 L volumes for outer membrane vesicle studies. The team cooperated in taking larger sample volumes (up to 118 L) while smaller samples of 3.5 L from individual depths was performed by

one team member. The samples were carried to the "Triple Lab" manually for the smaller water volumes and with the aid of a crane operated by the *IB Oden* crew for larger water volumes.

Measurements: Most measurements were made on whole seawater. Cross- or tangential flow filtrations were performed in some sub-projects. Start, end and post-processing of rate measurements required most personnel. Results from plankton and prokaryotic respiration measurements were collected, quality-assured, compiled and partly evaluated during the expedition. The work was divided into equal daily time units as much as possible. Staffing was planned in advanced but adjusted daily as sampling programme was decided by the expedition lead according to the prevailing weather and ice conditions. A number of control studies are performed to secure the accuracy of the measurements. The conversion factor from moles 3H-thymidine assimilated to cells produced was determined in batch culture experiments. The saturation level of added 3H-thymidine tracer to the natural thymidine pool was investigated in dilution experiments.
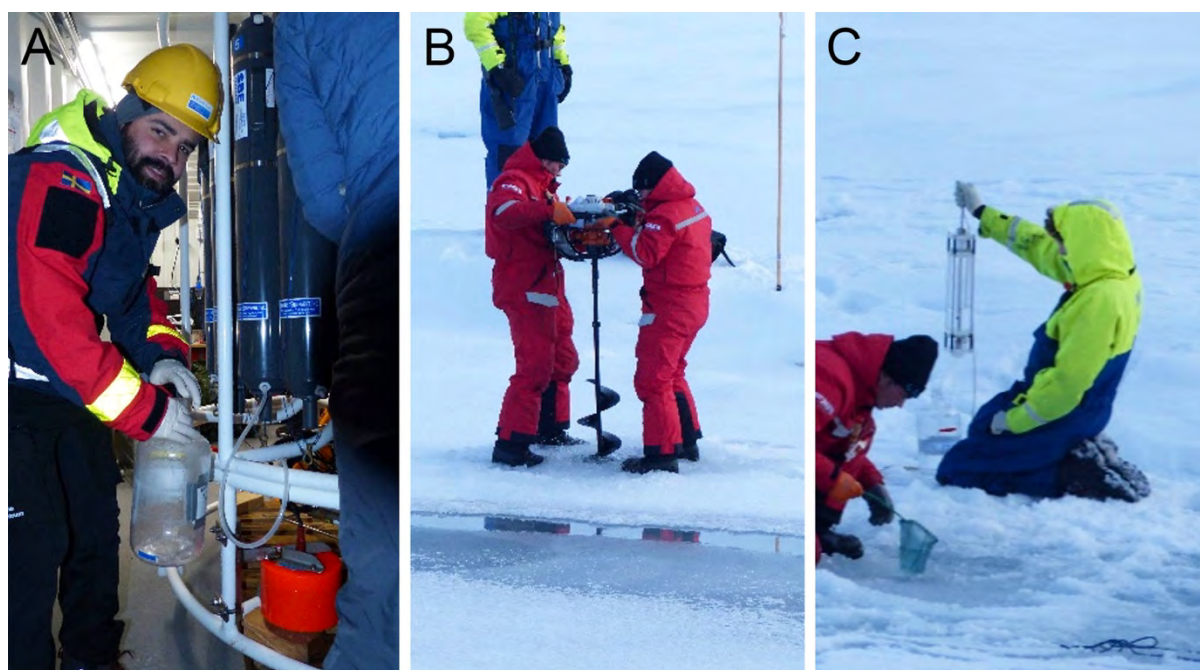
Figure 12.1: Water sampling for WP5. (A) Ashish Verma sampling from Niskin bottles on the CTD rosette. (B) Hauke Flores and Nicole Hildebrandt using an auger to make a hole in the ice for water sampling. (C) Dennis Amnebrink using a small Ruttner sampler for taking a water sample from the ice-seawater interface through the auger hole. ©Johan Wikner

Laboratory equipment used by WP5

Milli-Q water unit: The Milli-Q apparatus (*Figure 12.2A*) was crucial for the quality of analyses. Rinsing with Milli-Q is an important part of cleaning bottles and carboys between samplings. The availability of Milli-Q water was improved by bringing 20 L polycarbonate carboys with a tap. The carboy was recurrently filled when excess Milli-Q water was available. Despite this, Milli-Q water was recurrently in shortage on board because many WPs were in need of it.

Ventilated hood: The ventilated hood (*Figure 12.2B*) was extensively used for handling formaldehyde, glutaraldehyde, acridine orange, trichloroacetic acid and tritiated thymidine labelled material.

Temperature-controlled incubators: These consisted of a water bath with a thermostat and immersion cooler mounted (*Figure 12.2C*). Polyethylene glycol (40%) was used as medium, allowing incubation temperatures down to -1.8°C. This was used to incubate batch cultures of prokaryotes, measurements of prokaryotic biomass growth and incubation of oxygen consumption samples when the OPTOCS incubator had a failure.

Cold centrifuge for microtubes: A cold centrifuge was used to pellet cells precipitated with trichloroacetic acid at 16 000 × g and 4°C (*Figure 12.3 A*).

Optodes: Eight titanium optodes (Aanderaa™ , model 4330) were applied to measure oxygen consumption and respiration by both micro- and picoplankton and specifically picoplankton by 1.2 μm pre-filtration (*Figure 12.3 B*).

Oxygen consumption incubator: A high precision temperature-controlled incubator (OPTOCS incubator) with integrated magnetic stirring and external temperature sensors was used for measuring oxygen consumption (*Figure 12.3 C*).

Vacuum pumps: Two pumps constructed by technicians at the Umeå Marine Sciences Centre provided Vacuum (typically -13 kPa, -0.9 bar) was used to filter water samples and evaporate supernatant after TCA precipitation of cells (*Figure 12.4 A*). Several filter holders in one unit were used to prepare microscopic slides for counting the number of prokaryotes. The vacuum pumps were driven by the laboratory pressurized air system with 12 mm CEJN connections.
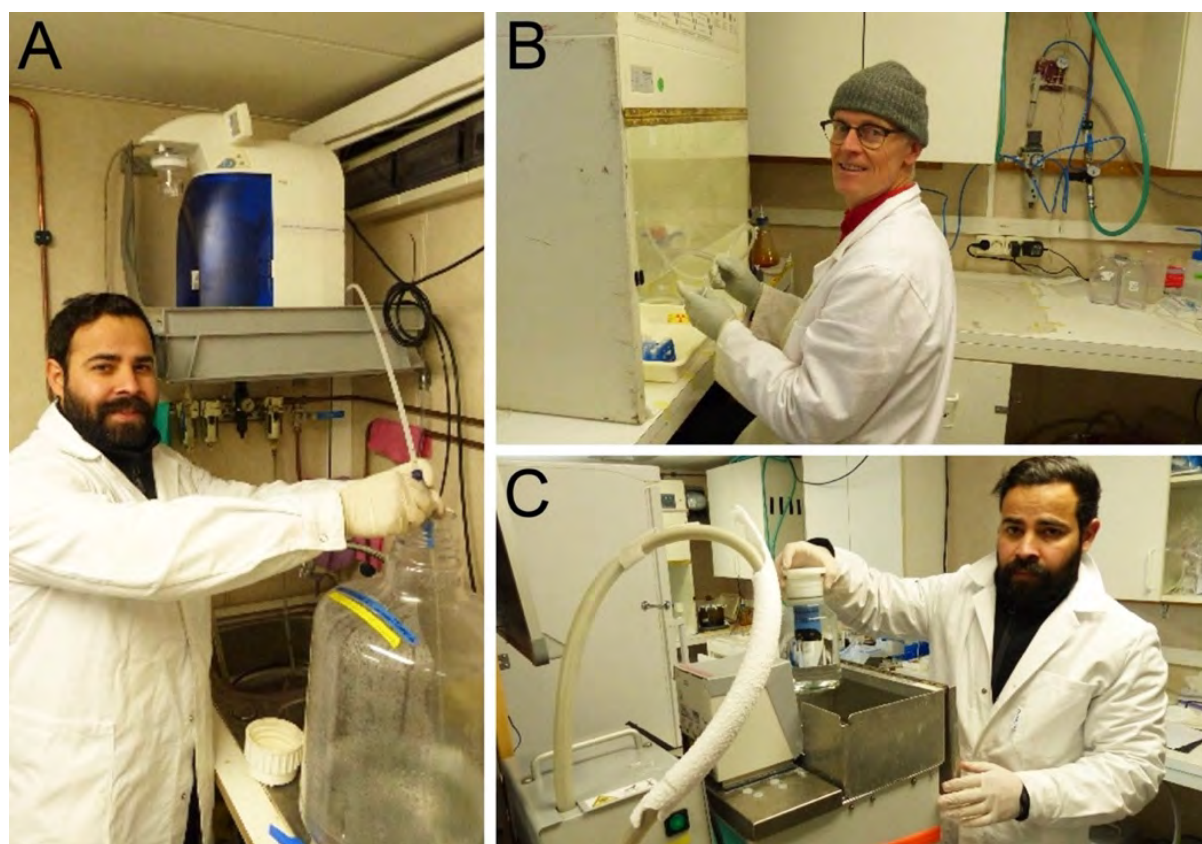
Figure 12.2: Laboratory equipment used by WP5. (A) Ashish Verma rinsing a bottle with Milli-Q water. (B) Johan Wikner aspirating samples for prokaryotic growth in the ventilated hood. (C) Ashish Verma using the thermostat bath with immersion cooler. (A,C) ©Johan Wikner, (B) ©Ashish Verma

Figure 12.3: Laboratory equipment used by WP5. (A) Cold centrifuge used to pellet TCA precipitated cells and concentrate samples for electron microscopy. (B) Optode oxygen sensor mounted in a stopper in a glass bottle. (C) Oxygen consumption incubator. ©Johan Wikner
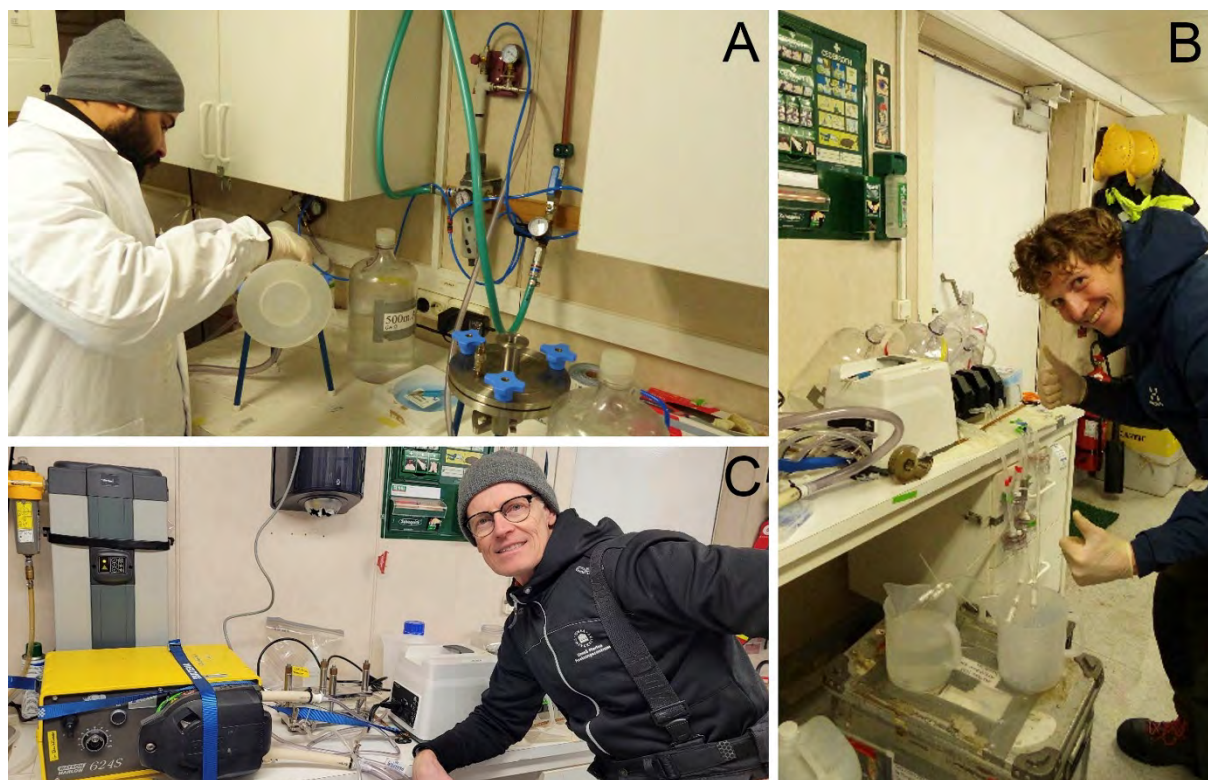
Figure 12.4: Laboratory equipment used by WP5. (A) Vacuum pumps for filtration and aspiration driven by pressurized air. (B) Peristaltic pumps used for collecting nucleic acids. (C) Tangential flow filtration system (metallic) with peristaltic pump (yellow). (A,B) ©Johan Wikner, (C) ©Ashish Verma

Peristaltic pumps: A low flow model used to collect DNA and RNA samples from sea water. A high flow model used for tangential flow filtration (300 kD) of 100 L water samples to less than 0.6 L in ca. three hours *Figure 12.4 B*).

Tangential flow unit: A holder for tangential flow filters with 300 kD cut off was used to concentrate outer membrane vesicles for analysis (*Figure 12.4 C*).

Epifluorescence microscope: The microscope was equipped with 10-100 × objectives and used to count the number of prokaryotes to find the correct sample volume for different depths and the expected low abundances in the Central Arctic Ocean (*Figure 12.5*). This microscope was also used by WP6 and WP9 to analyse the number of phytoplankton cells on chlorophyll-*a* filters and details of foraminifers, respectively.
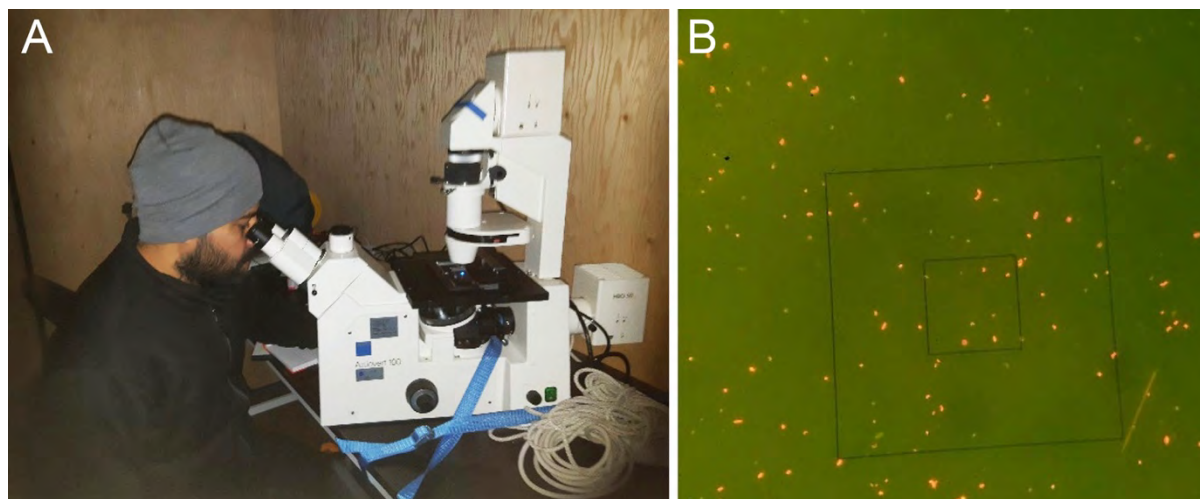
Figure 12.5: Epifluorescence microscopy. (A) Ashish Verma working at the microscope. (B) Arctic prokaryotes at 1000× magnification. ©Johan Wikner

Table 12.2: Summary of methods used by the WP5 (ASAP) project.

Parameter	Name
Prokaryotic abundance	SO21 SOP: prokaryotes (*Chapter 24*)
Prokaryotic biomass growth	SO21 SOP: prokaryotes (*Chapter 24*)
Plankton respiration	SO21 SOP: prokaryotes (*Chapter 24*)
Prokaryotic respiration	1.2 µm pre-filtered plankton respiration
Prokaryotic taxonomy	Prokaryotic taxonomy
Prokaryotic gene expression	RNA collection and sequencing
Prokaryotic morphology	Epifluorescence image analysis, scanning and transmission electron microscopy
Total dissolved N and P	Standard procedure[93]
Outer membrane vesicles	Electron microscopy, protein analysis, nucleic acid analysis[94]
Culturing of prokaryotes	Prokaryote isolation

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[93] Grasshoff K, et al., eds (1999) Methods of Seawater Analysis, Third Edition. Wiley
[94] Biller SJ, et al. (2014) Bacterial Vesicles in Marine Ecosystems. Science 343(6167):183–186

12.4 Summary of metadata collected

Collection of measurements and samples succeeded according to the planned sampling programme. A few samples for prokaryotic abundance were counted in the epifluorescence microscope to estimate a suitable sampling volume for slide preparation. These preliminary analyses showed that prokaryotic cells can be detected not only in the surface layer, but also in mesopelagic samples. Plankton and prokaryotic respiration could be measured above 50 m, but for samples from 100 m and deeper values were below the published detection limit of the method. Correction of data for systematic background trends to allow detection from 100 m and below will be investigated but is currently uncertain.

The metadata collected by WP5 (ASAP) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP5_ASAP" in the SND data repository and summarised in *Tables 12.3 and 12.4*.

Table 12.3: Overview of all measurements/samples collected by WP5 (ASAP) during the SAS-Oden 2021 expedition.

Parameter	Category	Total number of measurements/samples
Prokaryotic abundance	SAS Core Parameter	182
Prokaryotic biomass growth	SAS Core Parameter	749
Plankton respiration	SAS Core Parameter	96
Prokaryotic respiration	Project-specific	20
Prokaryotic taxonomy	Project-specific	20
Prokaryotic gene expression	Project-specific	322
Prokaryotic morphology	Project-specific	40
Total dissolved nitrogen	Project-specific	45
Total dissolved phosphorus	Project-specific	45
Total dissolved organic carbon	Project-specific	45
Outer membrane vesicles	Project-specific	5
Culturing of prokaryotes	Project-specific	58

Table 12.4: Overview of the water depths and number of stations from which the WP5 samples were taken during the SAS-Oden 2021 expedition. The sub-project PROMAC sampling was performed with duplicate Niskin bottles at each depth. ISI = ice-seawater interface

Depth (m)	SAS Core Parameters	Sub-Project PROMAC	Sub-project Respiration Q10	Sub-project TCF	Sub-project [3]HT dilution
ISI	8				
10	13	5	2	1	
ChlMax	13	5			
30	13			1	1
50	13				1
100	13				
200	13				
TempMax	13	5			1
500	13	5		1	

12.5 Summary of preliminary results

Prokaryotic abundance: The observed abundances of prokaryotic cells in the Central Arctic Ocean (*Figure 12.6*) were similar to those reported from an oligotrophic site in the Atlantic equatorial region with temperatures of 20°C[95]. The distribution with depth was typical for aquatic systems with higher values in the upper part of the water column. This occurred despite lower temperatures in the surface water, contrary to other aquatic environments. The standard error of cell counting showed that also the low abundances of prokaryotes in the mesopelagic zone are detectable when using a water sample volume of 25 mL.
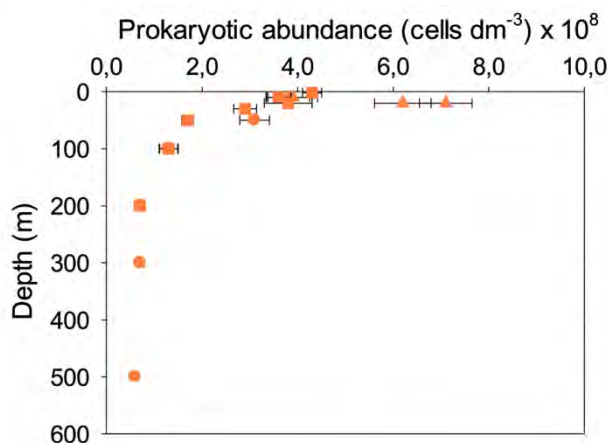
Figure 12.6: Example of the preliminary depth distribution of prokaryotic abundance composed from a few initial sampling profiles made during the SAS-Oden 2021 expedition. Error bars show 2× the standard error of the variation between microscope counting fields on the filter. Circles show samples from a SAS CTD sampling, squares from a SAS ice station sampling and triangles from a PROMAC sampling. ©Johan Wikner

Plankton respiration: Preliminary results of plankton respiration measurements in the CAO (*Figure 12.7*) were similar to published values from the north Atlantic Ocean[96]. Despite the lower temperature in the CAO, typically -1.7°C in the upper 100 m, plankton respiration was higher than in deeper water and slightly higher temperatures at the water-column temperature maximum (TempMax). Maximum plankton respiration occurred both in the ice-seawater interface, 10 m of depth and the chlorophyll maximum (ChlMax, depth depending on station investigated). Two experiments were performed using a temperature range of -1.7 to 0.6°C. The preliminary results suggested a negative effect of temperature in one case, while no effect of temperature on plankton respiration could be demonstrated in the other case.

Prokaryotic respiration: Apart from the depth profiles in the SAS-subproject, bacterial respiration data for analysing the level of maintenance respiration and associated activities were collected. Most values were above the detection limit. The depth profiles show a similar distribution as for plankton respiration but at a lower level.

Prokaryotic taxonomy and gene expression: Within the PROMAC sub-project, samples were collected for metagenomics and metatranscriptomics of the prokaryotic community from 1.2 µm pre-filtered samples (collected fraction 0.2-1.2 µm). These samples will be sequenced and bioinformatics analysed with bioinformatics in addition to – and for comparison with – the samples of the SO21 omics collaboration (collected fraction 0.2-200 µm).

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[95] Dufour PH, Torreton JP (1996) Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical north-eastern Atlantic Ocean. Deep Sea Research, Part I 43:1305–1320

[96] Reinthaler T, et al. (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnology & Oceanography 51:1262–1273

Thymidine conversion factor and thymidine isotope dilution: Three control experiments were conducted for estimating the thymidine conversion factor, and three control experiments were conducted for estimating the ³H-thymidine saturation concentration. These samples will be analysed as evaluated back in our laboratory.
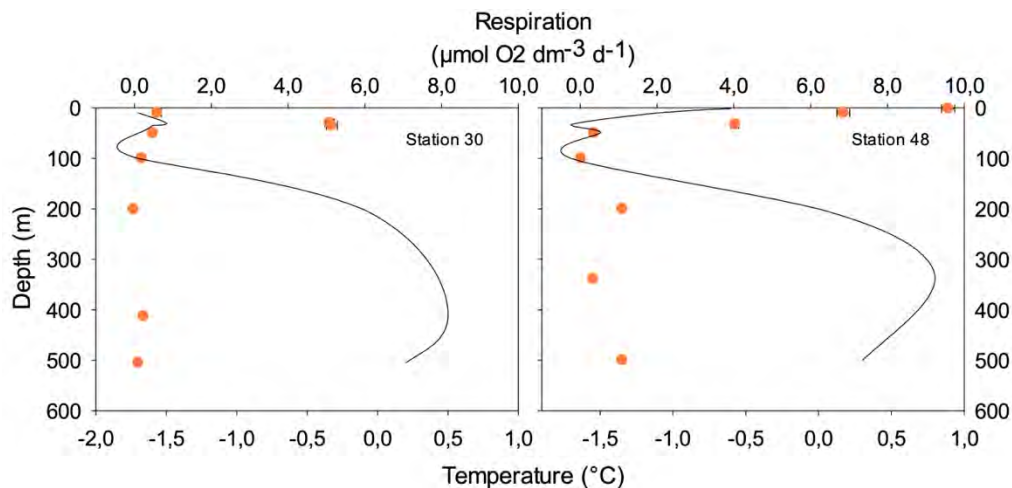
Figure 12.7: Plankton respiration (orange circles) and temperature (black line) in two depth profiles from the SAS-Oden 2021 expedition. 95% confidence intervals for the plankton respiration are hidden by the symbols in most cases. Note that duplicate sample bottles from ca. 30 m of depth on Station 30 were analysed (5 μmol O_2 dm^{-3} d^{-1}). ©Johan Wikner

12.6 Summary of post-cruise analyses and deliverables

Post-cruise analyses:
(1) Background corrections and calculations of plankton and prokaryotic respiration (Johan Wikner)
(2) Scintillation counting, quality assurance and calculations for prokaryotic biomass growth (Johan Wikner)
(3) Slide preparation, microscopic image analysis, quality assurance and calculations of prokaryotic abundance (Ashish Verma)
(4) Grid preparations, image analyses, quality assurance and calculations of prokaryotic morphology (Ashish Verma)
(5) Analysis of outer membrane vesicles (Sun Nyunt Wai)
(6) Sequencing and bioinformatics of the PROMAC samples (SciLifeLab Sweden, Dennis Amnebrink and Jarone Pinhassi)
(7) Nutrient analyses (Göran Bergqvist, Umeå Marine Sciences Centre)
(8) Culturing of prokaryotes (Dennis Amnebrink)

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals. At least three publications are planned: (1) Prokaryote ecology and its variation with depth and over the covered transect, (2) Carbon budget for the plankton food web in the investigated area and season. This would encompass flow of carbon through each trophic level, potential contribution from terrestrial carbon, mineralization to CO_2 and share of sedimentation to the seafloor, (3) Growth efficiency of the prokaryotes, extent of maintenance respiration and associated effects on cell morphology and gene expression.

13   WP6 (PICO)

Hanna Farnelid, Christien Laber

Project title: Ecosystem contributions of smallest primary producers in a changing Arctic Ocean (PICO)

13.1   Resources

External project funding: Anna-Greta and Holger Crafoord Foundation (grant number 2020-0881), the Linnaeus University Centre for Ecology and Evolution in Microbial model Systems (EEMiS), and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for two berths on the SAS-Oden 2021 expedition to Hanna Farnelid. All project participants are listed in *Table 13.1*.

Table 13.1: Overview of all onboard and onshore participants of the WP6 (PICO) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (berth)	Hanna Farnelid	hanna.farnelid@lnu.se	PI, WP Leader	LNU, Sweden
Yes (berth)	Christien Laber	christien.laber@lnu.se	Experiments and sampling	LNU, Sweden
Yes (WP7)	Lisa Winberg von Friesen	lisa.vonfriesen@bio.ku.dk	Chlorophyll-*a* analyses	UCPH, Denmark
Yes (WP10)	Marcus Sundbom	marcus.sundbom@su.se	Nutrient analyses experiments	SU, Sweden
No	Elin Lindehoff	elin.lindehoff@lnu.se	Uptake experiments	LNU, Sweden
No	Laura Bas Conn	laura.basconn@lnu.se	Lab work, DNA extractions, PCR	LNU, Sweden

13.2   Scientific motivation and specific research questions

Picophytoplankton (<2 µm) are the smallest primary producers in the world's oceans and contribute significantly to autotrophic carbon biomass and primary production globally. This research project investigates the diversity, controls and distribution patterns of picophytoplankton (picocyanobacteria and picoeukaryotes) species in the Central Arctic Ocean (CAO) ecosystem. The Arctic Ocean is one of the most rapidly changing seas on Earth. In the Arctic Ocean, picophytoplankton, mainly picoeukaryotes (e.g., *Micromonas* spp.), are thought to dominate the pelagic phytoplankton communities (60-90% of chlorophyll-*a*[97]), thus being largely responsible for carbon fixation and driving the microbial food chain.

In the Arctic Ocean, a community composition shift from larger phytoplankton to smaller and from picoeukaryotes to picocyanobacteria has been hypothesised. While the general understanding is that low temperatures limit picocyanobacterial distribution, several studies have reported that the cyanobacterium *Synechococcus* expands into the Arctic Ocean[98,99,100] and may eventually compete with or replace photosynthetic picoeukaryote populations. Such scenarios are likely to affect food

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[97] Metfies K, et al. (2016) Biogeography and photosynthetic biomass of arctic marine pico-eukaroytes during summer of the record sea ice minimum 2012. PLoS One 11:1–20

[98] Cottrell MT, Kirchman DL (2009) Photoheterotrophic microbes in the Arctic Ocean in summer and winter. Applied and Environmental Microbiology 75:4958–4966

[99] Nelson RJ, et al. (2014) Biodiversity and biogeography of the lower trophic taxa of the Pacific Arctic Region: sensitivities to climate change. In: Grebmeier JM, Maslowski W (Eds) The Pacific Arctic Region: Ecosystem Status and Trends in a Rapidly Changing Environment, pp. 269–336

[100] Paulsen ML, et al. (2016) *Synechococcus* in the Atlantic Gateway to the Arctic Ocean. Frontiers in Marine Science 3:191

quality, trophic processes and important ecosystem services[101] directly linked with the function of the biological pump. Whether *Synechococcus* is endemic to the Arctic or if it is transported by warmer water masses has not yet been resolved. A holistic view of the presence and abundance of picophytoplankton species in the Arctic Ocean is urgently needed to gain an understanding of the environmental boundaries governing their distribution.

Despite the significance of picophytoplankton in the Arctic Ocean, knowledge about these organisms forming the base of the microbial food web, is scarce. Studies of cold-adapted strains of *Synechococcus* are few[102,103,104], precluding an understanding of their physiological characteristics. Quantitative rate measurements controlling picophytoplankton abundances, needed for integrating them into ecosystem based models, are generally missing. Observations from the CAO, which has until recently been largely covered by sea ice, are rare[105,106,107]. Ultimately, studies integrating environmental factors as well as multiple trophic levels investigating grazing and viral controls on picophytoplankton populations may provide insights into both the abiotic and biotic factors controlling these significant but largely understudied groups.

A reoccurring theme over the last decade is reports of organisms from the Arctic Ocean previously not thought to occur there (e.g., diazotrophs and *Synechococcus*). A rapidly increasing rate of Atlantification has been suggested as a potential contributor through advection and potentially providing suitable conditions for more temperate organisms to prevail further north. By covering important parts of the north-flowing currents (Norwegian coastal current and the main Atlantic water inflow of the Arctic Ocean), this project provided a unique opportunity to spatially follow the Atlantic water mass during summer and autumn.

The specific research questions of the WP6 (PICO) project are:

(1) What is the biomass, distribution and composition of picophytoplankton in the CAO? (flow cytometry, chlorophyll-*a* and SO21 omics)
(2) How do grazing and viral lysis influence growth of individual picophytoplankton groups? (dilution experiments)
(3) Observed at a single cell level, how is metabolic activity and competition between populations influenced by available nitrogen species? (uptake experiments)
(4) How does bacterial and phytoplankton community composition change along a transect from the northern Atlantic Ocean to the CAO? (underway-sampling)

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[101] Vincent WF (2010) Microbial ecosystem responses to rapid climate change in the Arctic. The ISME Journal 4:1089–1091

[102] Powell LM, et al. (2005) Ecology of a novel *Synechococcus* clade occurring in dense populations in saline Antarctic lakes. Marine Ecology Progress Series 291:65–80

[103] Vincent WF (2010) Microbial ecosystem responses to rapid climate change in the Arctic. The ISME Journal 4:1089–1091

[104] Tang J et al. (2019) Complete genome sequence and comparative analysis of *Synechococcus* sp. CS-601 (SynAce01), a cold-adapted cyanobacterium from an oligotrophic Antarctic habitat. International Journal of Molecular Sciences 20:1–17

[105] Gradinger R, Lenz J (1995) Seasonal occurrence of picocyanobacteria in the Greenland Sea and central Arctic Ocean. Polar Biology 15:447–452

[106] Sherr EB, et al. (2003) Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. Deep Sea Research, Part I 50:557–571

[107] Zhang F, et al. (2015) Dominance of picophytoplankton in the newly open surface water of the central Arctic Ocean. Polar Biology 38:1081–1089

13.3   Summary of field work performed

SAS Core Parameters: WP6 was responsible for the SAS Core Parameters abundance and chlorophyll-*a* concentrations (Chl*a*) of autotrophic picophytoplankton. Flow cytometry (FCM) samples were collected according to "SO21 SOP: flow cytometry" (*Chapter 24*) in parallel with FCM samples for bacterial (WP2), viral (WP4), and phytoplankton (WP7) abundances. Chlorophyll-*a* samples of the 0.3-2 μm size fraction were collected on Advantec® glass fibre filters and measured on board (in duplicate) in parallel with duplicate Chl*a* samples of the size fraction 2-200 μm (WP7), and analysed according to "SO21 SOP: chlorophyll-*a*" (*Chapter 24*). WP6 also contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*).

Incubators: Deck-board incubators (*Figure 13.1*) were used for short (5 hour) and long (72 hour) incubation experiments. The incubators were designed to closely recreate the *in-situ* environment of the experimental sea water conditions. Hoses continuously pump seawater from 8 m into the incubators to keep temperatures similar to water-column conditions and to prevent freezing. Shading of the incubators also limited the light environment to levels observed in the surface ocean.

Figure 13.1: Hanna Farnelid and Christien Laber sampling incubated seawater from the deck-board incubators during the SAS-Oden 2021 expedition. ©Lisa Winberg von Friesen

Dilution experiments: The dilution experiments were carried out to explore mortality rates (grazing and viral lysis) of CAO picophytoplankton. These experiments were conducted at three stations during the expedition (Dil1, SO21 station 8; Dil2, SO21 station 26; Dil3, SO21 station 50; *Figure 1.1*). The sampling depth was at the chlorophyll maximum (ChlMax) as determined by a fluorometer on the CTD. The experimental design was adapted from Cram et al. (2016)[108]. The setup consisted of three dilution levels (0%, 50%, 90%). The experiments were sampled at three time points, at the start of the experiment, after 24 hours and after 72 hours (T0, T24, T72). All treatments were

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[108] Cram JA, et al. (2016) Dilution reveals how viral lysis and grazing shape microbial communities. Limnology & Oceanography 61:889–905

performed in triplicates. The community was diluted with bacteria-free water (0.2 μm-filtered) and virus-free water (100 kDa TFF filtered). Assuming that the grazing pressure was equal in the latter two treatments, the difference in mortality rates between the two treatments will be due to viral impacts on the picophytoplankton populations. The experiments were conducted in 4.7-L bottles which were incubated in deck-board incubators with running seawater flow from 8 m depth and PAR reduction of 55% situated on the front deck to minimize shading from the ship (*Figure 13.1*). Light and temperature were recorded using HOBO loggers. From each bottle, samples for HPLC, flow cytometry (FCM), epifluorescence microscopy, Lugol-conserved community composition, nutrients, POC/PON, POP, grazers and DNA were collected following the respective SO21 SOPs (*Chapter 24*) but with adjustments of volumes for the water budget of the sample bottle.

Nutrient-uptake incubations: The nutrient-uptake incubations were carried out to explore single-cell uptake rates by CAO picophytoplankton of different N substrates labelled with stable isotopes. The sampling depth was at the chlorophyll maximum (ChlMax) as determined by a fluorometer on the CTD. Dual ^{13}C-^{15}N incubations were performed to investigate nitrogen source preference and single cell uptake rates. Dissolved inorganic ^{13}C (DI^{13}C, NaHCO$_3$; 98% Sigma-Aldrich) in combination with either $^{15}NH_4^+$ (ammonium-^{15}N chloride; ≥98 atom% ^{15}N, Sigma-Aldrich) or $^{15}NO_3^-$ (sodium nitrate ^{15}N; 98% Cambridge Isotope Laboratories NLM-157-5) or dual labelled urea (urea-^{13}C,$^{15}N_2$; 99 atom% ^{13}C, 98 atom% ^{15}N, Sigma-Aldrich) or dual labelled amino acids (cell-free amino acid mixture - ^{13}C,^{15}N amino acid; Sigma 767964-1EA) were added. (Additions targeted ≤10% of ambient concentrations of DIC, NO$_3$, NH$_4$, urea and amino acids. The samples will later be analysed by IRMS (isotope-ratio mass spectrometry). The experiments were conducted six times during the expedition (UP1-UP6). The experiments were sampled at two time points, at the start of the experiment and after 5 hours (T0 and T5). T0 was represented by five replicated bottles without additions. All treatments were incubated in three replicate 2.3-L bottles in deck-board incubators with running seawater flow from 8 m depth and PAR reduction of 55% situated on the front deck to minimize shading from the ship (*Figure 13.1*). UP1, UP3, UP5 were run at the same time as the Dilution experiments (Dil1, Dil2, Dil3).

Underway-sampling: Bacterial and phytoplankton community composition was studied along a transect between Øresund and the Barents Sea. During transit between southern Sweden (Øresund) and the study area in the Central Arctic Ocean, *IB Oden* covered vastly different marine environments during two seasons. By sampling from the continuous seawater intake of the ship, bacterial and phytoplankton community changes could be followed over environmental gradients with a large spatial coverage. There is limited knowledge about how especially microbial, but also phytoplankton communities, gradually (or abruptly) change from the south into the Arctic Ocean. During the transit of *IB Oden* north in July, sampling took place every 8 hours, starting from outside of the exclusive economic zone of Norway (200 nm from Lofoten) and until reaching the sea ice north of Svalbard. During the transit south in September, sampling took place three times per day (sunrise, mid-day and sunset) starting during the last day in the sea ice north of Svalbard until southern Sweden (permit number: Norwegian Petroleum Directorate 802/2021). Samples were collected for DNA and RNA in triplicate, nutrients in duplicate and flow cytometry in five replicates according to "SO21 SOP: flow cytometry" (*Chapter 24*) at 37 stations. This is a collaboration with WP2 (MIME).

Isolation of picophytoplankton: To isolate cryophilic picophytoplankton strains from the CAO, batch incubations and serial dilutions were started using water from the ChlMax collected by the CTD. Seawater was pre-filtered (2.0 μm) to remove larger phytoplankton. Nutrients (NO$_3$ and PO$_4$), trace metals and vitamins were added to TFF (100 kDa) filtered seawater. The cultures were incubated in light incubators at 4°C shaded by two layers of mosquito net (approximately 30 μmol photons s^{-1} m^{-2} equivalent to between 30-40 m of depth).

Biological N₂-fixation at the chlorophyll maximum

In parallel with nutrient uptake incubations 2-6, larger volume stable isotope incubations (4.7 L) targeting biological N$_2$-fixation were performed with the same ChlMax water. These incubations are a collaboration with WP2, which is the SO21 project that is specifically targeting biological N$_2$-fixation. These incubations enable assessment of the coupling between phytoplankton and diazotrophs at the ChlMax. The incubations were performed five times during the expedition in triplicate (with ^{15}N$_2$ gas and NaH^{13}CO$_3$). At four of these five occasions (Up3-6), a second treatment with addition of organic carbon was performed in triplicate to investigate regulation of heterotrophic diazotrophs.

The N$_2$-fixation incubations took place in the on-deck incubators with continuous flow-through of seawater (intake at ~8 m) and an adjusted light level to 55% during 24 hours. Size-fractionated DNA/RNA samples were collected upon initiation of each incubation from where nifH amplicon sequencing will be performed to identify present and active diazotrophs. Additionally, samples from the two last incubations were collected for potential nanoSIMS analysis. The data generated by these incubations improve the resolution of biological N$_2$-fixation measurements in this previously under-sampled region of the Arctic Ocean, with a specific focus on the coupling between phytoplankton and diazotrophs at the ChlMax. The samples will be analysed at the University of Copenhagen and this is a collaboration with WP2 (MIME).

13.4   Summary of metadata collected

The metadata collected by WP6 (PICO) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP6_PICO" in the SND data repository and summarised in *Table 13.2*.

*Table 13.2: Overview of all measurements/samples collected by WP6 (PICO) during the SAS-Oden 2021 expedition. * = Collaboration with WP2 (MIME)*

Parameter	Number of stations / experiments	Category	Total number of measurements/samples
Cell density autotrophic picophytoplankton (FCM-pico)	18 SAS stations 12 depths (CTD bio)	SAS Core	216 (in triplicate)
Cell density autotrophic ice picophytoplankton (FCM-pico)	16 SAS stations 8 ice habitats	SAS Core	136 (in triplicate)
Chlorophyll-*a* (Chl*a*-pico) picophytoplankton (0.3-2 µm)	18 SAS stations 12 depths (CTD bio)	SAS Core	216 (in duplicate)
Chlorophyll-*a* (Chl*a*-pico) ice picophytoplankton (0.3-2 µm)	16 SAS Ice Stations 8 ice habitats	SAS Core	123 (in duplicate)
DIC	6 uptake experiments	Project-specific	90
DOC	6 uptake experiments	Project-specific	30
POC/PON	6 uptake experiments	Project-specific	30
POP	6 uptake experiments	Project-specific	30
IRMS (^{13}C and ^{15}N)	6 uptake experiments	Project-specific	95
Nutrients	6 uptake experiments	Project-specific	30
Urea/DFAA	6 uptake experiments	Project-specific	30
FCM	6 uptake experiments	Project-specific	180
Chl*a*	6 uptake experiments	Project-specific	24
Lugol	6 uptake experiments	Project-specific	30
DNA	6 uptake experiments	Project-specific	26
NanoSIMS	6 uptake experiments	Project-specific	101

POC/PON	3 dilution experiments	Project-specific	99
POP	3 dilution experiments	Project-specific	99
Nutrients	3 dilution experiments	Project-specific	99
FCM	3 dilution experiments	Project-specific	99
Epifluorescence microscopy	3 dilution experiments	Project-specific	99
Lugol	3 dilution experiments	Project-specific	99
HPLC pigments	3 dilution experiments	Project-specific	99
Grazers	3 dilution experiments	Project-specific	99
DNA	3 dilution experiments	Project-specific	99
IRMS_N2fix	5 N_2-fixation incubations *	Project-specific	36
DNA/RNA_N2fix	5 N_2-fixation incubations *	Project-specific	10
MIMS_N2fix	5 N_2-fixation incubations *	Project-specific	56
NanoSIMS_N2fix	5 N_2-fixation incubations *	Project-specific	14
Nutrients	37 underway stations *	Project-specific	74
FCM	37 underway stations *	Project-specific	185
DNA/RNA	37 underway stations *	Project-specific	167
Isolations batch incubations	3 SAS stations	Project-specific	6
Isolations dilution series	3 SAS stations	Project-specific	6

13.5   Summary of preliminary results

No preliminary results were obtained during the expedition.

13.6   Summary of post-cruise analyses and deliverables

Post-cruise analyses:

The FCM-pico samples will be analysed at LNU, including discrimination of phytoplankton groups (picocyanobacteria, picoeukaryotes, heterotrophic nanoflagellates), based on cell size and fluorescence properties. The Chl*a*-pico data is complete and will be quality-controlled and visualized at LNU after the expedition.

The project-specific DNA and RNA analyses will primarily target diazotrophs (*nifH* amplicon sequencing, qPCR of DNA and RNA targeting specific diazotrophs), *Synechococcus* and chosen picoeukaryotes.

DNA samples from the experiments will be extracted at LNU and 16S and 18S rDNA gene amplicons will be sequenced. RNA and DNA samples collected from the transit will be processed at the University of Copenhagen (Denmark). Cultures for isolations will be kept at LNU.

For the dilution experiments, grazing and influence of viral lysis will be calculated from the cell abundances measured using flow cytometry. HPLC and Lugol samples will be analysed with the same procedure as samples collected in WP7. POC/PON and POP analyses will be performed at LNU. The IRMS samples will be dried and packed at LNU (not acid-fumed), and sent to the UC Davis Stable Isotope Facility, University of California (USA) for analysis. NanoSIMS facility will be analysed at the Cameca NanoSIMS 50L Facility in Gothenburg. Nutrients for all experiments have been analysed onboard by Marcus Sundström.

Deliverables:

The project will produce scientific results contributing to the holistic approach of the SAS program. Specifically, WP6 will deliver:

- Density maps illustrating the abundance, community composition and diversity of picophytoplankton communities of the CAO and isolates of picophytoplankton species. These results allow for direct physiological characterization and investigation of abiotic factors controlling picophytoplankton populations and individual strains in the CAO.
- Rates of picophytoplankton growth, nanoflagellate grazing, and viral lysis. Such parameters are essential for integrating this organism group into ecosystem-based models.
- Metabolic activity and strategies, including carbon and nitrogen utilization rates and preferences of inorganic and organic sources on a single-cell level. These findings will be necessary to evaluate the success of picophytoplankton groups in a changing Arctic Ocean.

The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals. A short movie featuring the project will be produced in collaboration with the Communication Department of the Linnaeus University, Kalmar (Sweden).

14 WP7 (PHYTO)

Hanna Farnelid, Pauline Snoeijs-Leijonmalm, Clara Pérez Martínez, Lisa Winberg von Friesen

Project title: Responses of pelagic and sympagic primary producers to climate change in the Central Arctic Ocean (PHYTO)

14.1 Resources

External project funding: Anna-Greta and Holger Crafoord Foundation (grant number 2020-0881) and the Linnaeus University Centre for Ecology and Evolution in Microbial model Systems (EEMiS) to Hanna Farnelid, and the Swedish Research Council (VR, grant number 2018-04685) and the Swedish Research Council for Sustainable Development (FORMAS, grant number 2018-00509) to Pauline Snoeijs-Leijonmalm, and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for two berths on the SAS-Oden 2021 expedition to Hanna Farnelid and Pauline Snoeijs-Leijonmalm, respectively. All project participants are listed in *Table 14.1*.

Table 14.1: Overview of all onboard and onshore participants of the WP7 (PHYTO) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (WP6)	Hanna Farnelid	hanna.farnelid@lnu.se	Co-PI, WP Leader	LNU, Sweden
Yes (WP1)	Pauline Snoeijs-Leijonmalm	pauline.snoeijs-leijonmalm@su.se	Co-PI	SU, Sweden
Yes (berth)	Clara Pérez Martínez *	clara.perezmartinez@lnu.se	Primary production	SU, Sweden
Yes (berth)	Lisa Winberg von Friesen	lisa.vonfriesen@bio.ku.dk	Chlorophyll-*a* analyses	UCPH, Denmark
Yes (WP6)	Christien Laber	christien.laber@lnu.se	Chlorophyll-*a* analyses	LNU, Sweden
Yes (WP2)	Javier Vargas Calle	javier.vargas@slu.se	HPLC, POC, PON, POP	SLU, Sweden
Yes (WP10)	Marcus Sundbom	marcus.sundbom@su.se	Nutrient data	SU, Sweden
No	Laura Bas Conn	laura.basconn@lnu.se	Flow cytometry	LNU, Sweden
No	Sten-Åke Wängberg	sten-ake.wangberg@marine.gu.se	Advisory	GU, Sweden
No	Bengt Karlsson	bengt.karlson@smhi.se	Advisory	SMHI, Sweden
No	Anna Wranne	anna.wranne@smhi.se	Fluorometer sensor	SMHI, Sweden

* Employed at SU for the expedition, the regular affiliation of Clara Pérez Martínez is LNU (Sweden)

14.2 Scientific motivation and specific research questions

The PHYTO project will produce knowledge on the incorporation of inorganic carbon into organic carbon in the Central Arctic Ocean (CAO), and contribute to the understanding of the biological carbon pump that transports carbon from the air to the deep sea, a process that is important in biogeochemical and climate models. The CAO is annually experiencing rapid sea-ice thinning and loss that influences light penetration depth[109], oceanic CO_2 intake, and nutrient supplies[110] in the water column and therefore primary production both ice-associated (sympagic) and in the underlying water column (pelagic)[111]. Large parts of the CAO have limited or no previous measurements of algal dynamics within these habitats, hindering our effort to characterise productivity across the region and

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[109] Nicolaus M, et al. (2012) Changes in Arctic sea ice result in increasing light transmittance and absorption. Geophysical Research Letters 39:L24501

[110] Tremblay JÉ, et al. (2015) Global and regional drivers of nutrient supply, primary production and CO_2 drawdown in the changing Arctic Ocean. Progress in Oceanography 139:171-196

[111] Ardyna M, Arrigo KR (2020) Phytoplankton dynamics in a changing Arctic Ocean. Nature Climate Change 10:892-903

anticipate new dynamics in productivity that will accompany the changing climate. Along a large part of the SAS-Oden 2021 expedition route no previous on-site measurements of phytoplankton biomass and primary production and their relationship to environmental conditions exist[112]. Analysis of community composition will provide information on how far North-Atlantic phytoplankton species expand northward into the CAO today and provide a baseline for further climate change effects.

The specific research questions of the WP7 (PHYTO) project are:

(1) How does community composition, abundance, biomass and production of primary producers in sea ice habitats and the water column (0 - 500 m) vary along the SAS Oden 2021 expedition route?
(2) How does meltwater from sea ice affect primary producers in sea ice habitats and the surface seawater?

14.3   Summary of field work performed

SAS Core Parameters: WP7 was responsible for the SAS Core Parameters phytoplankton cell abundance, chlorophyll-*a* concentration (Chl*a*), particulate nutrients (POC, PON, POP), dissolved inorganic and organic nutrients of ice habitats, δ ^{13}C, δ ^{15}N, fat-soluble algal pigments (chlorophylls and carotenoids), taxonomic composition, and primary production. All parameters measured by WP7 are SAS Core Parameters. WP7 also contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*).

Water-column sampling: Seawater samples were taken at 18 stations along the expedition route (*Figure 1.1*) from the 12 standard CTD bio depths through the photic zone down to 500 m (10 m, ChlMax, 30 m, 40 m, 50 m, 75 m, 100 m, 125 m, 150 m, 200 m, TempMax, 500 m), two Niskin bottles per depth on the stern CTD bio (*Chapter 6.3*). The depth of the chlorophyll maximum (ChlMax) was determined by a fluorometer on the CTD bio and varied between 12 and 45 m of depth. The depth of the temperature maximum (TempMax) in the Atlantic water layer was determined by dual temperature sensors on the CTD bio and varied between 200 and 411 m of depth.

Ice-habitat sampling: Samples from sea ice, brackish brine, ice-seawater interface, melt ponds, and snow were taken at the 16 SAS Ice Stations along the expedition route (*Chapter 6.7*). All 16 SAS Ice Stations overlapped with a CTD bio for water-column sampling.

The following samples and measurements were collected from these environments:

Flow cytometry (FCM): Samples for FCM-phyto were collected according to "SO21 SOP: flow cytometry" (*Chapter 24*) in parallel with the FCM-prok (WP2), FCM-virus (WP4), and FCM-pico (WP6) samples (*Figure 14.1 A*). Flow cytometry rapidly and quantitatively measures optical characteristics of individual cells within a sample providing discrimination between members of the community, and additional cell staining methods further provide resolution for counting and discriminating specific members of the community.

Chlorophyll-*a* concentration (Chl*a*): As a pigment generally used by all phytoplankton, measuring the concentration of Chl*a* is established as an estimate for bulk phytoplankton abundance in the environment. It can also be useful for calibrating remote-sensing measurements of Chl*a*.

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[112] Nöthig EM, et al. (2020) Summertime chlorophyll a and particulate organic carbon standing stocks in surface waters of the Fram Strait and the Arctic Ocean (1991–2015). Frontiers in Marine Science 7:350

Duplicate samples of the 2-200 μm size fraction were collected on membrane filters by WP7, in line with duplicate Chl*a* samples of the size fraction 0.3-2 μm collected on Advantec® glass fibre filters by WP6 (*Figure 14.1 B*). Chl*a* was analysed on board according to "SO21 SOP: chlorophyll-*a*" (*Chapter 24*).

Figure 14.1: Laboratory work performed by WP7 (PHYTO) during the SAS-Oden 2021 expedition. (A) Hanna Farnelid preparing flow cytometry (FCM) samples. (B) Lisa Winberg von Friesen measuring chlorophyll-a concentrations. This laboratory work was performed in red-light conditions. (C) Lisa Winberg von Friesen preparing ice core sections for melting to later measure salinity. (A,B) ©Christien Laber, (C) ©SPRS

Particulate organic nutrients (POC, PON, POP): Samples of the 0.3-200 size fraction were collected on pre-combusted glass fibre filters (Advantec®, diameter 25 mm) by vacuum filtration according to "SO21 SOP: vacuum filtration" (*Chapter 24*) and immediately frozen at -20°C for later analyses of the particulate nutrient fractions, including δ ^{13}C, δ ^{15}N by isotope-ratio mass spectrometry (IRMS). These measurements are critical for biogeochemical and stoichiometric assessments of microbial biomass. POC and PON samples below 500 m were taken by WP10 but on glass fibre filters with larger diameter (47 mm) and a different type (Whatman® GF/F).

Dissolved nutrients: Extra water samples (45 mL) were taken from three ice habitats (melt pond, brackish brine, ice-seawater interface) for analysis of silicate and dissolved inorganic and organic N and P. These samples will also be used for double-checking the salinity of these three habitats.

Pigment concentrations (HPLC): Samples of the 0.3-200 size fraction were collected on pre-combusted glass fibre filters (Advantec®, diameter 25 mm) by vacuum filtration according to "SO21 SOP: vacuum filtration" (*Chapter 24*) flash-frozen in liquid nitrogen and stored at -80°C for later analyses of phytoplankton composition of chlorophylls and carotenoids with high-performance liquid chromatography (HPLC). These measurements provide the quantity and diversity of photosynthetic and photoprotective pigments utilized by the phytoplankton community. Different types of phytoplankton contain individual suites of pigments. Pigment composition analysis helps to further describe the phytoplankton community present by revealing the types of pigments present and the ratios of these pigments within the cells.

Water samples preserved with acid Lugol (Lugol): Samples were taken at 16 stations from the ChlMax, and the ice habitats melt pond, brackish brine, and ice-seawater interface. The samples were stained and preserved with an iodine solution (*Figure 14.2*) according to "SO21 SOP: phytoplankton community" (*Chapter 24*) for later analyses with light microscopy. Cell morphology will be used to determine the taxonomic composition and quantity of phytoplankton taxa present.

Figure 14.2: Samples for the analysis of taxonomic composition of the phytoplankton and ice-algal communities were preserved with acid Lugol's solution in 0.5-L dark glass bottles during the SAS-Oden 2021 expedition. ©Christien Laber
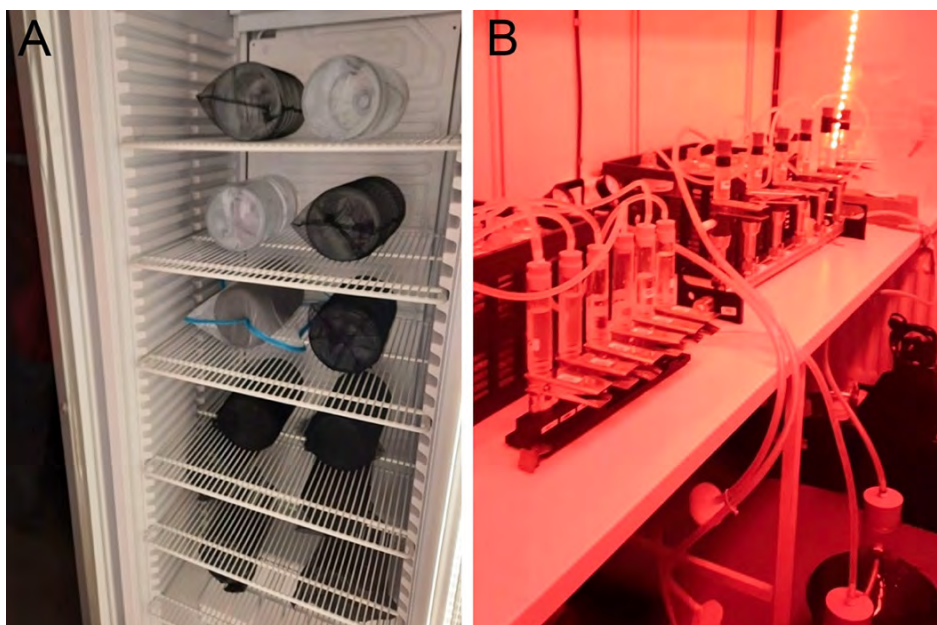
Figure 14.3: Primary production measurements during the SAS-Oden 2021 expedition. (A) Fridge with incubation bottles. (B) Filtration of the water after incubation in the "stable isotope" laboratory container (only used for isotope-enriched samples, never for field samples) in red-light conditions. (A) © Clara Pérez Martínez, (B) ©Javier Vargas Calle

Primary production (PP): Incubations with ^{13}C-bicarbonate (NaH^{13}CO$_3$) as tracer were performed in 2.3-L transparent polycarbonate bottles according to "SO21 SOP: primary production" (*Chapter 24*). The bottles were incubated for 24 hours in onboard fridges with glass doors, one dark fridge (dark treatment), and one with daylight LED-illumination outside the door (light treatment, *Figure 14.3 A*). A light gradient mimicking the light availability measured by the PAR light meter on the CTD bio at the 12 CTD bio depths was achieved by shading the incubation bottles with nets to different degrees according to *Figure 14.4*. After incubation the water was filtered on Advantec® glass fibre filters (*Figure 14.3 B*) for later analyses of ^{13}C with by IRMS (isotope-ratio mass spectrometry). Primary production measurements were also made for three ice-habitats: melt pond, brackish brine and ice-seawater interface, but not for melted ice or snow because after 24-48 hours the meltwater does not contain natural sympagic communities anymore. However, FCM, Chl*a*, HPLC, POC, PON, and POP will be measured from melted ice and snow.
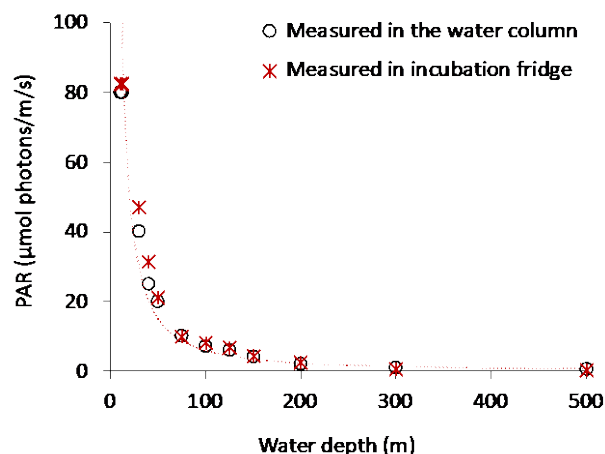
Figure 14.4: Photosynthetically Active Radiation (PAR) = light of wavelengths 400-700 nm, i.e., the portion of the light spectrum utilised for photosynthesis by primary producers measured in the water column and in the incubation fridge for measuring primary production during the SAS-Oden 2021 expedition. ©Clara Pérez Martínez

14.4 Summary of metadata collected

The metadata collected by WP7 (PHYTO) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP7_PHYTO" in the SND data repository and summarised in *Table 14.2*.

*Table 14.2: Overview of all measurements/samples collected by WP7 (PHYTO) during the SAS-Oden 2021 expedition from the water column from 12 depths (0-500 m) and 8 ice habitats at the ice stations. * = including FCM-pico, see Chapter 13, WP6) ** = reported in the excel file "SO21_Metadata_WP10_CATCHEM" in the SND data repository*

Parameter	Code	Category	CTD casts	Ice stations	Total number of unique measurements/samples
Phytoplankton density (triplicates) *	FCM-phyto	SAS Core	18	16	332
Chlorophyll-*a* concentration (duplicates)	Chl*a*-phyto	SAS Core	18	16	332
Underway chlorophyll-*a* fluorescence	Fluor-u	SAS Core	18	16	Continuous
Particulate organic carbon, including ^{13}C	POC	SAS Core	18	16	332
Particulate organic nitrogen, including ^{15}N	PON	SAS Core	18	16	332
Particulate organic phosphorus	POP	SAS Core	18	16	332
Dissolved nutrients three ice habitats	N, P, Si	SAS Core		16	48
Pigment composition	HPLC	SAS Core	18	16	332
Taxonomic composition (microscopy)	Lugol	SAS Core	16	16	64
Primary production (^{13}C enriched)	PP	SAS Core	18	16	664
Temperature at the ice stations	Temp	SAS Core		16	64 field, 282 (ice), 49 water
Salinity at the ice stations	Salin	SAS Core		16	64 field, 282 (ice), 49 water
Nutrient samples at ice stations**	Nut	SAS Core		16	345 (ice and water)

14.5 Summary of preliminary results

Most samples will be analysed after the expedition, and preliminary (uncorrected) results are only available for the onboard chlorophyll-*a* analyses. Preliminary, the 10-500 m average Chl*a* concentrations at the 18 CTD bio stations along the expedition route ranged from 6 mg m^{-2} at SO21 station 53 to 38 mg m^{-2} at SO21 station 3, with values below 20 mg m^{-2} Chl*a* at all but four stations (*Figure 14.5*). Chl*a* concentrations tended to be higher closer to the marginal ice zone (SO21 stations 3, 50, 57) than in the North Pole area (SO21 stations 16-26) and in the area between the North Pole and northern Greenland (SO21 stations 30-48).
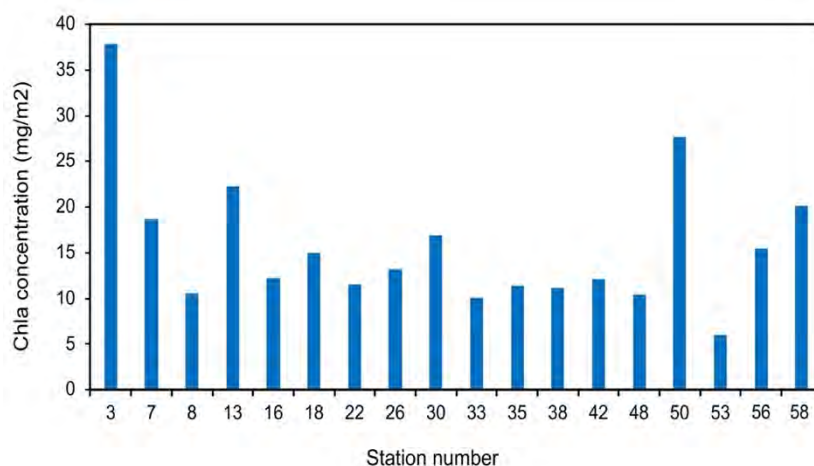
Figure 14.5: Preliminary (uncorrected) average chlorophyll-a concentrations (Chla) in the 10-500 m water column along the SAS-Oden 2021 expedition route. ©Christien Laber

14.6 Summary of post-cruise analyses and deliverables

The WP7 (PHYTO) project was led jointly by scientists from the Linnaeus University (LNU) and Stockholm University (SU) who also are responsible for the post-cruise analyses and publication of the results.

Post-cruise tasks Linnaeus University (LNU):
(1) Analysis of the FCM-phyto samples, including discrimination of phytoplankton groups based on cell size and fluorescence properties
(2) Quality control of the Chl*a*-phyto samples
(3) Quality control of the underway chlorophyll fluorescence data
(4) Drying and packing of the IRMS samples (not acid-fumed)

Post-cruise tasks Stockholm University (SU):
(1) IRMS analyses at the UC Davis Stable Isotope Facility, University of California (USA) – the POC and PON contents on the filters will also be available from these analyses
(2) Pigment composition analyses with HPLC (high-performance liquid chromatography) at DHI Lab, Denmark
(3) POP analyses at the accredited nutrient laboratory at the Department of Ecology, Environment and Plant Sciences (SU)
(4) Dissolved nutrients analyses at the accredited nutrient laboratory at the Department of Ecology, Environment and Plant Sciences (SU), including salinity measurements of these samples
(5) Taxonomic composition analyses (Lugol samples) in Poland or Lithuania

Expected results:
The results of the WP7 (PHYTO) will provide measurements of summertime biomass and primary production of phytoplankton in the upper water column (0-500 m) as well as in sympagic habitats of the CAO. As the base of the food web and local source of energy, these data will provide a basis for understanding the productivity of the CAO at all trophic levels. HPLC pigment analysis will, together with Lugol-fixed samples provide, taxonomic resolution of phytoplankton throughout the study region as well as information on photoadaptation of the phytoplankton within the water column. These will also help identify the incursion of North-Atlantic species into the CAO. Flow cytometry analyses will provide cell abundances of phytoplankton, as well as abundances of discrete populations of phytoplankton throughout the water column and ice environments. Collectively, these parameters will describe the current state of primary productivity throughout the CAO and help describe the relationship between ice and water column environments and how the changing ice conditions may impact phytoplankton production in these environments, as well as the efficiency of the biological carbon pump throughout the CAO. At least one research paper on primary productivity in the CAO is planned.

Deliverables:
The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA and RNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

15 WP8 (ZOO)

Emma Svahn

Project title: Unravelling biodiversity and production in zooplankton in one of the last blind spots of the Arctic Ocean (ZOO)

15.1 Resources

External project funding: The Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for one berth on the SAS-Oden 2021 expedition to Samuel Hylander. All project participants are listed in *Table 15.1*.

Table 15.1: Overview of all onboard and onshore participants of the WP8 (ZOO) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Samuel Hylander	samuel.hylander@lnu.se	PI	LNU
Yes (berth)	Emma Svahn	emma.svahn@lnu.se	WP leader, sampling & experiments	LNU

15.2 Scientific motivation and specific research questions

Zooplankton constitutes the link between primary production and higher trophic levels in aquatic environments. Two key zooplankton in the Arctic Ocean are *Calanus glacialis* and *Calanus hyperboreus*[113]. In preparation for diapausing and overwintering, these species accumulate lipids and are considered an important and high-quality source of food that supports higher trophic levels[114]. A third species of the same genus, *Calanus finmarchicus*, is encountered in the Arctic Ocean but has Atlantic-Boreal origin. *C. finmarchicus* is supporting much of the North Atlantic fisheries and its abundance has been shown to relate to the economically important Atlantic cod stock[115]. *C. finmarchicus* could potentially be transported to the Arctic Ocean by the inflow of Atlantic water but its expansion towards the Arctic may be limited by food and/or length of the productive season[116,117].

The implications of climate change (warming and decreasing ice cover) for the pelagic food web in the Arctic Ocean are unknown. The long-term predictions with climate change include alterations in the zooplankton community composition such as potential northward expansions of southerly species such as *C. finmarchicus*. Zooplankton biodiversity and production have been studied in some areas of the Central Arctic Ocean (CAO), but the area south of the North Pole towards Greenland is still largely a blind spot in our understanding[118]. Hence, investigations of community composition of both mesozooplankton and microzooplankton is required in order to better assess the food web and projections of possible future effects of climate change in the CAO.

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[113] Kosobokova KN, Hopcroft RR (2010) Diversity and vertical distribution of mesozooplankton in the Arctic's Canada Basin. Deep Sea Research II 57:96-110

[114] Kvile KØ, et al. (2019) Pan-Arctic depth distribution of diapausing *Calanus* copepods. The Biological Bulletin 237:76–89

[115] Beaugrand G, et al. (2003) Plankton effect on cod recruitment in the North Sea. Nature 426:661–664

[116] Friis Møller E, et al. (2016) *Calanus finmarchicus* egg production at its northern border. Journal of Plankton Research 38:1206–1214

[117] Weydmann A., et al. (2017) Warming of subarctic waters accelerates development of a key marine zooplankton *Calanus finmarchicus*. Global Change Biology 24:172–183

[118] Kosobokova KN, et al. (2011). Patterns of zooplankton diversity through the depths of the Arctic's central basins. Marine Biodiversity 41:29–50

The WP8 (ZOO) project specifically addresses the importance of zooplankton as vectors of microorganisms in the CAO. Copepods are associated with a rich bacterial community both on the exoskeleton and in the gut[119,120,121]. While a copepod is feeding, organic compounds are released into the surrounding water. This implies that the body of a copepod provides a nutrient-rich substrate and habitat for microbes in an otherwise nutrient-poor Arctic environment. In addition, the inside of a copepod, mainly the gut, also harbours a microbiome. Bacteria that survive gut passage exit with the faecal pellets and this process allows transfer and dispersal of microbes over different water layers due to the sinking of faecal pellets. The vertical migration of copepods is thus suggested to facilitate dispersal of both bacteria and viruses[122].

Due to their life strategies and by being an important constituent of the Arctic food web, copepods are major influencers of carbon cycling. The gut microbiomes of some copepods, in particular mid-latitude copepods, have been studied extensively (e.g., marine copepods[123,124,125,126], brackish copepods[127], and freshwater copepods[128]). These studies indicate a core microbiome consisting of bacteria adapted to the zooplankton and a transient microbiome more related to the ambient water or food source. However, very little research on Arctic copepod microbiomes has been performed, and thus the WP8 project will contribute with valuable information regarding geographical differences and potential differences/similarities among the microbiomes of two imperative zooplankton species of the CAO.

Together with WP1, WP8 will present the first recorded estimates of zooplankton abundance and biodiversity in the area between northern Greenland and the North Pole. It will also quantify the potential northward expansion of North Atlantic and Pacific zooplankton species as well as the importance of the zooplankton microbiome in the Arctic food web. The results of these studies will increase our knowledge of the pelagic food web in this area of the CAO.

The specific hypotheses of the WP8 (ZOO) project are:

(1) The ecologically and economically important North Atlantic species *C. finmarchicus* has spread into the Central Arctic Ocean but is not reproducing.
(2) The zooplankton community composition changes geographically depending on the inflow of North Atlantic water. There is also a vertical pattern in zooplankton community composition.
(3) The gut microbiomes of *C. glacialis* and *C. hyperboreus* consist of a core and a transient microbiome, the first is more stable and the latter is more closely related to food source and the ambient water microbial community.

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[119] Carman KR, Dobbs FC (1997) Epibiotic microorganisms on copepods and other marine crustaceans. Microscopy Research and Technique 37:116–135

[120] Tang KW (2005) Copepods as microbial hotspots in the ocean: Effects of host feeding activities on attached bacteria. Aquatic Microbial Ecology 38:31–40

[121] Grossart HP, et al. (2009) Bacterial diversity associated with freshwater zooplankton. Environmental Microbiology Reports 1:50–55

[122] Grossart HP, et al. (2010) Bacteria dispersal by hitchhiking on zooplankton. PNAS 107:11959-11964

[123] De Corte D, et al. (2014). Linkage between copepods and bacteria in the North Atlantic Ocean. Aquatic Microbial Ecology 72:215–225

[124] De Corte D, et al. (2018). Metagenomic insights into zooplankton-associated bacterial communities. Environmental Microbiology 20:492–505

[125] Moisander PH, et al. (2015). Stable associations masked by temporal variability in the marine copepod microbiome. PlosOne 10(9): e0138967

[126] Shoemaker KM, Moisander PH (2015) Microbial diversity associated with copepods in the North Atlantic subtropical gyre. FEMS Microbiology Ecology 91:fiv064

[127] Chae YJ., et al. (2021) Application of next-generation sequencing for the determination of the bacterial community in the gut contents of brackish copepod species (*Acartia hudsonica*, *Sinocalanus tenellus*, and *Pseudodiaptomus inopinus*). Animals (Basel) 11:542

[128] Grossart HP, et al. (2009) Bacterial diversity associated with freshwater zooplankton. Environmental Microbiology Reports 1:50–55

15.3 Summary of field work performed

SAS Core Parameters: Together with WP1 (EFICA), WP8 (ZOO) was responsible for sampling the SAS Core Parameters meso- and microzooplankton abundance and community composition using the multinet and bongo net (*Chapter 6.5*). WP7 also contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) as part of the SO21 omics collaboration (*Chapter 7.4*).

Mesozooplankton samples were taken with a 150 µm mesh size multinet 0-2000 m, and on two occasions also the water column below 2000 m was sampled (*Chapter 6.5*). The zooplankton organisms in the samples were concentrated on a 150 µm mesh sieve, washed down into a Kautex bottle and preserved in 20% formaldehyde solution buffered with hexamethylenetetramine to a final concentration of 4% for analyses after the cruise.

Microzooplankton samples were taken in the surface water layer (200-0 m) with a bongo net consisting of a 50 µm net and a 100 or 150 µm net (*Chapter 6.5*). The zooplankton organisms in the 50 µm cod end sample were concentrated on a 50 µm mesh sieve, rinsed down in a Kautex bottle and preserved with 20% formaldehyde solution buffered with hexamethylenetetramine to a final concentration of 4% for analyses after the cruise.

The 100 or 150 µm sample from the bongo net was used to collect species for different purposes for WP1 and for the WP8 project-specific ZOO gut microbiome experiments. Extra zooplankton samples for WP8 were collected at six stations for vitamin and/or pigment analyses after the cruise.

The ZOO gut microbiome experiments with the two most common *Calanus* species in the CAO, *C. C. glacialis* and *C. hyperboreus* (*Figure 15.1*) were performed at six stations (SO21 stations 8, 26, 35, 42, 50, 58; *Figure 1.1*). When possible, adult females were used, but due to variable abundances in the natural communities, different life stages were included in different experiments. *C. glacialis* and *C. hyperboreus* were collected from the bongo net with the largest mesh size (100 or 150 µm) from the upper 200 m of water column. From one cast per station half of the sample was immediately preserved in 96% ethanol while the other half of the sample was used for collecting animals for the experimental incubations. At two of the six stations two bongo net casts were performed due to low *Calanus* abundances in the natural communities, and the sample from one 100 µm cast was immediately preserved in 96% ethanol while the sample from the other 100 µm cast was used for collecting animals for the experimental incubations.

C. glacialis and *C. hyperboreus* were incubated in filtered (0.2 µm) seawater for four hours to allow gut evacuation to assess the core microbiome in an experimental set-up (*Figure 15.2*). The water was collected from the seawater intake in the "Main Lab" and sequentially filtered over 5-µm and 0.2-µm filters. The 5-µm filter will be used to assess the phytoplankton community and the 0.2-µm filter for the microbial community of the ambient seawater. After incubation, the *Calanus* individuals were collected and stored at -80°C preserved in 96% ethanol for assessment of the core microbiome. The incubation water of each tank was filtered onto a 0.2 µm filter to collect faecal pellets and monitor potential changes in microbial composition during the experiment. During the incubation time, the same number of individuals and species of copepods as used in incubation were collected to assess the ambient microbiome. The copepods were photographed and again preserved in 96% ethanol and stored at -80°C. The ethanol in all samples was exchanged with fresh 96% ethanol after at least 24 hours and stored again at -80°C.
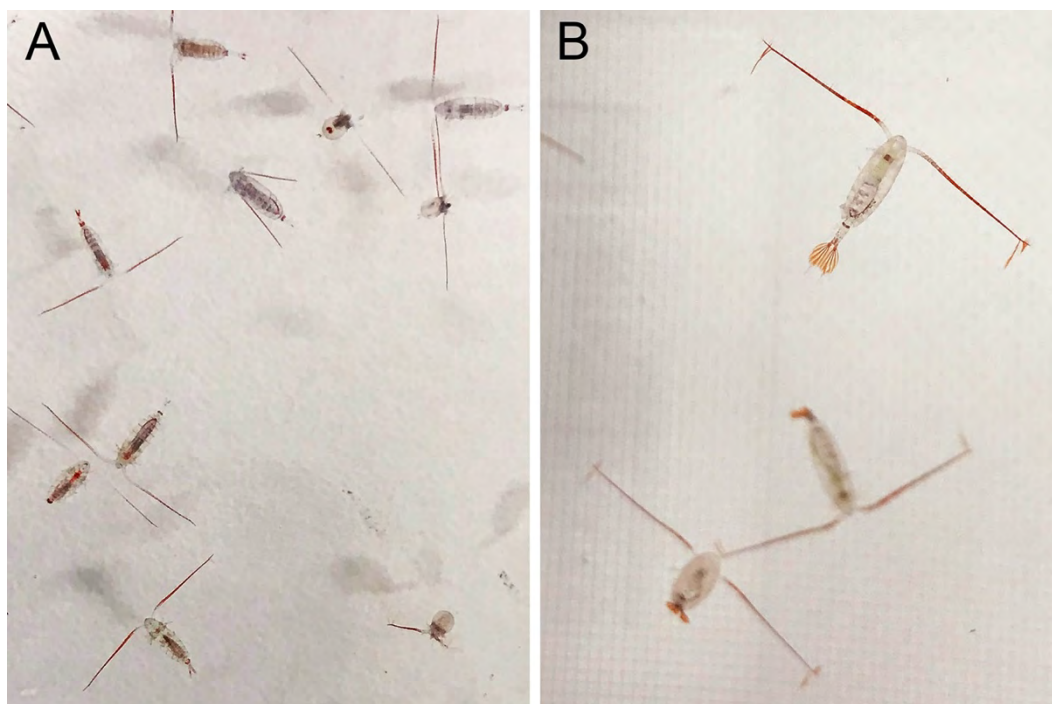
Figure 15.1: The two Calanus species used in the ZOO gut microbiome experiments performed by WP8 (ZOO) during the SAS-Oden 2021 expedition. (A) Calanus glacialis. (B) Calanus hyperboreus. ©Emma Svahn
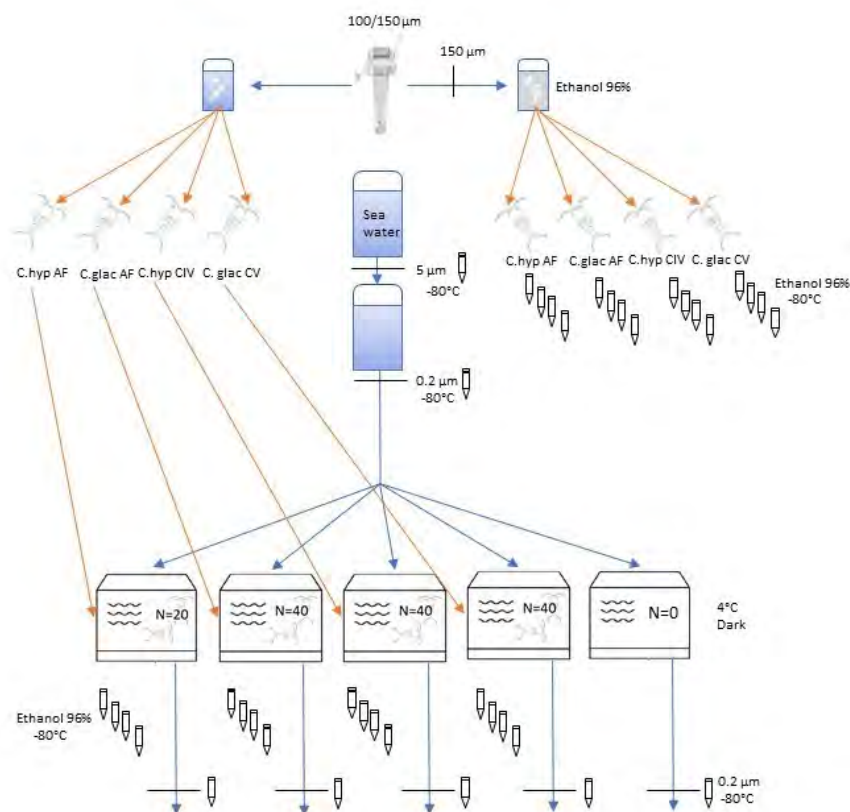

Figure 15.2: Experimental set-up of the ZOO gut microbiome experiments performed by WP8 (ZOO) during the SAS-Oden 2021 expedition. N = number of Calanus individuals. ©Emma Svahn

15.4   Summary of metadata collected

The metadata collected by WP8 (ZOO) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP8_ZOO" in the SND data repository and summarised in *Table 15.2*.

Table 15.2: Overview of all project-specific samples collected by WP8 (ZOO) during the SAS-Oden 2021 expedition. For the SO21 zooplankton community samples, see WP1 (Chapter 8).

Type of sample	Number of stations	Number of samples
Ambient phytoplankton community (5 μm filter)	6	7
Ambient bacterial community (0.2 μm filter)	6	7
C. hyperboreus – full gut	6	30
C. glacialis – full gut	5	21
C. hyperboreus – incubated	6	29
C. glacialis – incubated	5	21
C. glacialis filter (after incubation)	5	5
C. hyperboreus filter (after incubation)	6	7
Control filter (after incubation)	6	6
Bongo net community 100 or 150 μm (ethanol sample)	6	6
Zooplankton samples for vitamin and pigment analyses	6	53

15.5   Summary of preliminary results

Preliminary observations suggested that gut fullness was variable within and among species. No visible difference in gut evacuation of *C. hyperboreus* was observed between 4 and 16 hour incubations hence the shortest time (4 hours) was applied as a standard for the experiments. Filtration and other activities of the experimental species seemed a bit reduced in filtered seawater.

15.6   Summary of post-cruise analyses and deliverables

Mesozooplankton biomass, abundance and community composition of the field samples will be analysed by WP1 at the AWI, Germany (see *Chapter 8*). Microzooplankton biomass, abundance and community composition of the field samples will be analysed by WP1 or WP8 (still to be discussed). The samples from the gut microbiome will be analysed by WP8 at LNU, Sweden. Sequencing followed by genome-resolved metagenomics[129] will be used to investigate the type of food consumed as well as the microbiome and virome of the two *Calanus* species from different sampling stations. At least one research paper on zooplankton distribution in the CAO is planned together with WP1 (EFICA).

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[129] Kayani MR, et al. (2021) Genome-resolved metagenomics using environmental and clinical samples. Briefings in Bioinformatics 22:1-20

16 WP9 (FORAM)

Flor Vermassen, Clare Bird

Project title: Subpolar planktonic foraminifera invaders in the modern Arctic Ocean water column planktonic foraminifera biology (FORAM)

16.1 Resources

External project funding: Swedish Research Council (VR, grant number 2019-03757), the Bolin Centre for Climate Research (grants RA6_21_04 Arctic foraminifers and RA6_20_04 Bongo), and the Swedish Polar Research Secretariat (SPRS, implementation agreement Dnr 2020-119) for two berths on the SAS-Oden 2021 expedition to Helen Coxall. All project participants are listed in *Table 16.1*.

Table 16.1: Overview of all onboard and onshore participants of the WP9 (FORAM) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Helen Coxall	helen.coxall@geo.su.se	PI	SU, Sweden
Yes (berth)	Flor Vermassen	flor.vermassen@geo.su.se	WP Leader, field sampling & processing	SU, Sweden
Yes (berth)	Clare Bird	clare.bird2@stir.ac.uk	Field sampling, analyses	Univ. Stirling, UK
No	Matt O'Regan	matt.oregan@geo.su.se	Advisory	SU, Sweden
No	Martin Jakobsson	martin.jakobsson@geo.su.se	Advisory	SU, Sweden

16.2 Scientific motivation and specific research questions

The Greenland Ice Sheet is melting at an alarming rate and Arctic sea-ice extent is at an all-time low[130,131]. From a paleoclimate perspective, sea-ice retreat north of Greenland is not a unique consequence of modern warming. Multiple lines of evidence indicate that sea ice in this region was severely reduced during the early part of the present interglacial, the Holocene[132]. Moreover, recent studies of marine sediment cores find concentrations of invasive subpolar plankton fossils indicative of open water and thus severe reductions in sea ice during several previous interglacials[133,134]. The hypothesis is motivated by recent evidence for extended periods of ice-free conditions in Greenland during Pleistocene interglacials[135], characterized by peak atmospheric CO_2 concentrations. Therefore, it can be expected that ongoing climate change will invoke similar changes in planktonic foraminiferal biodiversity. However, little data exists on planktonic foraminiferal communities in the Central Arctic Ocean (CAO), and the region between Greenland and the Lomonosov Ridge has remained unexplored in this regard.

The first goal of the WP9 (FORAM) project is to survey the modern Arctic Ocean planktonic foraminiferal assemblages in the CAO to investigate the hypothesis that subpolar species are invading the Arctic Ocean in response to the drastic sea-ice retreat and rapid warming that has characterized

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[130] Stroeve JC, et al. (2012) Trends in Arctic sea ice extent from CMIP5, CMIP3 and observations. Geophysical Research Letters 39:1–7

[131] Trusel LD, et al. (2018) Nonlinear rise in Greenland runoff in response to post-industrial Arctic warming. Nature 564:104–108

[132] Funder S, et al. (2011) The Greenland ice sheet during the past 300,000 years: A review. Developments in Quaternary Sciences 15:699–713

[133] Nørgaard-Pedersen N, et al. (2007) Reduced sea ice concentrations in the Arctic Ocean during the last interglacial period revealed by sediment cores off northern Greenland. Paleoceanography 22:PA1218

[134] O'Regan M, et al. (2019) Stratigraphic occurrences of sub-polar planktic foraminifera in Pleistocene sediments on the Lomonosov Ridge, Arctic Ocean. Frontiers in Earth Science 7:1–18

[135] Schaefer JM, et al. (2016) Greenland was nearly ice-free for extended periods during the Pleistocene. Nature 540:252–255

Arctic climate over the last 30 years. The spatial and depth distribution of foraminifera species sampled from bongo nets and multinets near the ice edge and within the pack ice are studied. An integrated approach utilizing both morphological characteristics and DNA sequencing to identify foraminiferal species is being used to accomplish this goal.

The second goal is to improve the ability to interpret palaeoceanographic changes based on fossil occurrences of planktonic foraminiferal assemblages. This requires a better understanding of the biological preferences and environmental parameters that control the variability in the abundance of Arctic species, and their spatial and depth distribution. To achieve this, the microbiome of collected foraminifera will be analysed and coupled with the SO21 omics data set and the environmental conditions measured by other WPs at the same sampling sites. Another element of improving the palaeoceanographic toolbox will be to compare the morphological differences of live foraminifera in the water column with those of shells sedimented at the seafloor, the latter obtained by box core sampling. This allows us to investigate potential differences that may occur between live and fossil foraminiferal assemblages and could be used to better understand potential biases related to interpreting fossil records.

The third aim is to investigate the Holocene evolution of sea-ice and climate change in the CAO, in particular in the area north of Greenland. This sector of the Arctic Ocean is traditionally viewed as the last resort for multiyear sea ice, i.e., the final sector of the Artic ice pack that now becomes (seasonally) ice-free under warming conditions. However, the long-term evolution of the Arctic sea-ice cover is not well understood. While terrestrial studies have suggested ice-free conditions during the Early Holocene, marine studies from the Lomonosov Ridge have suggested a more stable, resilient sea-ice cover[136] (de Vernal et al., 2020). During this expedition, we targeted Holocene records located on topographic highs, with a special interest in sediment records located north of Greenland.

The specific research questions of the WP9 (FORAM) project are:

(1) Are subpolar planktic foraminifera invading the central Arctic Ocean in response to climate change?
(2) What are the environmental parameters controlling the spatial and depth distribution of Arctic planktonic foraminifera?
(3) What are the morphological differences between live populations in the water column and sedimented shells at the seafloor?
(4) How variable was the sea-ice cover in the central Arctic Ocean/north Greenland throughout the Holocene?

16.3   Summary of field work performed

SAS Core Parameters: WP9 contributed to sampling and/or filtering DNA and RNA according to "SO21 SOP: omics" (*Chapter 24*) and to sampling of DNA and RNA sediment cores from the box corer, as part of the SO21 omics collaboration (*Chapter 7.4*).

Project-specific sampling: To collect, quantify and identify planktonic foraminifera from both the water column and surface sediments, box core samples were taken at six stations: Stations 26, 38, 48, 50, 53, 60 (*Figure 16.1*). These stations were located at topographic highs where foraminiferal shell preservation at the seafloor would be optimal. Vertical 1000-0 m multinet casts with 55-µm mesh size

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[136] De Vernal A, et al. (2020) Natural variability of the Arctic Ocean sea ice during the present interglacial. PNAS 117:26069–26075

nets (*Chapter 6.5*) were performed at the same stations, and in one occasion at a nearby station (multinet Station 58, box core Station 60) because seafloor conditions proved to be inappropriate for box coring at Station 58. Additional 1000-0 m net casts for collecting foraminifers were performed with a bongo net (50-μm and 100-μm mesh) at three stations (Stations 7, 18, 26) and with the 55-μm mesh multinet at two stations (Stations 33, 42) (*Figure 16.1*).
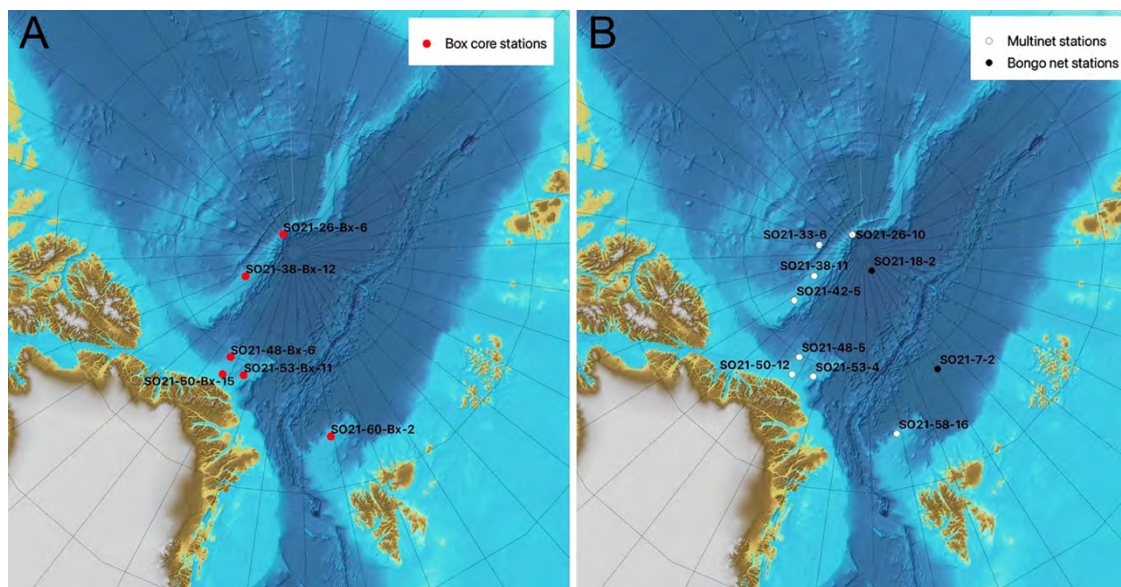
Figure 16.1: Map showing the sampling stations where project-specific samples were taken for WP9 (FORAM) were made during the SAS-Oden 2021 expedition. The Device Operation codes (Expedition-Station-Cast, cf. Table 5.3) are given next to the stations. (A) The six box core stations denoted by red circles. (B) The eight 55-μm mesh multinet stations denoted by white circles and two of the three 50-μm mesh bongo net casts denoted by black circles. At Station 26 both a multinet and a bongo net were taken. The latitude and longitude lines are spaced by 5 and 10 degrees, respectively. The background map was extracted from IBCAO[137].

Plankton net sampling: Five depth intervals (1000-500 m, 500-200 m, 200-100 m, 100-50 m, 50-0 m) were sampled with the multinet while the bongo net vertical hauls were from 1000 m to the surface (*Figure 16.2*). Where the bottom was shallower than 1000 m, the hauls started ca. 20 m above the seafloor. Foraminifers were picked from the nets under a stereo microscope (Zeiss Stemi 508) and transferred to micropalaeontological slides for assemblage counts. In some circumstances, where time was limited between nets, the sample was preserved in ethanol and the assemblage counts were performed on board later. At some sampling stations living foraminifers were selected for either genotyping with shell retention for later geochemical analysis, or for microbiome analysis (which destroys the shells). An additional subset of specimens was used in an isotope experiment (see below) or for rolling circle amplification (RCA)[138].

DNA and microbiome analysis: Foraminifers from the nets were picked into petri dishes containing 0.2 μm filtered seawater (*Figure 16.2 D*). Specimens were transferred to 200 μL of RNAlater in 0.5-mL tubes and stored at 4°C for later microbiome analysis to identify the trophic interactions of the foraminifers and thereby their role in the pelagic food web. Alternatively, specimens

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[137] Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

[138] An isothermal enzymatic process where a short DNA or RNA primer is amplified to form a long single stranded DNA or RNA using a circular DNA template and special DNA or RNA polymerases

were transferred to a micropalaeontological slide, air dried at room temperature and frozen for genotyping and shell analysis. Finally, 80 specimens of the red polar foraminifer *Neogloboquadrina pachyderma* from the 50-m surface layer were collected washed and kept in FSW for 36-48 hours prior to carrying out rolling circle amplifications using a GenomiPhi kit (Cytiva), to generate DNA for sequence investigations at a later date.

Isotope experiment: To assess kleptoplasty in *N. pachyderma*, specimens were collected from nets under low light and kept in FSW for 36 hours prior to the experiment. Specimens were exposed to 2 mM $NaH_{13}CO_3$ for 0 mins (control) 30 minutes, 6 hours or 12 hours prior to fixing in 2% PFA, 1% glutaraldehyde, 0.26 M sucrose and 0.1x PBS. These samples will later be processed through TEM and NanoSIMS.
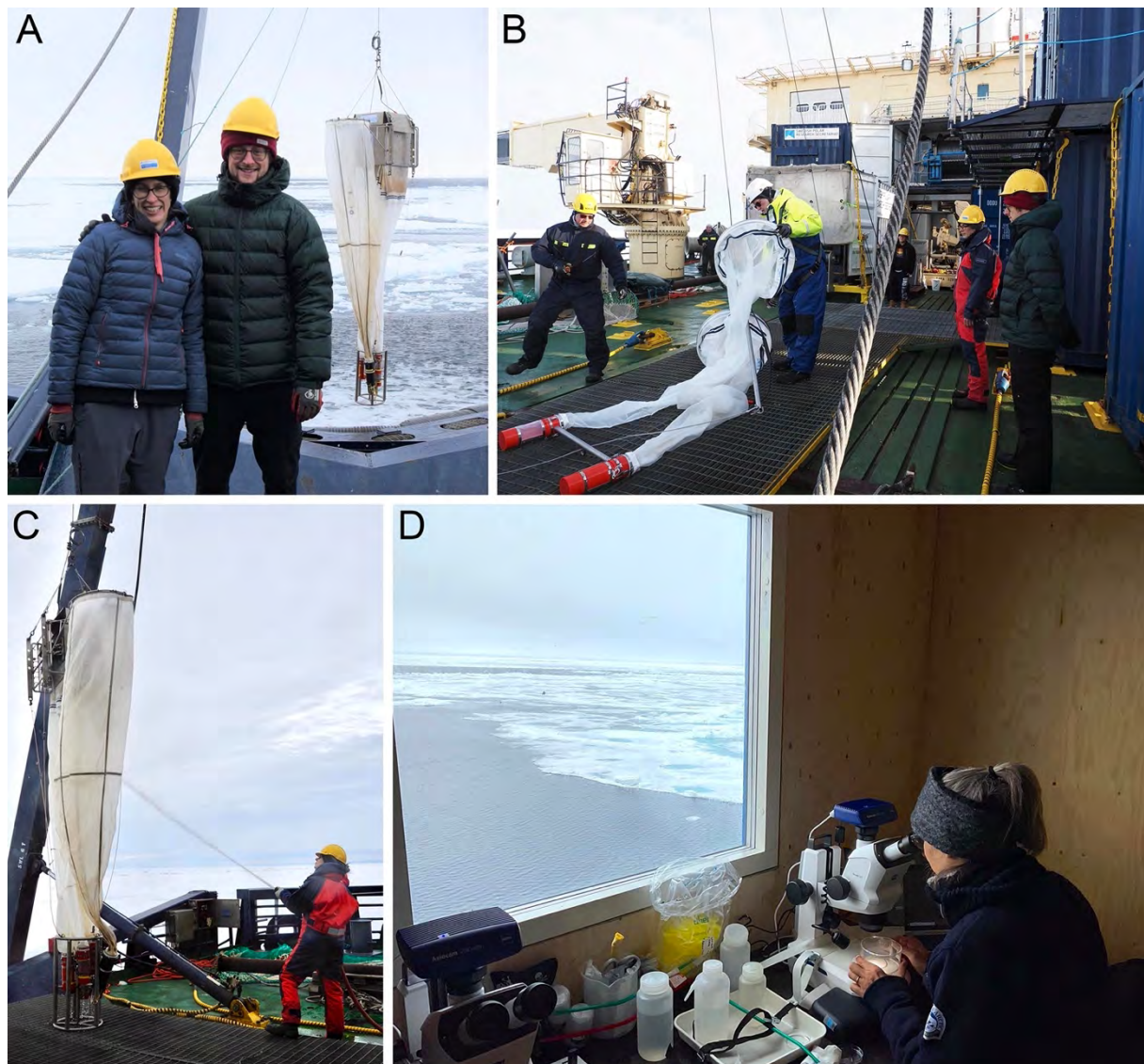
Figure 16.2: Sampling foraminifers for the WP9 (FORAM) project during the SAS-Oden 2021 expedition. (A) Clare Bird (WP9) and Flor Vermassen (WP9) with the multinet in the background. (B) Deployment of the bongo net. (C) Clare Bird hosing the multinet to collect remaining plankton into the cod ends. (D) Clare Bird analysing foraminifers with a stereomicroscope. (A) ©Hans-Jørgen Hansen, (B) ©SPRS, (C,D) ©Flor Vermassen

Box coring: Box coring was successfully performed at six stations during the SAS-Oden 2021 expedition (*Chapter 6.6*). Sediments were successfully subsampled by WP9 (FORAM) from seven box core samples (two at Station 50). The box core subsampling was performed by inserting core liners, scooping of the surface sediment, and by inserting U-channels. Two core liners (60 cm length, 8 cm diameter) were pressed into the box core sediment (*Figure 16.3*). The cores were extracted after the excess sediment surrounding the cores was removed. The first core was kept cool, the second core was sampled at 1 cm resolution using a plunger and custom-built core slicing set-up (*Figure 16.4*). Sliced core samples intended for IP25 analysis were frozen and sliced core samples intended for microfossil analysis were kept refrigerated. The two surface sediment samples were scooped from surface sediments (0.5 cm depth) and comprised ¼ the of the total surface sediment. Sampling the sediment by inserting U-channels without disturbing the stratigraphy proved to be challenging and was partially unsuccessful, resulting in a variable number of U-channels being obtained at the different box core stations. Seventeen sediment samples of box core SO21-26-Bx-6 were sieved using a 63-µm sieve, (*Figure 16.5*), subsequently air-dried and analysed under the microscope for foraminiferal assemblage analysis. Detailed information on box core subsampling is given in "SO21 SOP: box core" (*Chapter 24*).
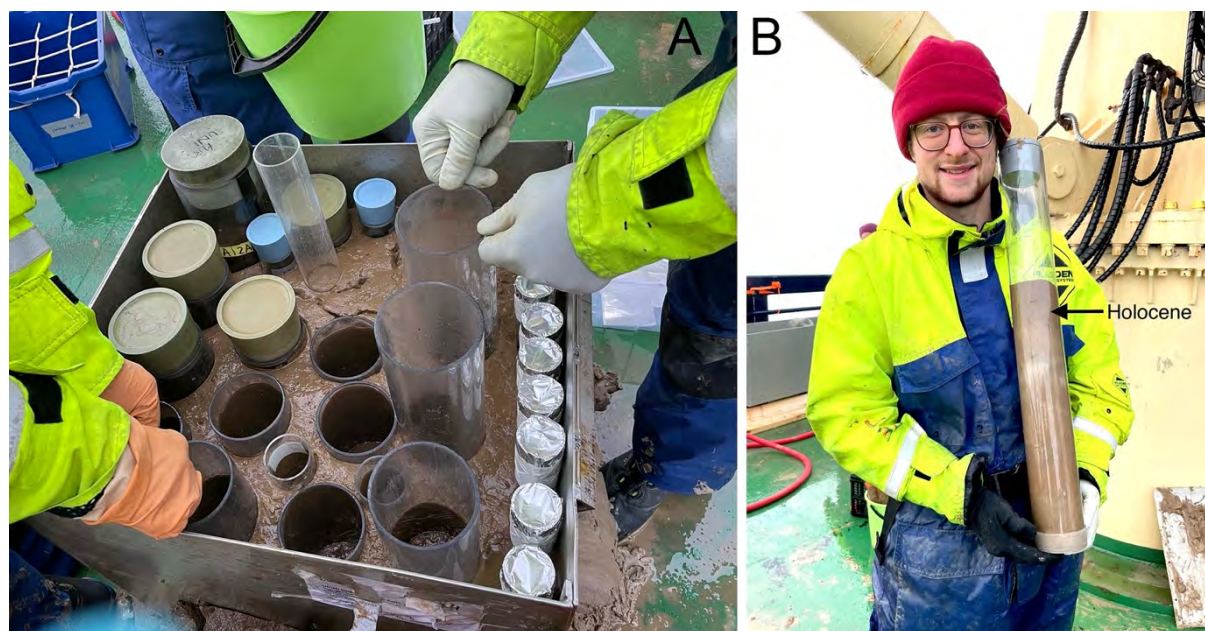
Figure 16.3: Subsampling from a box core sample during the SAS-Oden 2021 expedition. (A) Core liners are being inserted into the sediment. (B) Flor Vermassen holding a sediment core obtained from Station 50, located on the Morris Jessup Rise. The top, dark-brown layer likely comprises Holocene sediments. ©Pauline Snoeijs-Leijonmalm
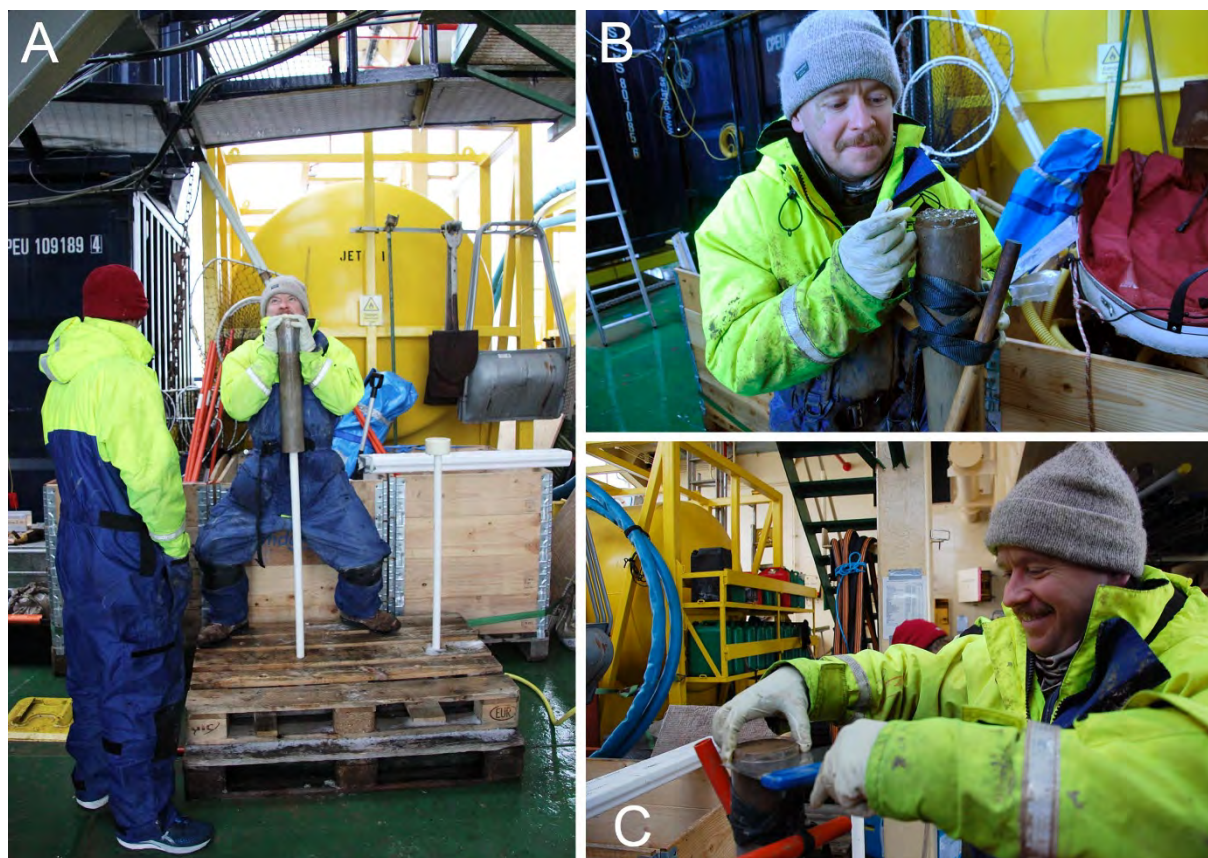
Figure 16.4: Plunger and custom-built core slicing set-up used for slicing sediment cores during the SAS-Oden 2021 expedition. (A,B) ©Emma Svahn, (C) ©Anna Lunde Hermansson

Figure 16.5: Deck work after box core sampling. (A) Flor Vermassen (WP9), Julek Chawarski (WP1) and Claudia Morys (WP1+2) taking care of samples and cleaning the deck. (B) Flor Vermassen sieving samples. ©SPRS

16.4   Summary of metadata collected

The metadata collected by WP9 (FORAM) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP9_FORAM" in the SND data repository and summarised in *Tables 16.2 and 16.3*.

Altogether, WP9 has collected 221 individual foraminifers into Eppendorf tubes for microbiome analysis or for TEM, and made 94 micropaleontological slides containing foraminifers picked from the water column assemblages for counts or DNA analysis.

Table 16.2: Overview of all project-specific subsamples collected from plankton net samples by WP9 (FORAM) during the SAS-Oden 2021 expedition.

Type of sample	Multinet Nr of stations	Multinet Nr of samples	Bongo net Nr of stations	Bongo net Nr of samples
Foraminiferal assemblages	8	40	3	3
TEM	5	7	1	1
DNA	3	3	2	2
Microbiome	5	8	1	1
Rolling circle amplification (RCA)	1	1		

Table 16.3: Overview of all project-specific subsamples collected from box core samples by WP9 (FORAM) during the SAS-Oden 2021 expedition.

Device Operation	WP9 Box core code	Location	Water depth (m)	Sedim. recovery (m)	WP9 Core 1	WP9 Core 2	WP9 nr of U-channels
SO21_26-06	SO21-26-Bx-6	Lomonosov Ridge	1318	0.39	Stored	Sliced	2
SO21_38-12	SO21-38-Bx-12	Lomonosov Ridge	1187	0.48	Stored	Sliced	1
SO21_48-06	SO21-48-Bx-6	Morris Jessup Rise	1537	0.46	Stored	Sliced	0
SO21_50-14	SO21-50-Bx-14	Morris Jessup Rise	889	0.29	Stored	Sliced	0
SO21_50-15	SO21-50-Bx-15	Morris Jessup Rise	900	0.20	Stored	Sliced	0
SO21_53-11	SO21-53-Bx-1	Morris Jessup Rise	1364	0.47	Stored	Sliced	1
SO21_60-02	SO21-60-Bx-2	Yermak Plateau	714	0.27	Stored	Sliced	0

16.5   Summary of preliminary results

Overall, we used an integrated approach using both biological and geological methods to provide the first comprehensive survey of modern planktonic foraminifera in the CAO. The produced dataset will serve as an important reference for comparison with future changes in the Arctic ecosystem, which are expected to occur under continued warming and sea-ice retreat. The multinet sites include areas where previously no information on the depth distribution of foraminifera existed, such as the North Pole area, the Greenland side of the Lomonosov Ridge and the Morris Jessup Rise. Similarly, the box cores obtained from the Morris Jessup Rise are the first sediment cores ever to be obtained in this area and have the potential to provide a unique insight into the Holocene development of sea ice and climate in the area north of Greenland.

Preliminary results from the water column and surface sediments suggest that the polar species *N. pachyderma* dominates the foraminiferal assemblage in the CAO. Our preliminary observations show no indications that subpolar species have invaded the CAO. While exceptional open water conditions were encountered just north of Greenland, the subpolar species *T. quinqueloba* was not observed in the water column during the expedition and appears not to yet have moved into the region. However, further analysis of the foraminiferal assemblage based on morphological characteristics and DNA analysis will be performed onshore to corroborate these initial results.

The dataset obtained from multinet sampling will advance the understanding of the environmental and biological preferences of *N. pachyderma*. Preliminary counting of foraminifer tests from multinets indicate that, the concentration of *N. pachyderma* was highest in the upper 50 m of the ice-covered ocean. Foraminifer abuncance was lower, but still relatively high, in the 50-100 m depth interval and subsequently decreased further with depth, with only a few specimens present in the 500-1000m depth interval. This trend was observed at nearly all stations (*Figure 16.6 A*). The maximum abundance of foraminifers coincided with the chlorophyll maximum (ChlMax) and the temperature minimum (TempMax) in the water column. A more in-depth post-cruise analysis will enable evaluation of the preferred niche of *N. pachyderma* including trophic interactions and preferred environmental conditions. A remarkable observation we made was that the majority of *N. pachyderma* specimens in the upper 50 m of the water column had red-coloured tests, whereas a switch to a higher proportion of yellow-coloured specimens occurred in the interval 100-50 m, and deeper intervals similarly comprised more yellow-coloured specimens (*Figure 16.6 B*). To our knowledge, yellow forms of *N. pachyderma* have not been described and the cause for these colour differences are unknown. Red and yellow specimens collected from the 100-50 m and 50-0 m depth intervals from two stations will be analysed for microbiome analysis. This could indicate if diet or potential symbionts are responsible for the observed differences in colour. Furthermore, a detailed comparison between the morphological characteristics of foraminiferal tests sampled from the water column and those found in the surface sediments will improve palaeoceanographic interpretations.
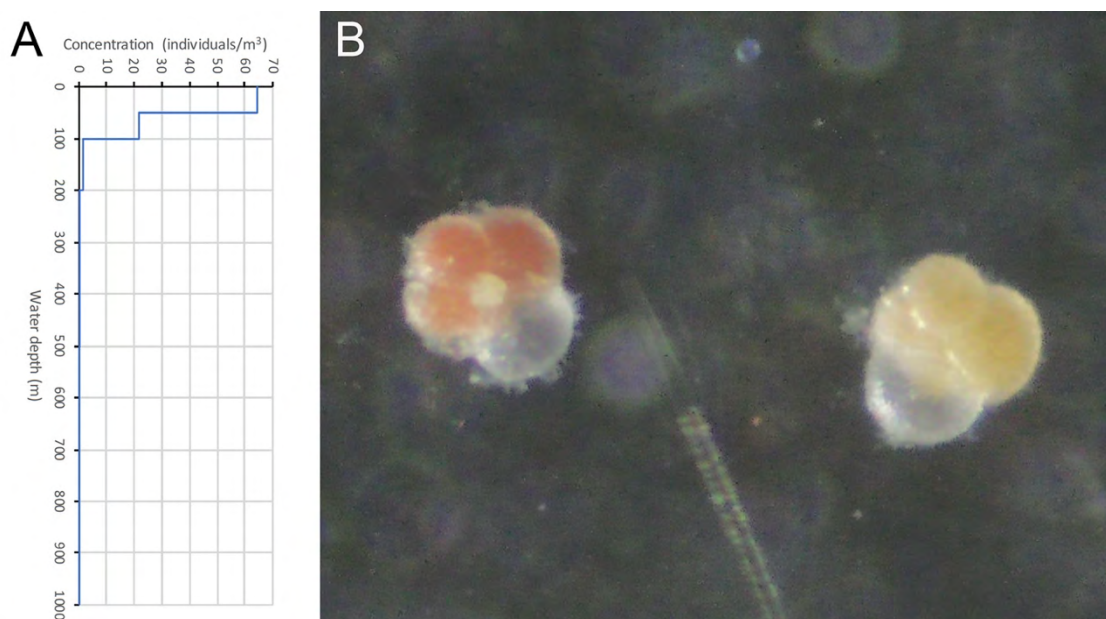
Figure 16.6: Preliminary results of WP9 (FORAM) obtained during the SAS-Oden 2021 expedition. (A) Depth distribution of foraminifers in the water column measured from a multinet at Station 26. (B) Microscope photograph showing Neogloboquadrina pachyderma with yellow and red cytoplasm. ©Clare bird

Preliminary observations regarding box core stratigraphy and comparison with previous core studies[139] indicate that the retrieved box core samples during the SAS-Oden 2021 expedition on the Lomonosov Ridge comprise the entire Holocene, and reach well into sediments from the Last Glacial. While the stratigraphy and chronology of sediments from the inner part of Morris Jessup Rise are virtually unknown, it is anticipated that the entire Holocene period was sampled by our box cores. Therefore, we anticipate that the by WP9 obtained box core subsamples are excellent material to generate valuable paleoclimate and/or sea-ice reconstructions, for example by measuring downcore variability of the sea-ice biomarker IP25.

16.6   Summary of post-cruise analyses and deliverables

Post cruise analyses:

The analyses will be carried out at Stockholm University (Sweden) and the University of Stirling (UK) as summarised in *Table 16.4*.

Table 16.4: Summary of the post-cruise analyses of WP9 (FORAM).

Type of sample	Post-cruise analysis
Assemblages dried on slides	Assemblage counts and morphometric analyses
Assemblages stored in ethanol	Assemblage counts
Sediment samples	Sieving, assemblage counts, IP25
Sieved sediment samples (>63 μm)	Assemblage counts
Foraminifers frozen on micropalaeontological slides	Genotyping and shell geochemistry
Foraminifers placed in RNAlater	Microbiome analysis (trophic preferences and symbiotic interactions)
Foraminifers placed in TEM fixative	TEM imaging
Rolling circle amplification (RCA)	Genome investigation
Foraminifers placed in TEM fixative after isotope experiment	TEM imaging and NanoSIMS to investigate potential kleptoplasty

Deliverables:

The following publications are planned: (1) Spatial distribution and environmental preferences of *Neogloboquadrina pachyderma* in the CAO, (2) Microbiome analysis of *Neogloboquadrina pachyderma*: implications for ecology and paleoceanography, (3) Holocene history of sea-ice variability in North Greenland. Collaborations include WP10 (nutrient concentrations, DIC, TA, calcium carbonate compensation depth; *Chapter 17*) and the SO21 omics collaboration (*Chapter 7.4*).

The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). DNA sequences will be archived in an international publicly available sequencing data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[139] O'Regan M, et al. (2019) Stratigraphic occurrences of sub-polar planktic foraminifera in Pleistocene sediments on the Lomonosov Ridge, Arctic Ocean. Frontiers in Earth Science 7:1–18

17 WP10 (CATCHEM)

Adam Ulfsbo, Marcus Sundbom, Amanda Nylund, Anna Lunde Hermansson

Project title: Carbon and tracer chemistry in the Arctic Ocean (CATCHEM)

17.1   Resources

External project funding: the Swedish Research Council for Sustainable Development (FORMAS, grant number 2018-001398), the European Union's Horizon 2020 Research and Innovation Programme[140] (Grant Agreement No. 820989), and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for four berths on the SAS-Oden 2021 expedition to Adam Ulfsbo, and the Hasselblad Foundation (Contract No. 2019-1218) to Adam Ulfsbo and Leif Anderson. All project participants are listed in *Table 17.1*.

Table 17.1: Overview of all onboard and onshore participants of the WP10 (CATCHEM) project.

On board	Name	e-mail address	Task in project	Affiliation
Yes (berth)	Adam Ulfsbo	adam.ulfsbo@marine.gu.se	PI, WP Leader	GU, Sweden
Yes (berth)	Marcus Sundbom	marcus.sundbom@aces.su.se	Co-PI, nutrients	SU, Sweden
Yes (berth)	Amanda Nylund	amanda.nylund@chalmers.se	Carbonate chemistry	Chalmers, Sweden
Yes (berth)	Anna Lunde Hermansson	anna.lunde.hermansson@chalmers.se	Carbonate chemistry	Chalmers, Sweden
No	Leif G. Anderson	leif.anderson@marine.gu.se	Co-PI, advisory	GU, Sweden
No	Toste Tanhua	ttanhua@geomar.de	CFC-12 and SF_6 (WP11)	GEOMAR, Germany
No	Agneta Fransson	agneta.fransson@npolar.no	Carbonate chemistry	Norwegian Polar Institute
No	Melissa Chierici	melissa.chierici@hi.no	Carbonate chemistry	IMR, Norway

17.2   Scientific motivation and specific research questions

The Arctic Ocean (AO) has a high potential for anthropogenic CO_2 uptake and storage relative to its size, a feature related to the intense ventilation of subsurface layers and high CO_2 solubility in low-temperature waters. Inherently, the AO is particularly sensitive to ocean acidification (OA), which has been shown to have detrimental effects on many forms of marine life and is expected to affect fish stocks, marine ecosystems and fisheries of the Arctic. Critical OA thresholds have already been passed in some regions of the AO such as the upper halocline of the Canada Basin and the shallow East Siberian Sea.

Within the next few decades most other regions will follow suit with potentially serious impacts on marine organisms. Yet, the actual ecosystem impacts are still virtually unknown. The present and future state and impacts of anthropogenic carbon invasion and ocean acidification need to be quantified to accurately understand and manage future Arctic ecosystems. Increasing anthropogenic CO_2 from the atmosphere at an increasingly ice-free surface ocean, increased freshwater storage from sea-ice melt and riverine input, increasing terrestrial organic carbon inputs from rivers, thawing

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permafrost and coastal erosion, as well as changes in wind and ocean circulation patterns are critical processes to consider as the Arctic Ocean is an integrated part of the global overturning circulation where changes at high latitudes propagate to lower latitudes and vice versa, but it is also interconnected across domains where shifts in the physical state of the water masses impact the ecosystems and carbon cycle. In turn, any major perturbation of the carbon cycle will feed back on the climate and the physical domain and ultimately to the marine ecosystem.

The goal of WP10 (CATCHEM) is to improve our understanding of the driving processes and factors underlying anthropogenic changes in carbon storage and ocean acidification by concurrent collection of hydrographic, chemical and ecosystem data along the expedition route. The gradual uptake of anthropogenic CO_2 by the ocean from the atmosphere can, in principle, be quantified as the ensuing gradual rise of dissolved inorganic carbon (DIC) at the surface and eventually within the ocean interior. The large and complex natural ocean background provides difficult challenges to assess any trends and to distinguish the small anthropogenic component. Several different methods have been developed to estimate the total anthropogenic CO_2 inventory since the preindustrial era and to quantify the increase in storage. In this work package, the transit-time distribution (TTD) approach will be employed to determine the anthropogenic carbon storage in the Arctic Ocean and the extended Multiple Linear Regression (eMLR) approach for estimating the decadal change.

The current state of ocean acidification and the underlying drivers for changes of this state needs to be analysed concurrently with changes in physical and biogeochemical processes, such as: sea ice conditions (extent, concentration, melt/freeze, drift), air-sea gas exchange, freshwater input, ocean circulation, wind fields, and biological production and remineralization processes. It is possible to estimate the change in ocean acidification from the change in anthropogenic CO_2 through thermodynamic relationships, steady-state assumptions, and explicit buffer factors, which also provide means to compare the degree of buffering in different regions at different times, and to gain insight into the buffering mechanisms. Hydrographic data (salinity, temperature) and quasi-conservative chemical tracers such as nutrients, dissolved oxygen, total alkalinity (TA), dissolved organic carbon (DOC), and transient tracers (CFCs, SF_6) will be used to quantitatively define water mass distributions, their frontal boundaries both horizontally and vertically, and the pathways that define their structures and residence times.

The specific research questions of the WP10 (CATCHEM) project are:

(1) What is the input and fate of organic, inorganic, and anthropogenic carbon?
(2) What is the magnitude, drivers, and impacts of ocean acidification?
(3) What is the distribution, structure, and residence time of the water masses?

17.3   Summary of field work performed

SAS Core Parameters: WP10 (CATCHEM) was responsible for sampling the SAS Core Parameters dissolved oxygen, inorganic nutrients (NO_3/NO_2, PO_4, SiO_3), dissolved inorganic carbon (DIC), total alkalinity, pH, $\delta^{18}O$ of H_2O, dissolved organic carbon (DOC), particulate organic carbon (POC), and coloured dissolved organic matter (CDOM) from the bow CTD (*Chapter 6.2*).

Sampling strategy

To address the main research questions of WP10 (CATCHEM) , the main focus of the field work was to sample the water column at predefined depths from surface to bottom (*Table 6.1*) at as many stations as possible along the expedition route. Sea ice was also sampled at a number of stations. In addition, when conditions allowed, the Secchi depth was measured by lowering a white standard disc (*Figure 17.1 A*) into the water and recording the depth at which the disc disappears from view.

Water sampling

Water samples were collected using a SBE rosette system equipped with 22 Niskin bottles, each having a volume of 12 L, from the bow of the ship. The number of bottles was reduced to 22 because of the mounting of the LADCP for WP14 (*Chapter 20*). The bottles were closed at predefined depths during the return of the CTD rosette package from the bottom to the surface according to the depths defined by the international SAS programme (10, 20, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 400, 500, 700, 1000, 1500, 2000, 2500, 3000, 3500, 4000, bottom-50, bottom; *Table 6.1*). It should be noted that *IB Oden* mixes the water down to at least 10 m of depth when drifting at a sampling station by using the bow seawater ice flushing system to keep ice floes away from the CTD wire. Water samples for all variables were drawn immediately after the rosette was secured in the CTD container. The order of sampling was determined by the risk of contamination, i.e., CFC-12 and SF_6 (WP11), O_2 (WP10), CH_4/N_2O (WP 12/13), DIC, pH/TA, nutrients, $\delta^{18}O$ of H_2O, DOC, and POC (WP10).

Oxygen samples were drawn from the Niskin bottles into iodometric flasks, equipped with glass or teflon stoppers using silicon tubing and filled from below in order to avoid bubbles. The volume of the bottles was approximately 150 mL and they were overfilled with about two flask volumes. The oxygen was directly fixated by the addition of 1 mL each of concentrated manganese chloride (3 M) and alkaline (NaOH 8 M) sodium iodide (4 M) solutions to form manganese oxyhydrodoxide precipitate. Samples for the determination of DIC, pH and total alkalinity were collected as soon as possible as atmospheric CO_2 can impact the former two. Two 250-mL Pyrex® bottles having tight plastic screw caps were filled to the rim in order to minimize contamination. All samples were stored at temperatures between 10 and 15°C before analysis within hours of collection.

Water samples for nutrients were carefully drawn directly from the Niskin spigots, without intermediary tubing, into 100-mL plastic bottles (PE, Kautex). The pre-washed bottles (laboratory machine dishwashing followed by six-hour soaking in 50% HCl at 40°C) were rinsed three times with sample water before being filled without headspace and immediately stored in the dark at 4°C until onboard measurement.

Samples for determination of $\delta^{18}O$ of H_2O were taken directly from Niskin bottles into new 2-mL borosilicate glass vials or 12-mL exetainer vials that were rinsed 2-3 times before being filled using a gentle flow rate and sealed with gas proof caps without leaving any head space.

From a subset of stations, WP10 sampled particulate organic carbon (POC) at depths deeper than 500 m from the CTD (*Figure 7.1 B*). WP7 sampled for POC analysed from 12 depths in the 10-500 m depth interval att 18 CTD bio stations by WP7 (*Chapter 14*). Thus, the SAS POC samples were taken from different CTD casts. Whole-water samples were drawn from the Niskin bottles via silicone tubing into 5.5 L carboys (PE, Kautex) rinsed with sample water. A vacuum filtration station (Model 22.300, KC Denmark) was used to filter up to five samples in parallel through 47 mm Whatman® GF/F filters under a gentle vacuum (-0.05–0 bar). The filters had beforehand been individually packed in aluminium foil sleeves and combusted at 450°C for 4-6 hours. The whole sample volume, typically ca. 5.5 L was let to pass the filter whereafter the filter was retrieved, folded, and put back into its aluminium sleeve. Occasionally, visible fibres from ambient air or larger zooplankton were observed

and removed using a fine-tipped tweezer before the filter was folded and packed. The packed filters were immediately frozen at -80°C inside a zipped plastic bag until further on-shore preparation and analysis.

Dissolved organic carbon (DOC) samples were prepared using two different methods. 1) At stations where POC samples were taken, a subsample of the GF/F filtrate (see above) was collected after ca. 1 L of water had passed the filter. Then the vacuum was turned off and a 40-mL EPA glass vial (Cole-Parmer™ EPA Pre-Cleaned TOC Vial) was attached to the filtrate outlet (*Figure 17.1 C*). By slow gravity filtration, the vial and the PTFE-coated closure were washed three times with filtered sample water before being filled with ca. 30 mL filtrate. At all other stations water samples for DOC analysis were filtered using 30 mm syringe filters with 0.45 µm pore size (Whatman® Puradisc Aqua CA 30/0.45). These single-use cellulose acetate filters are produced especially for DOC analysis and are thus pre-cleaned. However, at low natural DOC levels, such as those expected in the deep Arctic Ocean, any trace carbon leaking from filters must be avoided. Therefore, prior to sampling, the filters were first soaked in MQ water for at least 4 hours and then 2×60 mL of MQ was pushed through he filters. When sampling from the rosette, EPA vial and the all-plastic syringe (Henke-Ject) were rinsed three times with sample water directly from the Niskin bottle before the syringe, with attached filter, was filled with ca. 70 mL sample water and sealed with its plunger. The filter was further washed with ca. 40 mL sample water before the remaining water in the syringe was gently pressed through filter into the EPA vial. After sampling, the EPA vials were immediately frozen at -20°C for later on-shore analysis.

The bow CTD was equipped with a Turner Cyclops 6K CDOM fluorometer. For post-calibration of the sensor, CDOM samples were taken by WP10 (CATCHEM) at seven stations using the same procedures as described above for DOC.
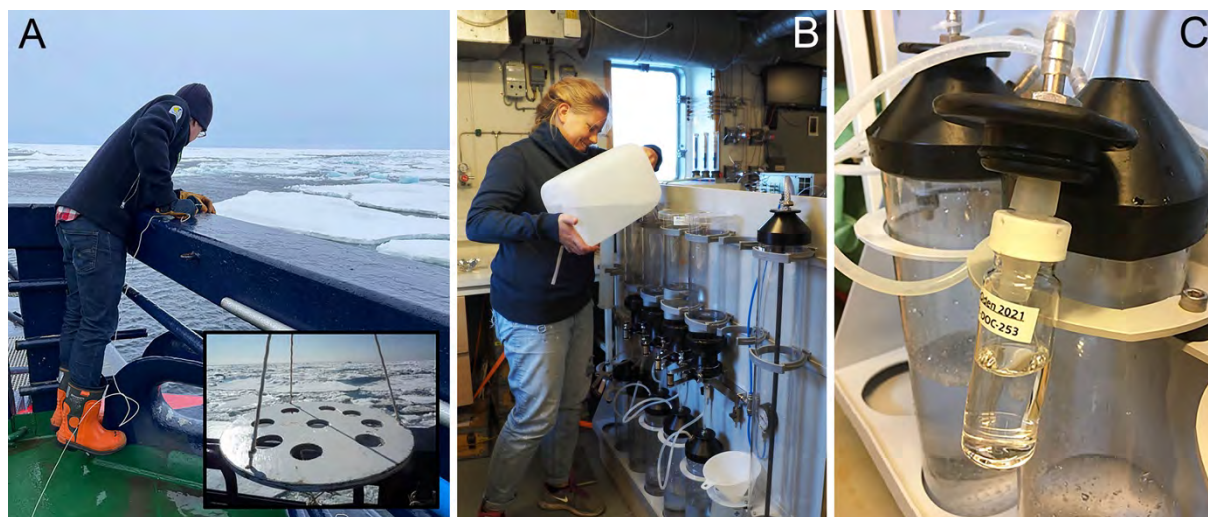
Figure 17.1: Field and laboratory work carried out by WP10 (CATCHEM) during the SAS-Oden 2021 expedition. (A) Deployment of a 50-cm diameter Secchi disc deployed from the port aft of IB Oden. *(B) Anna Lunde Hermansson performing filtrations for particulate organic carbon (POC) analyses. (C) In-line gravity filtration for dissolved organic carbon (DOC) analyses. (A) ©Anna Lunde Hermansson, (B) ©SPRS, (C) ©Marcus Sundbom*

Ice sampling

Ice cores were collected at eight ice stations using a 9-cm Kovacs corer (*Chapter 6.7*). The cores were typically sectioned with a stainless-steel saw from the top on site. Core sections were immediately placed in individual 5-L PVF Tedlar® bags and sealed with plastic clips (*Figure 17.2*). Excess air was removed with an electric vacuum pump (VWR Mini Vacuum Pump) via the Tedlar® bag valve and sections were allowed to melt in the dark at laboratory temperature. Upon melting, usually after 24 hours, the bulk ice melt was carefully transferred to 250-mL borosilicate glass bottles using Tygon® tubing. The DIC/TA cores were collected at the same site as the SAS cores for salinity, temperature, and nutrients (*Chapter 6.7*).

In addition, sectioned ice cores and water from ice habitats (melt pond, brackish brine and ice-seawater interface) taken in collaboration with other work packages were sampled for analysis of dissolved inorganic nutrients and total organic carbon (TOC). Ice core sections in plastic zip-lock bags were melted at room temperature and then subsampled for onboard analysis of nutrients. Ice water for TOC was transferred to MilliQ-washed Falcon tubes and kept frozen at -20°C until later analysis on shore.

Figure 17.2: Packing sea-ice sections into Tedlar® bags on the ice by WP10 (CATCHEM) during the SAS-Oden 2021 expedition. ©Anna Lunde Hermansson

17.4   Analytical methods

Total dissolved oxygen (on board)

Dissolved oxygen was determined using an automatic Winkler titration setup with UV detection (Scripps Institute of Oceanography (SIO) Oxygen Titration System version 2.35m, *Figure 17.3*). Reagents were prepared following Langdon[141]. Before each titration session the system blanks were

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[141] Langdon C (2010) Determination of dissolved oxygen in seawater by Winkler titration using amperometric technique. In Hood EM, et al. (Eds.): The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. Version 1, (p. 18). IOCCP Report Number 14; ICPO Publication Series Number 134 [http://www.go-ship.org/HydroMan.html]

determined by addition of reagents to MilliQ water. The titrations were performed in the same iodometric flasks used for the collection of the samples from the rosette after the manganese oxyhydroxide precipitate was dissolved by sulphuric acid (5 M). MilliQ water was added to the conical glass top to prevent contamination from air and samples were stored in the dark. Sets of samples from 1-3 stations were analysed in one run. Sodium thiosulphate (~0.22 N) solutions were prepared onboard for the titration and the concentration was set each day using pre-weighed potassium iodate (0.0123xxx N) standard. Precision was determined using replicates from the same depth and was better than 0.5 µmol kg⁻¹.
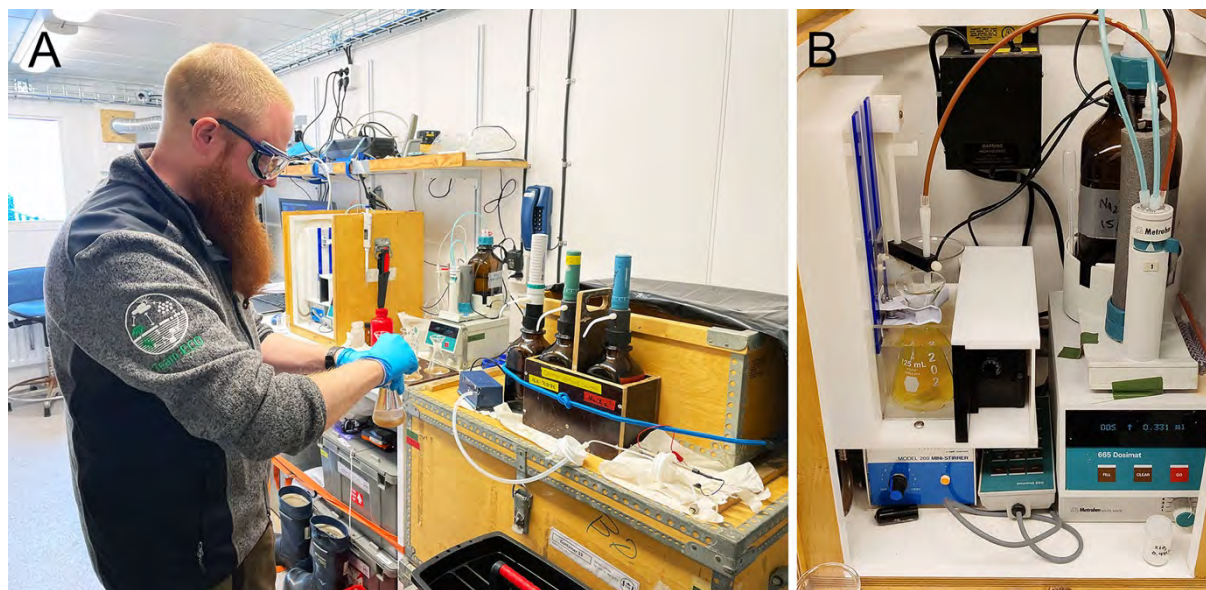
Figure 17.3: Measurement of oxygen concentration in seawater during the SAS-Oden 2021 expedition. (A) Adam Ulfsbo at work in the "Main Lab" of IB Oden. (B) Setup of the oxygen titration rig used during the expedition. (A) ©SPRS, (B) ©Adam Ulfsbo

Seawater pH (on board)

Seawater pH was determined on the total scale employing a spectrophotometric method using the indicator m-Creosol Purple (mCP)[142,143]. Purified mCP[144] was purchased from the laboratory of Eric Achterberg, GEOMAR, Kiel, Germany. A 2 mM indicator solution was prepared by dissolving pre-weighed mCP indicator in 0.5 L filtered (0.45 µm) seawater of ca. 34 salinity. The indicator was adjusted to a pH in the same range as the samples, approximately ± 0.2 pH units, by adding a small volume of 1 M HCl or NaOH. Before running a set of samples, the pH of the indicator was measured using a 0.02 cm cuvette. The measurements were performed on board within hours from sampling and samples were thermostated to 25°C in a water bath 30 minutes prior to analysis. An automatic system[145] was used (*Figure 17.4*) where the sample and indicator were mixed in a syringe (Kloehn V6)

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[142] Clayton TD, Byrne RH (1993) Spectrophotometric seawater pH measurements: total hydrogen results. Deep Sea Research, Part I 40:2115–2129 [https://doi.org/10.1016/0967-0637(93)90048-8]

[143] Carter BR, et al. (2013). An automated system for spectrophotometric seawater pH measurements. Limnology & Oceanography, Methods 11:16–27 [https://doi.org/10.4319/lom.2013.11.16]

[144] Liu X, et al. (2011) Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements. Environmental Science and Technology 45:4862–4868 [https://doi.org/10.1021/es200665d]

[145] Fransson A, et al. (2013) Development and Optimization of a Labview program for spectrophotometric pH measurements of seawater, pHspec ver 2.5, University of Gothenburg.

before injected to a 1-cm cuvette of a diode array spectrophotometer (Agilent 8453), where the absorbance was measured at wavelengths 434, 578, and 730 nm, the latter accounting for background absorbance. Indicator corrections were made according to the recommendations in Chierici et al.[146]. The pH values are corrected to 25°C on the total scale. The accuracy is determined by the pureness of the indicator and was also checked by the determination of certified reference material (CRM batch #181 and #191). The latter measurements indicate that it should be well below 0.01 pH unit. The precision as determined by replicates from the same sample bottle was in the range of ±0.001 pH unit.
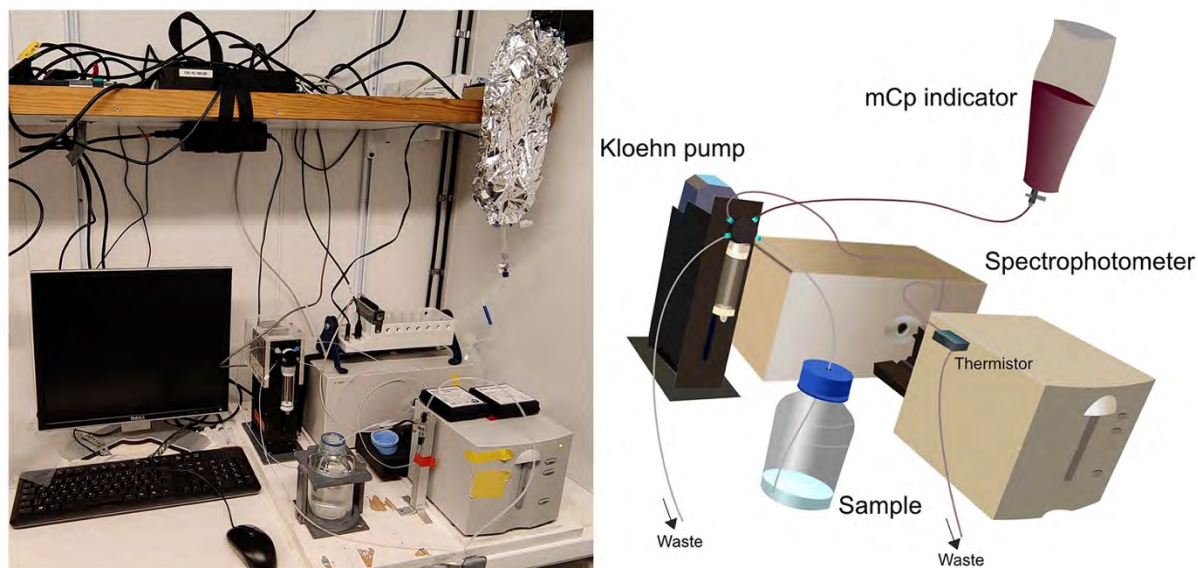
Figure 17.4: Setup of the spectrophotometric equipment for the measurements of seawater pH used during the SAS-Oden 2021 expedition. ©Anna Lunde Hermansson

Total alkalinity (on board)

Total alkalinity (TA) was determined using a semi-open cell potentiometric (Orion ROSS 8102BN) titration (Metrohm Dosimat 665) method using a 5-point Gran evaluation[147]. The system measures alkalinity in μmol L^{-1} using the nominal hydrochloric acid (HCl) concentration of 0.05 mol L^{-1} and 0.65 mol L^{-1} sodium chloride (NaCl). The sample is transferred from the sample bottle by pressure to a thermostated water-jacketed volumetric pipette before dispensing in to the titration cell. Certified reference material (CRM Batch #181 and #191), purchased from A. Dickson, Scripps Institution of Oceanography, was used to determined accuracy[148]. For all samples and CRM analysis the alkalinity in μmol/kg was calculated using the salinity (from the CTD bottle file and the certified salinity, respectively) and the temperature measured at the beginning of the titration. Sample results were then multiplied with the factor determined from the CRM measurements at each individual station, and the correction was always below 0.5%. The given precision was computed as standard deviations of duplicate analyses performed continuously during the cruise. Duplicates were run from the same sample bottle since total alkalinity is not sensitive to atmospheric contamination with the results typically not deviating more than 2 μmol kg^{-1}.

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[146] Chierici M, et al. (1999). Influence of m-cresol purple indicator additions on the pH of seawater samples: Correction factors evaluated from a chemical speciation model. Marine Chemistry 65:281–290 [https://doi.org/10.1016/S0304-4203(99)00020-1]

[147] Haraldsson C, et al. (1997). Rapid, high-precision potentiometric titration of alkalinity in ocean and sediment pore waters. Deep Sea Research, Part I 44:2031–2044 [https://doi.org/10.1016/S0967-0637(97)00088-5]

[148] Dickson AG, et al. (2007). Guide to best practices for ocean CO$_2$ measurements. PICES Special Publication 3 (Volume 3). [https://cdiac.ess-dive.lbl.gov/ftp/oceans/Handbook_2007/Guide_all_in_one.pdf]

If the correlation coefficient of the linearity of the Gran points was <0.9990, the sample was analysed again as both duplicates and triplicates if necessary. At Station 28, the correlation coefficient was consistently low, and the precision of the analysis was not satisfactory. An extensive troubleshooting was performed where the pH electrodes, the tubing, the valves, the acid and temperature were considered as potential explanations to the sudden deviations. All of which were tested and adjusted individually but without the desired results. Finally, replacement of the built-in magnetic stirrer to a separate stirrer resulted in high-precision analysis and correlation coefficients >0.99990 and the remaining samples were analysed with the new setup. No further investigation was made on the specific issue of the original magnetic stirrer, but it appears as if the magnetic field might have interfered with the potentiometric measurements. Shaking while breaking ice might have damaged the motor itself or the connection to the stirrer.

Total dissolved inorganic carbon (on board)

Dissolved inorganic carbon (DIC) was determined from one of the 250-mL Pyrex® bottles collected from the Niskin bottles using a coulometric (UIC 5011) titration method (*Figure 17.5*) based on Johnson et al.[149] using the MIDSOMMA system (Marianda, Kiel, Germany). A known volume of seawater, using a pipette having a volume of 15.250 mL at 25°C, was added to a stripping cell containing 0.5 mL 8.5% phosphoric acid. Pure N_2 gas (150 mL min^{-1}) was used to extract the CO_2 formed from the DIC and transferred it to a coulometer cell (UIC cathode and anode solutions). The gas is bubbled through a reagent containing ethanolamine, which reacts with the CO_2 to produce hydroxyethylcarbamic acid. The latter is coulometrically titrated by the hydroxide ions generated at the cathode and the pH in the reagent solution is monitored coulometrically through the indicator thymolphthalein. At the anode, silver is oxidized. The number of electrons produced corresponds to the amount of CO_2 in the sample and can thus be converted to concentration by dividing by the sample volume. The precision was typically ca. 2 μmol kg^{-1}, with the accuracy set by calibration against certified reference materials (CRM Batch #181 and #191), supplied by A. Dickson, Scripps Institution of Oceanography (USA).

The N_2 gas of the system both act as a carrier of the CO_2 to the coulometer cell and as a pressure regulator to empty the sample from the volumetric pipette to the stripping cell. The gas flow is measured continuously and large variability in flow suggested in a faulty system where the sample analysis could not reach blank within the predetermined 20 minutes. All tubing and valves prior the coulometric cell were carefully investigated and, when necessary, replaced. In addition, the sample inlet to the coulometric cell was clogged and had to be bypassed with an additional tubing. Finally, the gas flow returned to stable values and the analysis could commence. Clogging and wearing of the tubing might have developed successively but calibration with certified reference material and running of sample replicates supports the analysis results and will be used for QA and QC. The cathode (platinum electrode) was regularly cleaned with concentrated (63%) HNO_3 to ensure adequate performance.

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[149] Johnson KM, et al. (1985) Coulometric TCO$_2$ analyses for marine studies; an introduction. Marine Chemistry 16:61–82 [https://doi.org/10.1016/0304-4203(85)90028-3]
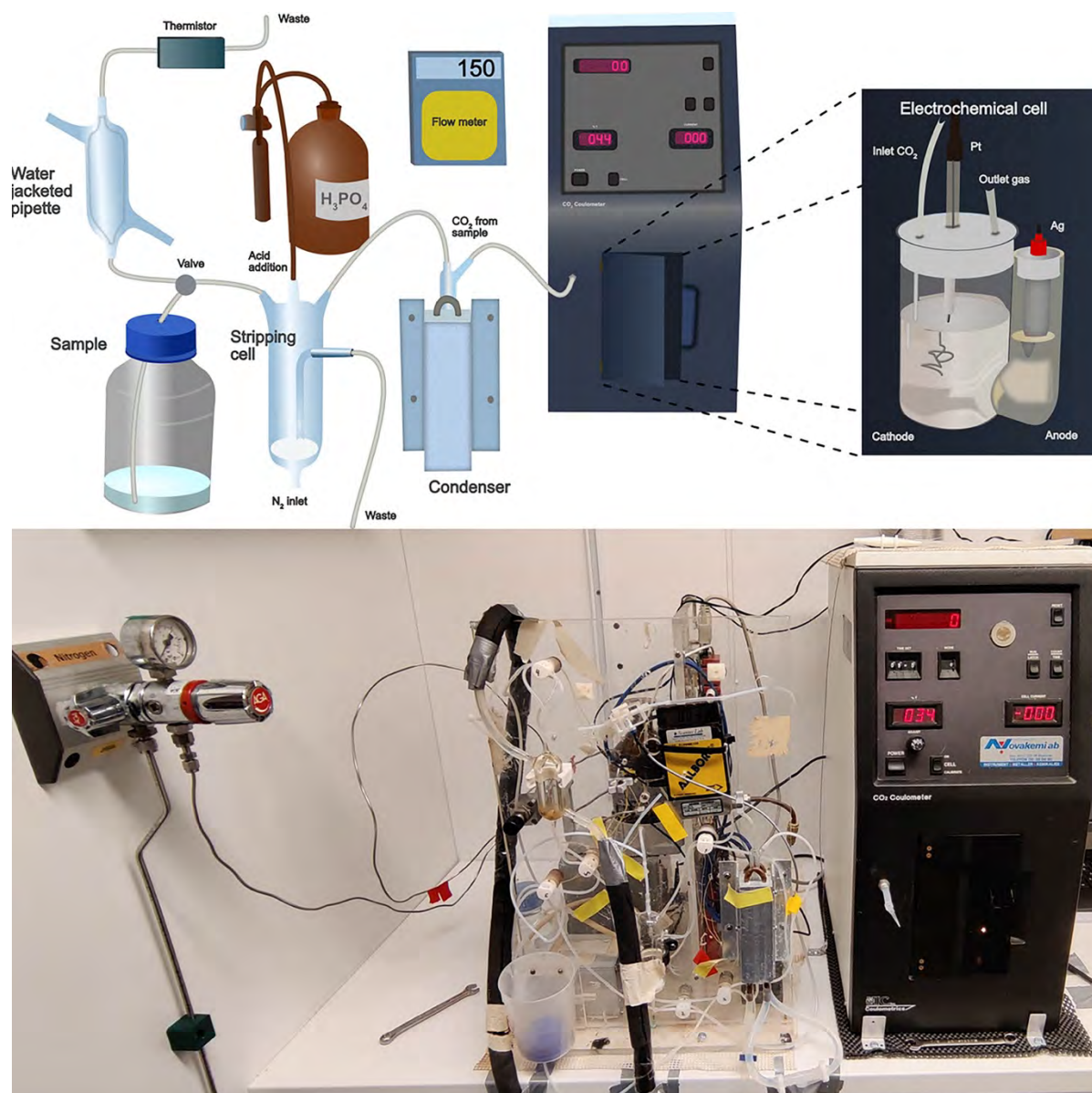
Figure 17.5: Setup of the MIDSOMMA coulometric titration system used for measuring dissolved inorganic carbon (DIC) during the SAS-Oden 2021 expedition. ©Anna Lunde Hermansson

Dissolved inorganic nutrients (on board)

The macro-nutrients phosphate, nitrate+nitrite, ammonium, and silicate (PO_4, NO_3+NO_2, NH_4, and SiO_4) were determined colorimetrically on board as soon as possible after sampling using a four-channel continuous flow analyser (QuAAtro039, SEAL Analytical, *Figure 17.6*). The instrument was set up to use QuAAtro Methods No. Q-064-05 Rev. 8, Q-119-11 Rev. 2, Q-069-05 Rev. 8 and Q-066-05 Rev. 5 for PO_4, NO_3+NO_2, NH_4, and SiO_4, respectively. These methods largely correspond to standard methods SS-EN ISO 15681-2:2018, SS-EN ISO 13395:1996, SS-EN ISO 11732:2005 and SS-EN ISO 16264:2004. The nutrient analyser was connected to an autosampler and was controlled by a computer with AACE software (version 7.11, SEAL Analytical).

Each analysis run was preceded by a calibration using standards prepared from stock solutions of K_2HPO_4, KNO_3, NH_4Cl, and a solution of a commercial stable silica compound solution ($SiCl_4$ in NaOH, Merck Titrisol Silicon Standard). For sea water samples, artificial seawater with 35 ‰ salinity

was used for preparing working standards and for system wash solution, whereas a 2 ‰ salinity solution was or ice water analyses. Usually, each sample was measured twice by repeating the sample run in the tray protocol in the software. Each run included samples for correcting for any drift as well as commercially available certified reference materials for nutrients in seawater (VKI QC SW4.1B for NH_4 and NO_3; VKI QC SW4.2B for PO_4 and Si). If analytical problems occurred, manifested as abnormal peaks, bad calibration or low sensitivity, the sample batch was re-analysed immediately after the problem was located and fixed.
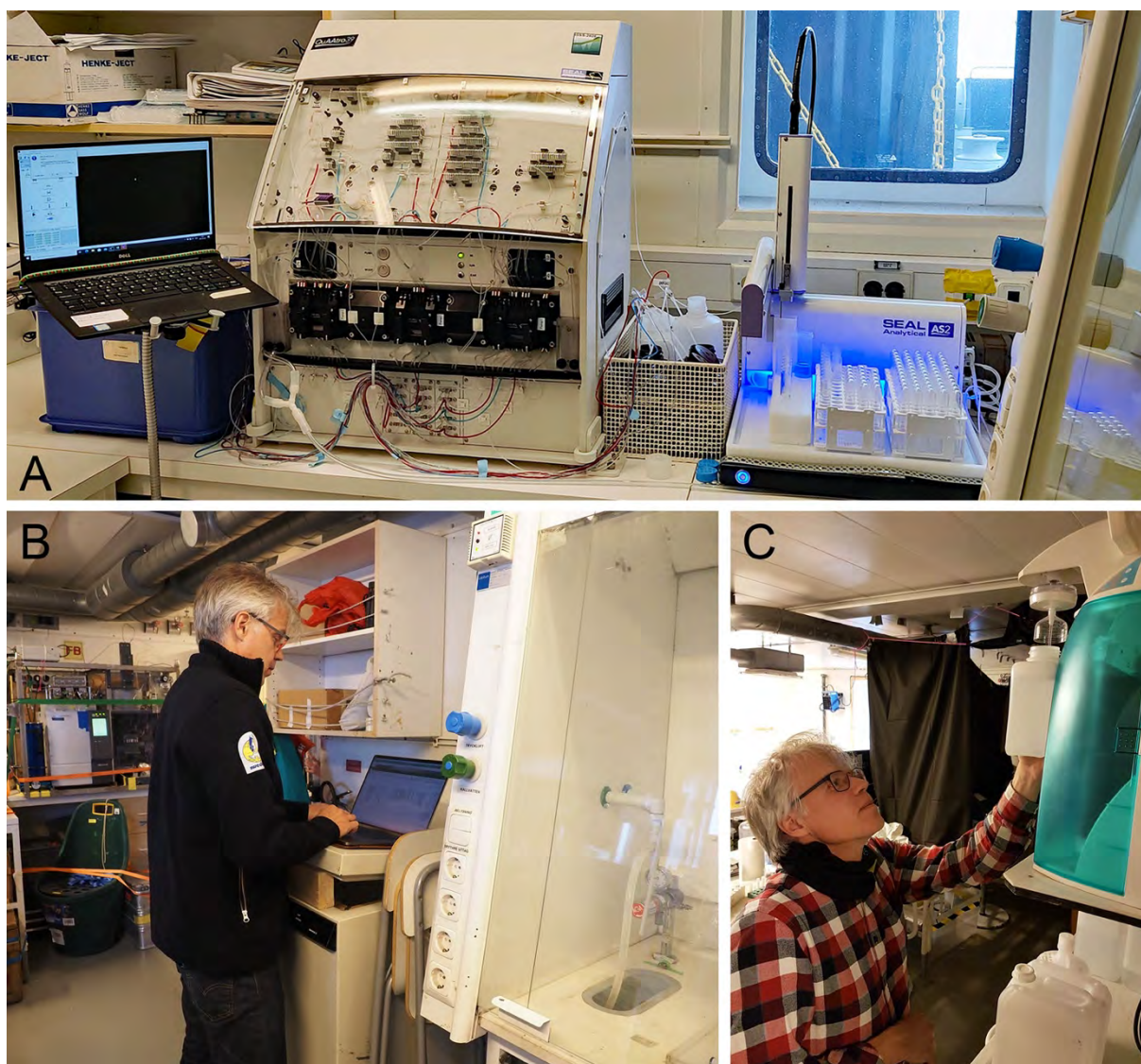
Figure 17.6: Measurements of dissolved inorganic nutrients during the SAS-Oden 2021 expedition. (A) Setup of the QuAAtro39 system in the clean laboratory in the "Main Lab" of IB Oden (starboard side), used for measuring dissolved inorganic nutrients during the SAS-Oden 2021 expedition. (B,C) Marcus Sundbom at work in the "Main Lab" of IB Oden during the expedition. (A) ©Adam Ulfsbo, (B) ©SPRS, (C) ©Yannis Arck

Underway seawater biological oxygen saturation (O₂/Ar ratios)

Biological oxygen supersaturation was measured continuously by Equilibrator Inlet Mass Spectrometry (EIMS) provided by Nicolas Cassar, Duke University, USA, a method previously described by Cassar et al.[150]. Briefly, seawater from the ship's underway system was pumped through a gas equilibrator, the headspace of which was connected to a quadrupole mass spectrometer for continuous elemental O₂/Ar ratio measurements. The ion current ratio was calibrated by periodically sampling ambient air. From the O₂/Ar supersaturation, a gas exchange rate, and the oxygen concentration at saturation, the net biological oxygen flux across the ocean surface may be estimated. However, the oxygen optode (Aanderaa 4835) stopped working during the initial setup phase, as did the computer-controlled flow (100 mL min^{-1}) of the gear pump. Unfortunately, this makes the collected underway measurements of O₂/Ar difficult to evaluate for the intended purpose of deriving the biological oxygen saturation and estimating rates of net community production.

Underway seawater pH

Seawater pH (total scale) was measured continuously every 10 minutes from the ship's underway system using a Kongsberg Contros HydroFIA pH[151]. Seawater was pumped from the same container as the O₂/Ar measurements. The flow-injection measurements were thermostated to 25°C and pH was calculated at constant salinity (35). Two measurements were done every 10 min initially, then one measurement per 10 min. Reagent cartridges with meta-cresol purple (0.01 mol L^{-1}) and HCl (0.1 mol L^{-1}) were purchased from 4H-JENA, Germany. Post-processing will include salinity correction using the ship's underway salinity measurements (SBE45).

Underway seawater total alkalinity

Total alkalinity was measured continuously every 10 minutes from the ship's underway system using a Kongsberg Contros HydroFIA TA[152]. Seawater was pumped from the same container as the O₂/Ar measurements. The flow-injection measurements were thermostated to 25°C and pH was calculated at constant salinity (35). The HydroFIA TA was calibrated with repeat calibrations with certified reference material (CRM Batch #181 and #191) during the expedition. Reagent cartridges with bromocresol green (0.002 mol L^{-1}) and HCl (0.1 mol L^{-1}) were purchased from 4H-JENA, Germany. Post-processing will include salinity correction using the ship's underway salinity measurements (SBE45).

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[150] Cassar N, et al. (2009) Continuous high-frequency dissolved O₂/Ar measurements by Equilibrator Inlet Mass Spectrometry. Analytical Chemistry 81:1855–1864 [https://doi.org/10.1021/ac802300u]

[151] Aßmann S, et al. (2011) Spectrophotometric high-precision seawater pH determination for use in underway measuring systems. Ocean Science 7:597–607 [https://doi.org/10.5194/os-7-597-2011]

[152] Seelmann K, et al. (2019). Characterization of a novel autonomous analyser for seawater total alkalinity: Results from laboratory and field tests. Limnology & Oceanography: Methods, 17:515–532 [https://doi.org/10.1002/lom3.10329]

17.5   Summary of metadata collected

The metadata collected by WP10 (CATCHEM) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP10_CATCHEM" in the SND data repository and summarised in *Table 17.2*.

Table 17.2: Overview of all measurements/samples collected by WP10 (CATCHEM) during the SAS-Oden 2021 expedition.

Parameter	Nr of samples	Analysis	Data owner
Dissolved oxygen	672	Onboard	Adam Ulfsbo
DIC	806	Onboard	Adam Ulfsbo
TA	806	Onboard	Adam Ulfsbo
pH	672	Onboard	Adam Ulfsbo
Dissolved inorganic nutrients	1225	Onboard	Marcus Sundbom
$\delta^{18}O$ of H_2O	421	Post-cruise (SU)	Marcus Sundbom
DOC	665	Post-cruise (SU)	Marcus Sundbom
POC	180	Post-cruise (SU)	Marcus Sundbom
CDOM	72	Post-cruise (DTU)	Marcus Sundbom
Salinity	60	Post-cruise (GEOMAR)	Toste Tanhua
TA underway	2877	Onboard	Adam Ulfsbo
pH underway	3975	Onboard	Adam Ulfsbo
Secchi depth	21	Onboard	Marcus Sundbom

17.6   Summary of preliminary results

A total of 673 water samples were collected from the CTD rosette and were analysed for dissolved inorganic nutrients, DIC, TA, pH, and oxygen onboard. Preliminary results are presented as depth profiles for DIC, TA and pH (*Figure 17.7*) and inorganic nutrients (*Figure 17.8*). Our results are overlain observations from the US[153] (GN01; HLY1502) and German[154] (GN04; PS94) Arctic GEOTRACES surveys in 2015 from the central Arctic Ocean for reference. The GN01 survey transects the Amerasian Basin from the Chukchi Sea to the North Pole and the GN04 survey transects the Eurasian Basin from the shelf between Svalbard and Franz Josef Land to the North Pole (*Figure 17.9*). Both cruises are included in GLODAPv2.2020[155]. Deep-water (>300 m) concentrations of DIC, TA, DO, nutrients and pH values during agree well with previous observations from the Arctic Atlantic Water of the central Arctic Ocean. There was a clear signal of the upper halocline (75–100 m) from the Makarov Basin, along the Lomonosov Ridge, to the Morris Jesup Plateau with maxima in DIC and nutrients and minima in oxygen and pH.

[153] Woosley RJ, et al. (2017) Internal consistency of the inorganic carbon system in the Arctic Ocean. Limnology & Oceanography: Methods 15:887–896 [https://doi.org/10.1002/lom3.10208]

[154] Jones EM, Ulfsbo A (2017) Seawater carbonate chemistry (TA, DIC, pH) measured on water bottle samples during POLARSTERN Cruise PS94 (ARK-XXIX/3) [https://doi.org/10.1594/PANGAEA.875883]

[155] Olsen A, et al. (2016) An internally consistent data product for the world ocean: the Global Ocean Data Analysis Project, version 2 (GLODAPv2). Earth System Science Data Discussions 1–78 [https://doi.org/10.5194/essd-2015-42]
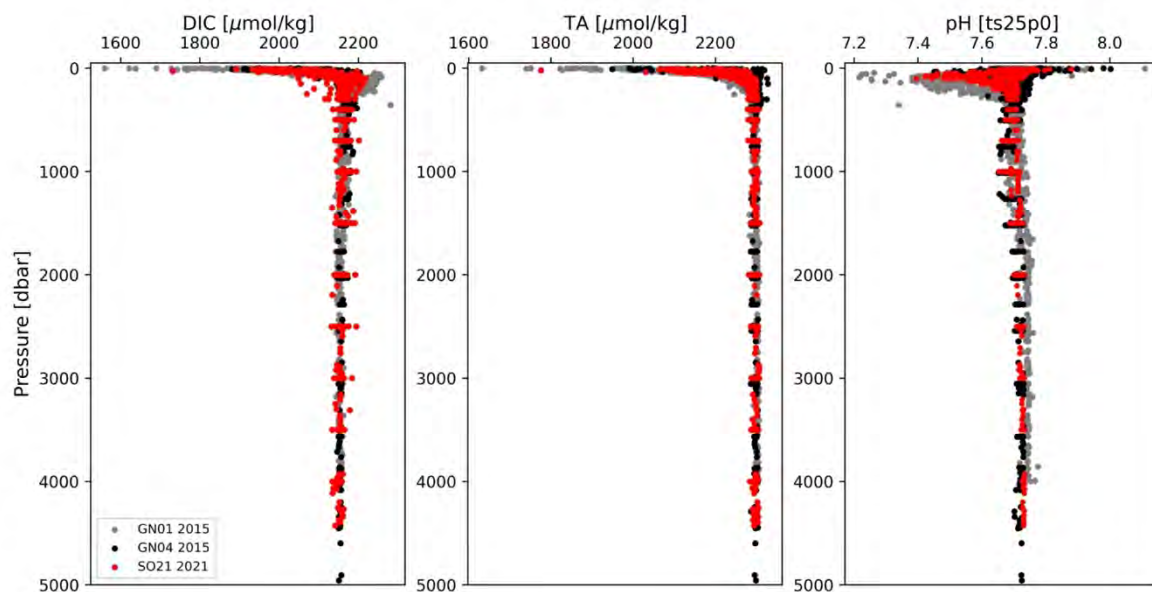
Figure 17.7: Depth profiles of dissolved inorganic carbon (DIC), total alkalinity (TA), and seawater pH (total scale, 25°C, 0 dbar pressure). Shown are our preliminary results from the SAS-Oden 2021 expedition (red) overlaying observations from the GN01 (grey) and GN04 (black) Arctic GEOTRACES surveys from the central Arctic Ocean in 2015. ©Adam Ulfsbo
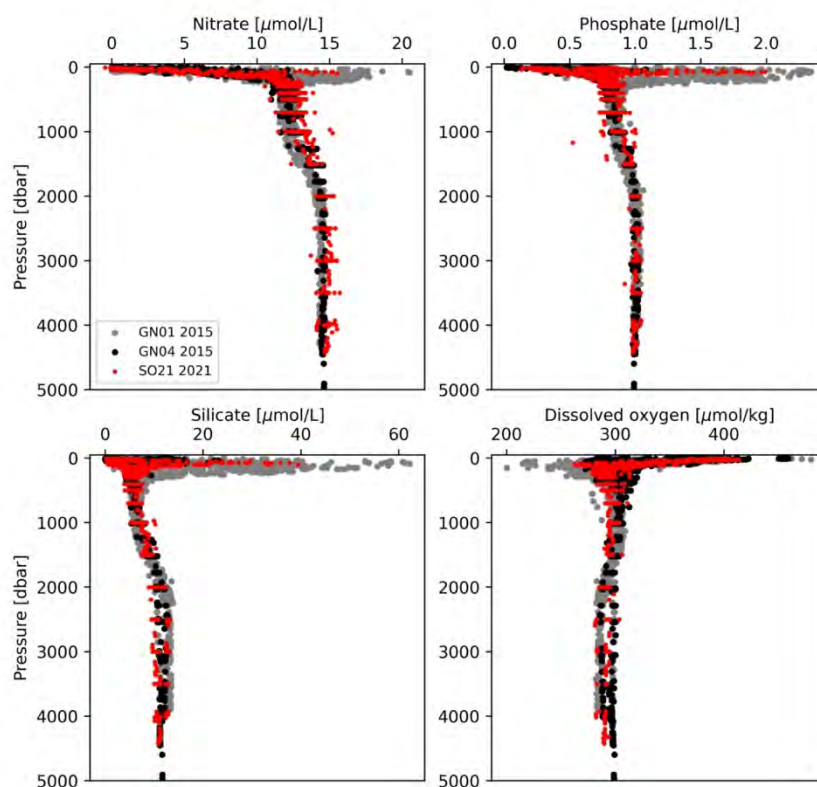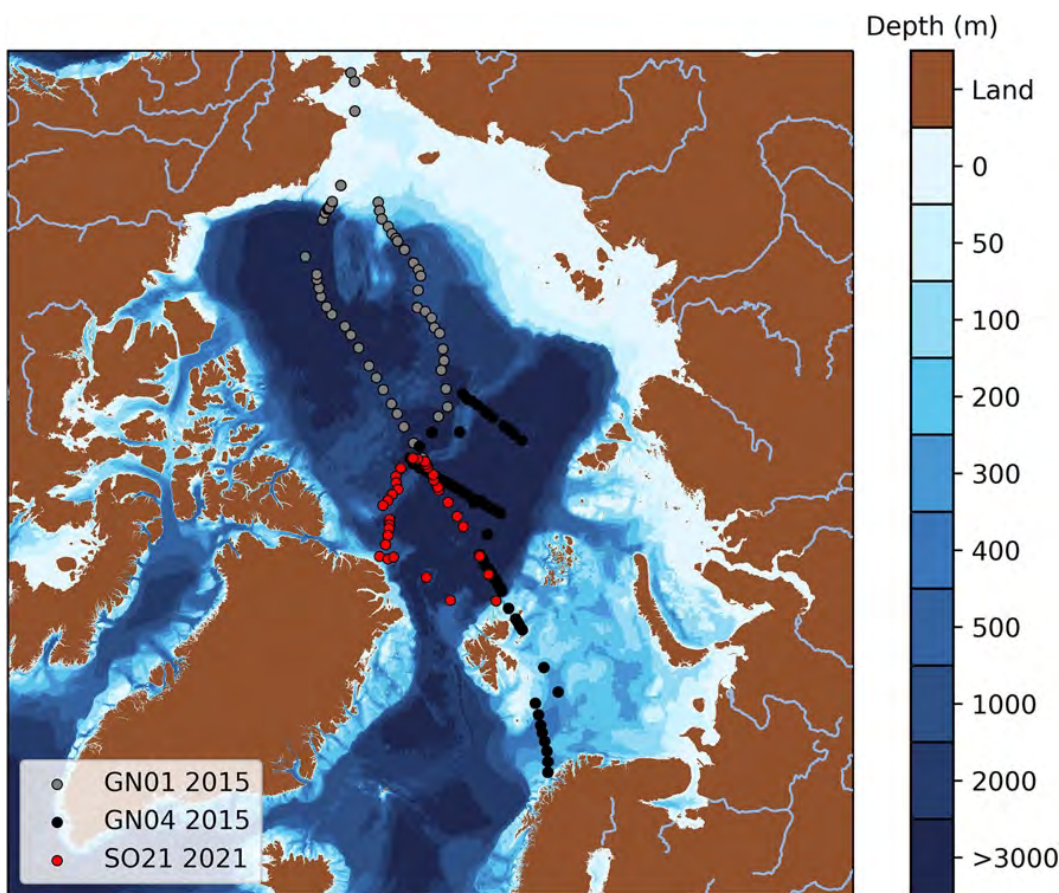

Figure 17.8: Depth profiles of dissolved inorganic nitrate+nitrite, phosphate, silicate, and dissolved oxygen Shown are our preliminary results from the SAS-Oden 2021 expedition (red) overlaying observations from the GN01 (grey) and GN04 (black) Arctic GEOTRACES surveys from the central Arctic Ocean in 2015. ©Marcus Sundbom

Figure 17.9: Map showing the CTD stations sampled by WP10 (CATCHEM) during the SAS-Oden 2021 expedition. Shown are also the stations during the 2015 Arctic GEOTRACES cruises GN01 (grey) and GN04 (black) for reference. ©Adam Ulfsbo

17.7   Summary of post-cruise analyses and deliverables

All measurements made onboard will undergo quality assurance (QA) and quality control (QC) after the expedition.

The $\delta^{18}O$ of H_2O, DOC, POC, and CDOM samples will be analysed post-cruise by Marcus Sundbom (SU, Sweden).

Sixty deep-water salinity samples were collected from 10 CTD stations and will be analysed post-cruise using a salinometer (Guildline Autosal) and IAPSO standard seawater[156] by Toste Tanhua (GEOMAR, Germany). The samples may be used to calibrate the two SBE 4 sensors on the bow CTD.

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[156] Kawano T (2010) Method for salinity (conductivity ratio) measurement. In: The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. Version 1. IOCCP Report Number 14, ICPO Publication Series Number 134 [http://www.go-ship.org/HydroMan.html]

18 WP11 (VACAO)

Lennart Gerke, Yannis Arck

Project title: Ventilation and anthropogenic carbon in the Arctic Ocean (VACAO)

18.1 Resources

External project funding: The Deutsche Forschungs Gesellschaft (DFG) for the project "Der arktische Ozean 2020 – Ventilationszeitskalen, anthropogener Kohlenstoff und Variabilität in einer sich verändernden Umgebung" (TA 317/8-1 and AE 93/21-1), the Arctic Research Icebreaker Consortium (ARICE, EU[157]), grand number 730965, including two berths on the SAS-Oden 2021 expedition from the Swedish Polar Research Secretariat (SPRS, Implementations Agreement Dnr 2020-119) to Toste Tanhua, and the Hasselblad Foundation (Contract No. 2019-1218) to Adam Ulfsbo and Leif Anderson. All project participants are listed in *Table 18.1*.

Table 18.1: Overview of all onboard and onshore participants of the WP11 (VACAO) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Toste Tanhua	ttanhua@geomar.de	PI	GEOMAR, Germany
Yes (berth)	Lennart Gerke	lgerke@geomar.de	WP Leader, sampling & analyses	GEOMAR, Germany
Yes (berth)	Yannis Arck	yannis.arck@iup.uni-heidelberg.de	Sampling & analyses	Univ. Heidelberg, Germany
Yes (WP10)	Adam Ulfsbo	adam.ulfsbo@marine.gu.se	Water chemistry (WP10)	GU, Sweden
No	Leif G. Anderson	leif.anderson@marine.gu.se	Water chemistry (WP10)	GU, Sweden
No	Céline Heuzé	celine.heuze@gu.se	Oceanography (WP15)	GU, Sweden
No	Werner Aeschbach	aeschbach@iup.uni-heidelberg.de	Noble gases, ^{39}Ar	Univ. Heidelberg, Germany
No	Markus Oberthaler	markus.oberthaler@kip.uni-heidelberg.de	Measurement of ^{39}Ar	Univ. Heidelberg, Germany
No	Norbert Frank	nfrank@iup.uni-heidelberg.de	Measurement and analysis of ^{14}C	Univ. Heidelberg, Germany
No	Núria Casacuberta	ncasacuberta@phys.ethz.ch	Measurement and analysis of ^{129}I, ^{236}U	ETH, Zürich, Switzerland

18.2 Scientific motivation and specific research questions

The first goal of WP11 (VACAO) is to obtain and constrain the timescales of ventilation of the Arctic Ocean. ^{39}Ar is an essential tracer when estimating the ventilation time scales of deep and intermediate water masses in the Arctic Ocean. It covers much longer time scales of 50-1000 years compared to CFC-12 and SF$_6$. The latter two tracers are limited to young water masses that are either close to the surface or in high ventilated deep waters. The new measurement technique of ^{39}Ar, namely Argon Trap Trace Analysis (ArTTA), now allows for standard oceanographic application due to the low amount of water needed for one sample (5-10 L) compared to the ~1000 L per sample using the Low Level Counting method. With ^{39}Ar and the measurement of the Tracers CFC-12 and SF$_6$ we are able to determine the ventilation timescales of the complete water column from surface to bottom in every part of the Arctic Ocean. Ventilation time scales are given by the mean age of transit time distributions that are constrained by a multi tracer approach.

The saturation of all transient tracers is determined by surface conditions as well as by interior mixing processes. Measurements of stable noble gases (He, Ne, Ar, Kr, Xe) are used to determine possible saturation anomalies that arise during ice formation, water subduction and interior mixing. One focus is set to measure surface saturation during ice-formation. A second one concerns subduction/

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convection processes. Analysing the saturation distortion for these different surface boundary conditions is of key importance for the correct input function of the tracers in polar waters and with this essential for constraining the ventilation timescales in the Arctic Ocean. The corrected input functions will reduce the uncertainty of the age distributions and improve the ocean circulation models.

Comparing the new tracer observations with historical data enables us to identify changes in the ventilation. This will be complemented by analysing biogeochemical processes regarding oxygen utilization rates and respiration processes such as formation of nutrient maxima in cooperation with the WP10 (CATCHEM) and WP15 (WAOW).

The second goal of WP11 (VACAO) is to estimate the anthropogenic carbon content of the Arctic Ocean. The precise mean age is essential for the calculation of the anthropogenic carbon content of the Arctic. Here we use the constrained distribution function and the biogeochemical data from WP10 (such as DIC, TA, etc.) to back-calculate the distribution of anthropogenic carbon in the ocean. The obtained concentrations of anthropogenic carbon are then used to determine the column inventory of the Arctic Ocean, which provide new estimates on the carbon uptake capacity. The analysis of the current and historic tracer data then provides information on changes in the carbon inventory and thus about the carbon uptake rates during the last 40 years. These results are essential to quantify the buffer effect of the ocean for the increasing concentrations of greenhouse gases as well as climate damaging compounds in the atmosphere.

The specific research questions of the WP11 (VACAO) project are:

(1) What are the timescales of ventilation in the Arctic Ocean?
- Validation and application of the noble gas techniques
- Application of ventilation tracers
- Tracer inter-comparison and synthesis
(2) How large is the anthropogenic carbon content of the Arctic Ocean?
(3) What does the surface circulation in the Arctic Ocean look like and where is the interface between the Pacific and Atlantic waters entering the Arctic Ocean?

18.3   Summary of field work performed

SAS Core Parameters: WP11 (VACAO) was responsible for sampling and analysing the SAS Core Parameters CFC-12 and SF_6 from the bow CTD (*Chapter 6.2*).

Sampling and analysis of CFC-12 and SF_6 (on board)

During the SAS-Oden 2021 expedition a gas chromatographic (GC) - electron capture detector (ECD) system was used in connection with a purge and trap unit (GC-ECD/PT5) for the measurements of the transient tracers CFC-12 and SF_6 (*Figure 18.1*). The system is a modified version of the set-up normally used for the analysis of CFCs[158]. This modified set-up is described in detail by Tanhua et al.[159,160]. This set-up allowed efficient and simultaneous analysis of both tracers.

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[158] Bullister JL, Weiss RF (1988) Determination of CC13F and CC12F2 in seawater and air. Deep Sea Research 35:839–853

[159] Tanhua T, et al. (2004) A first study of SF_6 as a transient tracer in the Southern Ocean. Deep Sea Research, Part II 51:2683–2699 [https://doi.org/10.1016/j.dsr2.2001.02.001]

[160] Tanhua T, et al. (2005) Spreading of overflow water from the Greenland to the Labrador Sea. Geophysical Research Letters 32:L10605 [https://doi.org/10.1029/2005GL022700]

The trap consisted of 100 cm 1/16 inch tubing packed with 70 cm Heysep D kept at temperatures between -60 and -70°C during the purge and trap process. The trap was desorbed by heating to 100°C and injected onto a pre-column of 30 cm Porasil C followed by 60 cm Molsieve 5A in a 1/8 inch stainless steel tubing. The main column consisted of 1/8" packed stainless steel tubing with 200 cm Carbograph 1AC (60-80 mesh) and a 20 cm Molsieve 5A post-column. All columns were kept isothermal at 50°C. Detection was performed with the electron capture detector (ECD).

Samples were drawn from Niskin bottles using 250-mL ground glass syringes, of which an aliquot of ca. 200 mL was injected to the purge-and-trap system. The sampling strategy was based on full depths profiles with up to 24 specific depths. The sampling depths were chosen due to the SAS Core Parameters to cover the most prominent features in the water column such as biological features and characteristics of certain water masses.

Injecting small volumes of gaseous standard containing CFC-12 and SF_6 performed standardization. This working standard was prepared by the company Deuste-Steiniger (Germany). The CFC-12 and SF_6 concentrations in the standard had been calibrated vs. a reference standard obtained from R.F Weiss group at SIO. Another calibration of the working standard will take place in the lab after the cruise. Calibration curves were measured roughly once every 2 weeks in order to characterize the non-linearity of the system, depending on work load and system performance. Point calibrations were always performed before and after each station to determine the short term drift in the detector. Replicate measurements were taken on several stations for data statistics. The final processing and calibration of the obtained transient tracer data will be performed onshore at the GEOMAR in Kiel.

Figure 18.1: Setup of the GC-ECD/PT5 system used for measuring CFC-12 and SF_6 during the SAS-Oden 2021 expedition. ©Lennart Gerke

Table 18.2: Detection limit and precision of PT5 system.

Parameter	CFC-12	SF_6
Limit of detection	0.2 fmol kg^{-1}	0.04 fmol kg^{-1}
Precision	0.02 pmol kg^{-1} / 1.5%	0.03 fmol kg^{-1} / 2.6%

Sampling for analysis of ³⁹Ar

For the analysis of ³⁹Ar, 5 to 10 L of water sample is needed due to its extremely rare abundance. Therefore, a complete Niskin bottle (12 L) was reserved on the CTD deep VACAO for this sample only. Similar to the stable noble gas isotopes, an exchange of the dissolved gases in the sampled water with the atmosphere must be avoided at any time. A helium leak-tight procedure has to be deployed while sampling and storing the water.

Regular industry propane gas bottles commonly used for camping activities have been proven to be suitable containers for ³⁹Ar samples. On the SAS-Oden 2021 expedition mainly fabric-new propane gas bottles of 12-L volume and also some smaller ones of 6-L volume were used to take samples from each depth of the VACAO stations, as well as some additional samples taken at the slope of the Lomonosov Ridge towards the Amundsen Basin near the North Pole.

Prior to the expedition the propane gas bottles were prepared to exclude any previous contamination caused by the sampling container itself. They were evacuated and flushed with pure nitrogen twice in Heidelberg before being shipped with a filling of 1.2 bar of pure nitrogen. Just before sampling, a special 3-way adapter was mounted onto the bottle's valve and the nitrogen was pumped out from the bottle through the adapter by a vacuum pump. The bottle and the adapter were then closed, so that the vacuum remained inside, thus excluding any contamination by the atmosphere. For sampling, the inlet of the 3-way adapter was connected via silicon tubing to the Niskin bottle and the system was rinsed for several seconds. The propane gas bottle was placed onto a regular analogue bathroom scale to monitor the fill level of water inside the bottle (*Figure 18.2 A*). To start the filling, the bottle's valve was opened and the 3-way adapter was turned towards the bottle and the water from the Niskin bottle could enter. After the bathroom scale showed ca. 9 kg of additional weight, the bottle was closed and the sampling was completed. The bottle was not filled completely, because the remaining water in the Niskin bottle might have started some equilibration with the atmospheric gas already and a certain head space is needed inside the propane gas bottle to facilitate subsequent degassing in the lab in Heidelberg.

Figure 18.2: WP11 (VACAO) sampling seawater from the CTD rosette at the bow during the SAS-Oden 2021 expedition. (A) Filling propane bottles for ³⁹Ar analysis. (B) filling aluminum bags for ¹⁴C analysis. ©Yannis Arck

Sampling for ²³⁶U and ¹²⁹I analyses

Sampling for later ²³⁶U analyses was performed at the six VACAO stations (Stations 5, 8, 16, 20, 28, 46; *Figure 1.1*) by filling 3-L cubitainers with seawater. The cubitainers were filled at eight different depths to cover the upper water column. Sampling for later ¹²⁹I analyses was performed at the same six

stations from the same depths by filling plastic bottles with a volume of 250 mL seawater. Additionally, samples were taken at 10 other stations from the upper water column.

Sampling of Medusa samples

Sampling of Medusa samples (HCFC-22, HCFC-141b, HCFC-142b, HFC-134, …) was performed at the six VACAO stations by filling glass ampoules with a volume of 1.1 L. The ampoules were filled with a specific steel construction and rinsed by letting the volume capacity run over 2 to 3 times. The ampoules were flame-sealed after sampling under continuous flow of N_2-gas.

Sampling for analyses of noble gases

The sampling of water for the analysis of the most abundant stable noble gas isotopes (He, Ne, Ar, Kr, Xe) was performed at the six VACAO stations from 11 depths. Some additional samples were taken at the slope of the Lomonosov Ridge towards the Amundsen Basin near the North Pole, as well as two deep samples at the station closest to Greenland (Station 50). During sampling and storage, a complete isolation of the seawater sample from the surrounding atmosphere has to be ensured at all times. Helium is the smallest and most volatile gas, and it can pass through any tiny leak and falsify the results. To cope with these high demands, a construction of an aluminium rail with two stainless steel clamps at both ends was used with a copper tube of ca. 50 cm length and 10 mm diameter. During sampling, the copper tube was connected to the Niskin bottle via regular silicon tubing and the copper tube was rinsed for several seconds (*Figure 18.3*). To eliminate any air bubbles inside the system, the tubing was squeezed especially at the connecting points and with the nut runner, which is used to close the clamps, hard knocks were applied to the aluminium rail, to loosen any air bubbles inside the copper tube. After this process, the water sample was sealed by closing the clamp at the outflow of the copper tube first, and then closing the clamp at the inflow. This process presses the copper very close together, comparable to helium leak-tight cold welding. Ca. 20 mL of seawater was taken twice (A and B samples) from each Niskin bottle.

Sampling for analysis of ¹⁴C

A new sampling method for the analysis of ¹⁴C in DIC was deployed during the SAS-Oden 2021 expedition. The samples were not poisoned after sampling, but instead biological activity was prevented by freezing them at -20°C. This posed new challenges to the sampling and storage routine. A regular gas-tight aluminium foil bag designed for gas sampling, with a total capacity of 1 L, was modified before sampling the water. The special valve to ensure that only gas can enter the bag was removed, and only a small stainless steel tube opening to the bag was left. Shortly before sampling, the sample bag was connected to a vacuum setup to flush the bag with pure nitrogen once, followed by an evacuation with a designated pump. This ensures no significant atmospheric intrusions inside the bag prior to sampling, and a lower resistance of the bag while the water is entering.

A short piece of silicon tubing was connected to the stainless steel tube opening of the bag, and the silicon tube's other end was connected to a Niskin bottle. The bag was filled about half-way to contain ca. 500 mL seawater sample (*Figure 18.2 B*). The silicon tube was detached from the Niskin bottle and the sample bag was squeezed by hand so that the air bubble that had entered during sampling could escape from the bag. Once only water flowed out of the silicon tube, it was quickly bent and sealed by a plastic clip commonly used to close bread bags. The samples were stored in a -20°C freezer and transported to the home lab after the expedition.

Figure 18.3: WP11 (VACAO) sampling seawater from the bow CTD during the SAS-Oden 2021 expedition. (A) Lennart Gerke sampling water for SF$_6$ and CFC-12 analysis. (B) Yannis Arck sampling water for SF$_6$ and CFC-12 analysis. (C) Yannis Arck sampling water from a Niskin bottle into copper tubes for the analysis of noble gases. (A,B) ©Anna Lunde Hermansson, (C) ©Yannis Arck

18.4   Summary of metadata collected

The metadata collected by WP11 (VACAO) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP11_VACAO" in the SND data repository and summarised in *Table 18.3*.

Table 18.3: Overview of all measurements/samples collected by WP11 (VACAO) during the SAS-Oden 2021 expedition.

Parameter	Nr of stations	Samples per station	Total nr of samples
CFC-12 and SF_6	34	7 to 26	709 (including replicates)
^{129}I	18	8 to 21	168
^{236}U	6	8	48
Medusa	6	7	41 (one sample broke)
Noble gases	9	11(A+B), 2-5 extra	144
^{39}Ar	8	11, 5 extra	76
^{14}C	6	11	65 (one sample broke)

18.5   Summary of preliminary results

The distribution of CFC-12 and SF_6 along a few stations from the North Pole, crossing the Lomonosov Ridge describes some specific ventilation pattern of different water masses (*Figure 18.4*). The different distribution of both tracers is based on their different atmospheric histories so that CFC-12 already covers the deeper and less ventilated water masses. The shallow and intermediate water masses show the characteristic tracer gradients from equilibrated or even oversaturated concentrations at the surface, which monotonically decline towards the bottom of each basin. Some significance is visible in the bottom water of the Makarov Basin, where the partial pressure of SF_6 can only be measured until a depth of 2000 m as compared to 3500 m in the Amundsen Basin. This indicates slow ventilated deep water and thereby old water in this basin. It is also visible in the CFC-12 data, where the detection is still possible but the partial pressure is lower as compared to the other basins.

18.6   Summary of post-cruise analyses and deliverables

CFC-12 and SF_6: The final processing and calibration of the obtained transient tracer data will be performed on shore at GEOMAR in Kiel (Germany). The running standard will be calibrated, as it was before the cruise and all the data will undergo quality control (QC). The preliminary data will be processed by, e.g., using the calibrated salinity and oxygen data from the sensors on the CTD. Additionally, the measurements and results from the noble gas analyses will give information on the saturation biases in polar waters and thereby help gain the correct input functions of the tracers in polar waters which is essential for constraining the ventilation time scales in the Arctic Ocean. The measurements of ^{39}Ar in addition to CFC-12 and SF_6 will help to determine the ventilation time scales of the complete water column from surface to bottom. Also, this will bring up another tracer to being used with the transit-time distribution (TTD) to gain more information on anthropogenic carbon uptake.
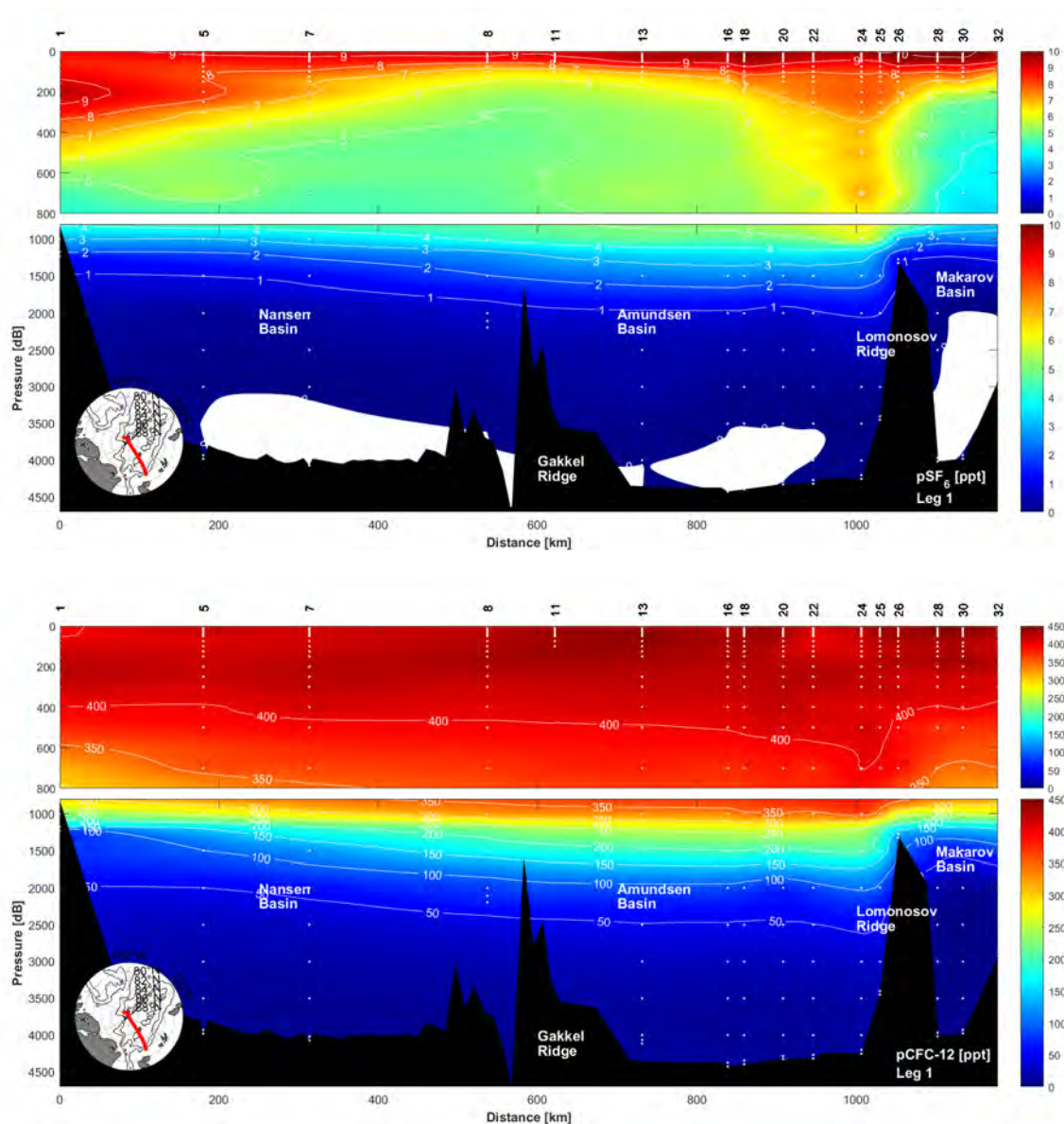
Figure 18.4: Preliminary distribution of SF$_6$ (upper panel) and CFC-12 (lower panel) partial pressure in ppt (parts per trillion) over Leg 1 of the SAS-Oden 2021 expedition. ©Lennart Gerke

[39]Ar: The still largely unknown and new technique of Atom Trap Trace Analysis (ATTA) is applied to measure the [39]Ar/Ar ratio dissolved in the water. Since it is a very rare radioactive isotope produced in the atmosphere it is assumed to be equally distributed throughout the globe. Its half-life of 269 years poses difficulties when analysing it with low level counting, hence large water masses and a long analysis time was required. With ATTA, the single decays are not counted anymore, but the [39]Ar atoms in the sample are slowed down by a laser system in a vacuum setup. A magneto-optical trap can catch and detect single atoms, and compared to the count rate of a known reference standard, the [39]Ar ratio in the sample can be deduced. Thus, the mean residence time of the water is given by the amount of [39]Ar atoms which have decayed since its last contact and equilibration with the atmosphere. For the delicate ATTA system, a purified argon sample of a few mL of gas has to be provided. This is obtained by a special purification setup which processes the water samples from the propane gas bottles. The bottle is connected to the setup and the water is degassed, while all gas is absorbed onto a trap. It is then sent through a titanium sponge getter, which reacts with all gases except the noble gases. Eventually, a few mL of pure argon are obtained which can then be analysed by the ATTA system.

236U and 129I: The samples will be processed and analysed at the Physical Oceanography Group and the Laboratory of Ion Beam Physics at Eidgenössische Technische Hochschule (ETH) in Zurich, Switzerland.

Medusa samples: The samples will be analysed with the help of the Medusa measuring instrument as soon as the samples arrive at GEOMAR in Kiel (Germany). The analytic system consists of a purge and trap unit attached to a gas chromatograph (GC) and a mass spectrometer (MS) for detection. This MS has to be used as the measured HCFC's and HFC's have low responses and large uncertainties when they are measured by an electron capture detector (ECD).

Noble gases: The seawater samples for stable noble gas isotopes will be analysed at the Institute of Environmental Physics at the University of Heidelberg (Germany). A well-established mass spectrometer system is capable of processing and analysing the special samples with high accuracy. The sample will be attached to the system, opened and degassed, while the noble gases are frozen onto two separate cold traps, one for He and Ne and one for Ar, Kr and Xe. To measure the individual concentrations of each noble gas isotope in the sample, the traps are subsequently heated to a certain temperature in order to only release one noble gas, which is then analysed by either a faraday cup, or an electron multiplier, depending on its abundance. Hence, by knowing the original water amount of the sample, the total amount of each dissolved noble gas isotope is investigated which gives rise to many different processes during the last equilibration at the water surface and also during interior mixing, causing possible saturation anomalies.

14C: The analysis of 14C will be performed at the University of Heidelberg (Germany), and will include two steps. First, the samples are melted and degassed to release all the dissolved inorganic carbon of the water into a preparation setup. A graphitization process is applied to capture the small carbon within the sample. Afterwards, it is sent to the Curt-Engelhorn-Zentrum Archäometrie gGmbH in Mannheim to measure the 14C content via accelerator mass spectrometry.

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

19 WP12 (TRACE) & WP13 (TGB)

Ian Brown, Lina Holthusen

Project title: Trace gases cycling in the Arctic marine ecosystem (TRACE)
Project title: Central Arctic Ocean trace gas biogeochemistry (TGB)

Note: The two projects WP12 (TRACE) with focus on nitrous oxide (N_2O) and WP13 (TGB) with focus on methane (CH_4) are presented together in this chapter because these two greenhouse gases were always measured from the same samples during the SAS-Oden 2021 expedition. One person from WP12 (TRACE) withdrew from the SAS-Oden 2021 expedition a few weeks before the start of the expedition. This berth was filled with Per Lundgren, an extra resource person from the Swedish Polar Research Secretariat (SPRS), to especially assist WP12 with field sampling when necessary.

19.1   Resources

External project funding of WP12 (TRACE): The Arctic Research Icebreaker Consortium (ARICE, EU[161]), grant number 730965, including two berths on the SAS-Oden 2021 expedition from the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) to Damian L. Arévalo-Martínez. All project participants are listed in *Table 19.1*.

Table 19.1: Overview of all onboard and onshore participants of the WP12 (TRACE) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Damian L. Arévalo-Martínez	darevalo@geomar.de	PI	GEOMAR, Germany
Yes (WP13)	Ian Brown	iaian2@pml.ac.uk	WP Leader, sampling and analyses	Plymouth Mar. Lab., UK
Yes (berth)	Lina Holthusen	lina.aleke.holthusen@uol.de	Sampling and analyses	GEOMAR, Germany

* Lina Aleke Holthusen was employed by GEOMAR (Germany) during the expedition, her new affiliation of is Carl von Ossietzky University of Oldenburg, Germany

External project funding of WP13 (TGB): The Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for one berth on the SAS-Oden 2021 expedition to Brett Thornton. All project participants are listed in *Table 19.2*.

Table 19.2: Overview of all onboard and onshore participants of the WP13 (TGB) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Brett Thornton	brett.thornton@geo.su.se	PI, post-cruise analyses	SU, Sweden
Yes (berth)	Ian Brown	iaian2@pml.ac.uk	WP Leader, sampling and analyses	Plymouth Mar. Lab., UK
No	Patrick Crill	patrick.crill@geo.su.se	Post-cruise analyses	SU, Sweden

19.2   Scientific motivation and specific research questions

N_2O and CH_4 are biogenically produced trace gases whose atmospheric concentrations are increasing at a rate in the order of 0.7 ppbv y^{-1}. Both gases are radiatively active, contributing approximately 6%

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[161] https://arice-h2020.eu

and 15% of "greenhouse effect", respectively, whilst N_2O contributes to stratospheric ozone depletion and CH_4 limits tropospheric oxidation capacity.

Globally, the ocean is considered to act as a net source of atmospheric N_2O while for CH_4 the ocean is close to equilibrium relative to the atmosphere. However, the Arctic Ocean is exceptionally susceptible to climate change. Recent studies have shown that surface seawater is warming faster than in other oceans and the documented retreat of sea-ice will increase light penetration, including UV. These environmental parameters are highly likely to act as stressors and alter the Arctic Ocean ecosystem structure and function, which in turn will feedback on climate. One of the key feedback mechanisms is derived from the cycling of climatically active trace gases whose production and consumption pathways are closely associated with several physical and biological processes.

The project address the biogeochemical cycling of the climate-relevant trace gas N_2O at the sea-air and ice-seawater interfaces in the Arctic Ocean (AO). Although the ocean is generally acknowledged as an overall source of this gas, at regional and basin-wide scales there is a large range of variability in terms of their sources and sinks, which in turn, poses challenges to the accurate assessment of their role in the marine nitrogen and carbon cycles. Environmental changes such as warming and decrease in sea ice coverage are expected to affect production/consumption pathways of both N_2O, but the direction of the future trends is highly uncertain. In response to the particular sensitivity of the AO to climate change, we will conduct TRACE, a study of pathways and emissions of this greenhouse gas within the context of the SAS-Oden 2021 expedition, as an important contribution to the Synoptic Arctic Survey (SAS). TRACE aims to fill the gaps of both data coverage and process understanding with respect to the marine cycling of N_2O in the AO.

During the expedition, we conducted a comprehensive sampling program focused on: (1) the assessment of the magnitude and spatial variability of sea-air fluxes of N_2O over variable ice conditions, and (2) deciphering the water column distribution and major formation pathways of this gas. To this end, the working program included underway and water column measurements. Yet, an evaluation of the fluxes of trace gases through the sea-ice-atmosphere interface can only be partially resolved in the absence of direct measurements within sea ice. Moreover, the dynamics of water directly in contact with bottom ice, where biogeochemical rates are expected to be high, are hard to disentangle with traditional underway methods since they, by necessity, mix the upper ~10 m of the water column as the ships breaks through the ice. In order to account for this gap and to improve our mechanistic understanding of the role of sea-ice variability in the production and emission of N_2O, this gas was added to the core parameters sampled at ice stations during the expedition,

Considering the large gaps of data coverage and process understanding with respect to the marine cycling of N_2O and CH_4 in the Arctic marine ecosystem, the major goals are to:

(A) Ascertain the magnitude and spatial variability of sea-air fluxes of N_2O to the atmosphere over variable ice conditions in the central Arctic Ocean.
(B) The annual emission of CH_4 from the Arctic Ocean remain uncertain; (current estimate at 2.1 Tg CH_4/year (high uncertainty); 0.3 5% of the global emissions).
(C) CH_4 emissions from the central Arctic Ocean are relatively unknown, where near surface production of CH_4 are the likely source. Under ice measurements have shown supersaturations of CH_4. We want to quantify summertime emissions in the central Arctic Ocean, when polynyas and ice melt provide emission pathways.
(D) Elucidate the water column distribution of N_2O and CH_4 in the central Arctic Ocean.
(E) Determine N_2O and CH_4 exchange in sea ice melt ponds, a relatively unquantified part of the ecosystem which may become more common in a warmer Arctic (cooperation with WP16, ACAS).

(F) Measure any sea air emissions of CH_4 collocated with seafloor gas seeps (cooperation with WP14, MWA).

The specific research questions of the WP12 (TRACE) and WP13 (TGB) projects are:

(1) Is the CAO a net source or sink of atmospheric N_2O and CH_4? (Ascertain the magnitude and spatial variability of the N_2O and CH_4 fluxes across the sea ice air continuum over variable ice conditions in the central Arctic Ocean)
(2) Do the canonical pathways for N_2O cycling also take place within sea ice? Are the biological communities alike? (Elucidate the major pathways involved in N_2O production/consumption across the water column)
(3) Will increased sea ice melting lead to enhanced emissions of N_2O to the atmosphere? (Assess the potential effects of future ocean warming in the biogeochemical cycling and emissions of N_2O)

19.3   Summary of field work performed

SAS Core Parameters: WP12 (TRACE) was responsible for measuring N_2O concentrations, and WP13 (TGB) for measuring CH_4 concentrations in seawater and different ice habitats.

Discrete sea water samples were collected from Niskin bottles on a CTD rosette with clean Tygon® tubing into 500-mL borosilicate bottles (*Figure 19.1*). Samples were overfilled with three times the bottle volume to eliminate air bubbles and poisoned with 200 µL of a saturated mercuric chloride solution. They were then transferred to a water bath at $25 \pm 0.1°C$ and temperature equilibrated for a minimum of one hour before analysis. Further discrete samples were also collected from the ships underway supply.

Figure 19.1: Lina Holthusen from WP12 (TRACE) taking a seawater sample from a Niskin bottle on the CTD rosette during the SAS-Oden 2021 expedition. ©Anna Lunde Hermansson

Sea ice was sampled by extracting an ice core at the SAS Ice Stations (*Figure 19.2 A*, *Chapter 6.7*). the ice core was sliced into 15-cm sections, each packed into a 2-L gas-tight Tedlar® bag (*Figure 19.2 B*) which allows the remaining air to be manually evacuated. The sealed bag was then transported to the ship where mercuric chloride was added to preserve the sample and the air was evacuated. All slicing was performed on the ice. Once on board, ice samples are allowed to thaw under ambient temperature. Upon full melting, resulting water was transferred to 500-mL glass bottles and analysed on board.

In order to measure N_2O and CH_4 concentrations in waters directly beneath (and in contact with) sea ice under undisturbed conditions, a Ruttner sampler was deployed below the bottom of the sea ice (*Figure 19.3*). The system consisted of a sampling bottle open at each end and closed by sending a messenger to close the sampler once the required depth is achieved. The sample was transferred to a glass bottle, preserved with mercuric chloride and analysed on board.

Samples from melt ponds and leads were collected by directly filling the bottle at the water surface. Profiles were collected in the leads using the Ruttner sampler to a depth of 1 m below the ice bottom every 50 cm. All samples were preserved with mercuric chloride and analysed on board.

Samples were analysed for CH_4 and N_2O by single-phase equilibration gas chromatography using a flame ionisation detector and electron capture detector similar to that described by Upstill-Goddard et al.[162]. Samples were typically analysed within 12 hours of collection and calibrated with the three standards. (*Figure 19.4*)
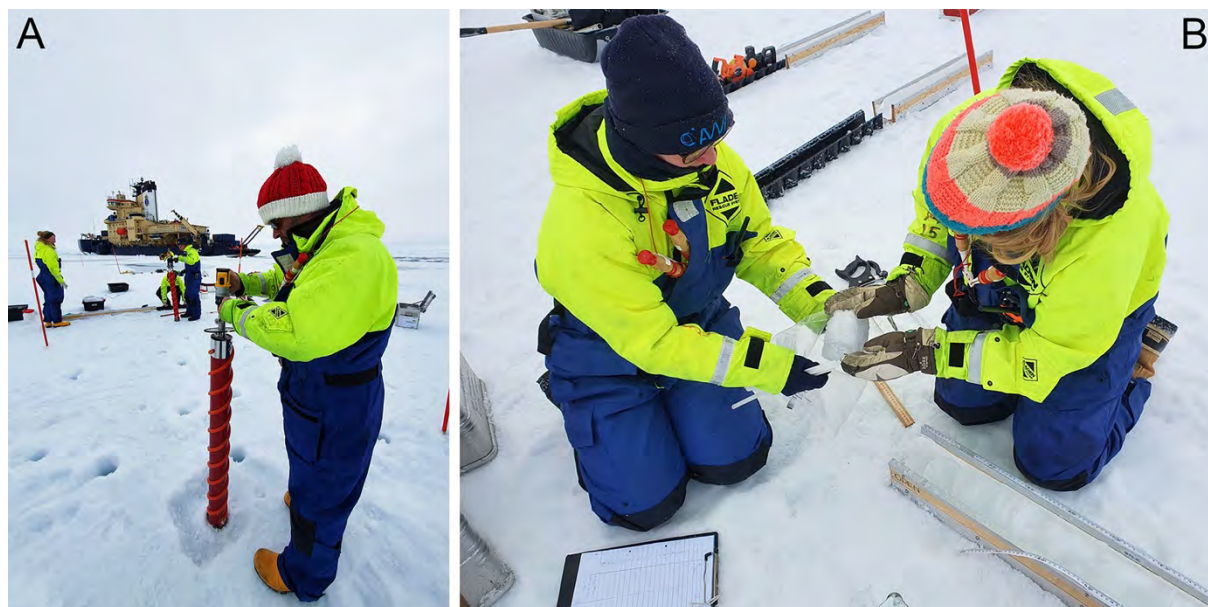
Figure 19.2: WP12 (TRACE and WP13 (TGB) sampling ice samples during the SAS-Oden 2021 expedition. (A) Ian Brown from WP12 (TRACE) and WP13 (TGB) deploying a 9-cm diameter Kovacs ice corer operated by a battery-driven drilling engine. (B) Lina Holthusen from WP12 (TRACE) and Anna Lunde Hermansson from WP10 (CATCHEM) packing ice sections in a Tedlar® bag. (A) ©Anna Lunde Hermansson, (B) ©Amanda Nylund

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[162] Upstill-Goddard RC, et al. (1996) Simultaneous high-precision measurements of methane and nitrous oxide in water and seawater by single phase equilibration gas chromatography. Deep Sea Research, Part I 43:1669-1682 [https://doi.org/10.1016/S0967-0637(96)00074-X]

Figure 19.3: Ian Brown from WP12 (TRACE and WP13 (TGB) taking a water sample from the ice-seawater interface with a Ruttner sampler. ©Anna Lunde Hermansson

Figure 19.4: Ian Brown from WP12 (TRACE) and WP13 (TGB) elaborating measurements in the chemistry lab at IB Oden *during the SAS-Oden 2021 expedition. ©Anna Lunde Hermansson*

19.4   Summary of metadata collected

The metadata collected by WP12 (TRACE) and WP13 (TGB) during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP12+13_TRACE+TGB" in the SND data repository and summarised in *Table 19.3*.

Table 19.3: Overview of all measurements/samples collected by WP12 (TRACE) and WP13 (TGB) during the SAS-Oden 2021 expedition.

Parameters	Sample type	Number of stations	Total nr of measurements
N_2O and CH_4	Underway surface water	120	120
N_2O and CH_4	Seawater from CTD	33	605
N_2O and CH_4	Ice and water from ice stations	10	151

19.5 Summary of preliminary results

Preliminary results of the water-column concentrations of N_2O and CH_4 are shown in *Figure 19.5* and preliminary results of the underway surface-water concentrations of CH_4 are shown in *Figure 19.6*.

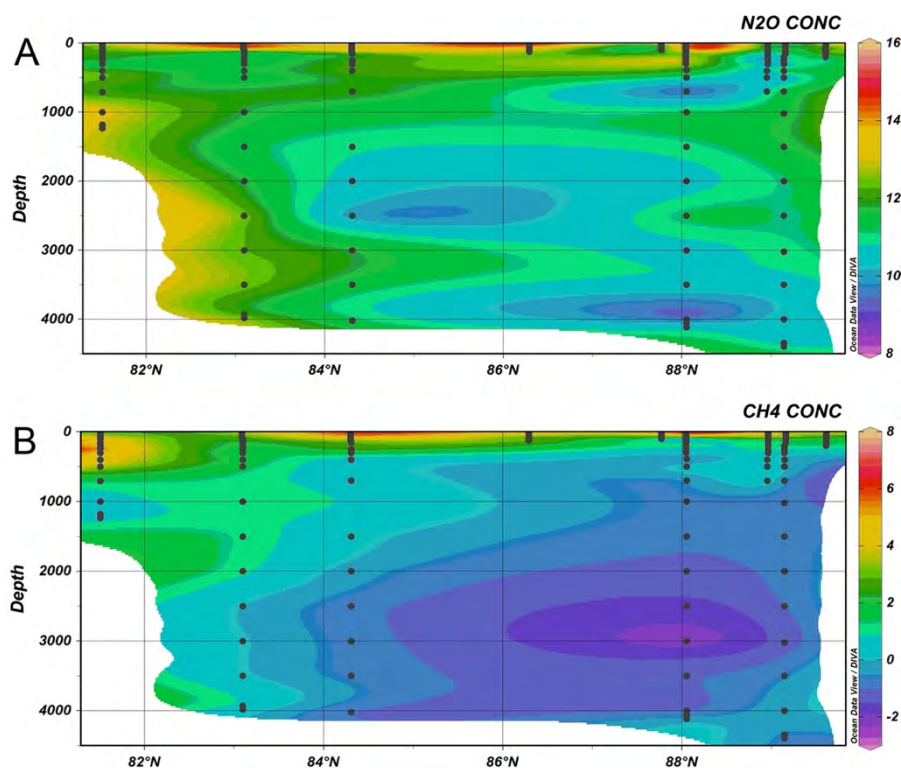*Figure 19.5: Preliminary results of the water-column concentrations of (A) N_2O and (B) CH_4, measured by WP12 (TRACE) and WP13 (TGB), respectively, during the SAS-Oden 2021 expedition. ©Ian Brown*

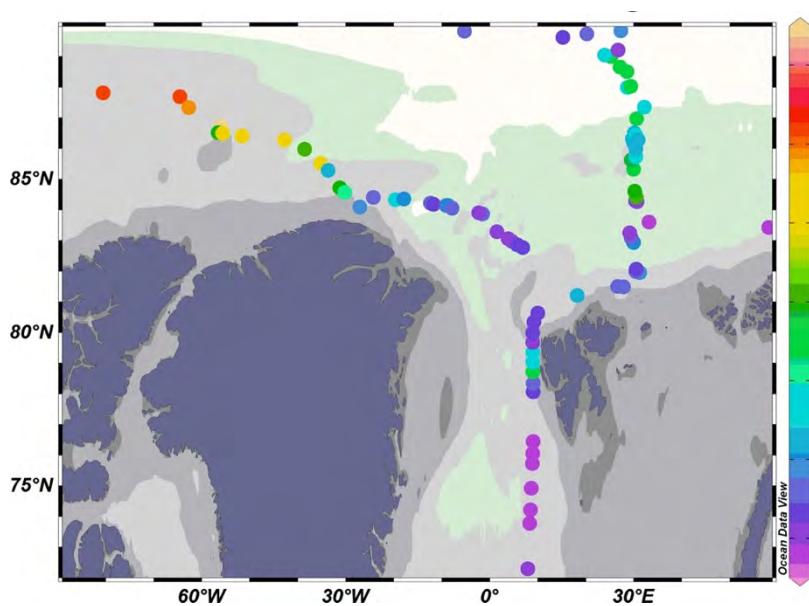*Figure 19.6: Preliminary results of the underway surface-seawater concentrations of CH_4 measured by WP13 (TGB) during the SAS-Oden 2021 expedition. ©Ian Brown*

19.6   Summary of post-cruise analyses and deliverables

Further data processing will continue and a final processed and quality-checked data set will be produced.

Collaboration is anticipated between GEOMAR (Germany), Stockholm University (Sweden), and Plymouth Marine Laboratory (UK), in combining with previous and subsequent data sets for publication. Collaboration is also anticipated with WP16 (ACAS).

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). The data will also be submitted to The British Oceanographic Data Centre (BODC). The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

20 WP14 (MWA)

Julia Muchowski, Alexandra Padilla, Caroline Bringensparr, Carlo Castro

Project title: Midwater acoustics (MWA)

20.1 Resources

External project funding: Swedish Research Council (VR, grant number 2018-04350) and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for four berths on the SAS-Oden 2021 expedition to Christian Stranne. JM was funded during the expedition by EFICA (WP1). The participation of CC and CB was financed by the Nippon-Foundation-GEBCO-Seabed 2030 project. All project participants are listed in *Table 20.1*.

Table 20.1: Overview of all onboard and onshore participants of the WP14 (MWA) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Christian Stranne	christian.stranne@geo.su.se	PI	SU, Sweden
Yes (berth)	Julia Muchowski*	julia.muchowski@geo.su.se	WP Leader, CTD, acoustics	SU, Sweden
Yes (berth)	Alexandra Padilla	apadilla@ccom.unh.edu	CTD, acoustics	Univ. New Hampshire, USA
Yes (berth)	Caroline Bringensparr	caroline.bringensparr@geo.su.se	Seafloor acoustics	SU, Sweden
Yes (berth)	Carlo Castro	carlos.castro@geo.su.se	Seafloor acoustics	SU, Sweden
No	Martin Jakobsson	martin.jakobsson@geo.su.se	Advisory	SU, Sweden
No	Lars Arneborg	lars.arneborg@smhi.se	Advisory	SMHI and GU, Sweden

20.2 Scientific motivation and specific research questions

In this project we combine acoustic observations of the water column and the seafloor with in-situ CTD measurements to learn about:

Thermohaline stratification and vertical mixing: Broadband acoustic observations provide insights into where and when mixing occurs in the open ocean, which has important implications for our understanding of vertical heat transport in the Arctic Ocean, and the sensitivity of the Arctic sea ice cover to temperature changes in the inflowing Atlantic surface waters.

Ocean surface mixed layer depth (MLD) and its variability: Our knowledge of high-frequency temporal and spatial variability of the MLD is very limited. With the new breed of wideband acoustics, we can increase the spatial and temporal resolution of MLD variability by several orders of magnitude compared to traditional CTD measurements. The MLD is an important parameter controlling, to a large extent, ocean-atmosphere gas fluxes and ocean primary production.

Methane gas plumes in the water column: There is concern that in a warming climate, methane hydrates stored in marine sediments in the Arctic Ocean will melt. When methane hydrate melts, free methane gas is formed within the sediments. If large quantities of methane gas reach the atmosphere, they can potentially reinforce climate warming. Using acoustics, we try to identify potential gas vents in the Arctic Ocean – this is also important for future assessments of changes in seafloor methane gas escape. This component can be matched ocean atmosphere flux measurements of methane gas, carried out by WP16.

Assessment of the entire water column (in collaboration with WP1): A combination of wideband echo sounders with different frequency ranges (WBAT, mounted on the CTD operated from the stern, max. depth 1000 m and ship-mounted EK80), an Underwater Vision Profiler (UVP, mounted on the CTD operated from the bow, max depth seafloor), plankton nets and turbidity/fluorescence data from CTD sensors, can provide a unique picture of the oceanographic and biological processes in the water column.

Seafloor and sub-bottom habitats: The Arctic Ocean north of Greenland is one of the least mapped regions in the world. This project includes seafloor mapping of uncharted waters along the expedition route and provides bathymetric data to the International Bathymetric Chart of the Arctic Ocean (IBCAO)[163] under the auspices of the "Seabed 2030" project, which has the goal of having all of the World oceans mapped by 2030. The multibeam sonar and chirp sub-bottom profiler of *IB Oden* collected depth data and information about bottom hardness and the uppermost 50-200 m of the seafloor sediments. This geophysical mapping component provides the crucial geospatial framework for all measured and sampled parameters of the water column and seabed. It also serves as a base for a bottom habitat assessment. In addition, the mid-water multibeam data were logged as a complement to the wideband acoustics.

The specific research questions of the WP14 (MWA) project are:

(1) Can we observe stratification, vertical mixing, and the mixed layer depth with acoustics to estimate the vertical transport of heat and nutrients which are important for local ecosystems as well as the large scale circulation?
(2) Can we detect gas seeps in deeper areas? How abundant are hydrate systems and seafloor gas escape in the Arctic Ocean today? This is particularly interesting near the continental shelf slope north of Greenland.
(3) Do the echosounder data indicate the presence of persistent bottom currents at the Greenland margin? Are there drift deposits along the slopes of the Lomonosov Ridge and/or North? Sediment drifts provide direct information about prevailing bottom current activities, and are prime coring targets holding high-resolution paleoceanographic information.

20.3   Summary of field work performed

Note: The part of the field work performed by WP14 (Julia Muchowski and Alexandra Padilla as members as the CTD team) is reported in *Chapter 21*. Together with WP15 (WAOW), WP14 (MWA) was responsible for the organization of the CTD casts and CTD data retrieval in two daily 12-hour shifts: WP14, 2 persons during day time 8:00-20:00; WP15, 1 person during night time 20:00-8:00. Adam Ulfsbo (WP10) was responsible for the water budgets of the Niskin bottles of the bow CTD. All CTD deck sheets and water budgets were scanned and are available in the SND data repository.

SAS Core Parameters: Together with WP15 (WAOW), WP14 (MWA) was responsible for measuring the SAS Core Parameters pressure (depth), salinity, and temperature in CTD depth profiles from the bow CTD (62 casts) and the stern CTD (50 casts) (*Chapters 6.2 and 6.3*). The CTD team also contributed to the acquisition of data from the following sensors mounted on the two CTDs: PAR, turbidity meter, fluorometer, oxygen, CDOM as well as a LADCP (*Figure 20.1 A,B*), a magnetometer (IMP, *Figure 6.1 D*), an additional light sensor (*Figure 20.1 D*), and from the stand-alone measuring

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[163] Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

devices WBAT (*Figure 8.7*) and UVP (*Figure 20.1 B*) of WP1 (EFICA). WP14 (MWA) was also responsible for running the EK80 echosounder together with WP1 (EFICA).

Figure 20.1: Overview of CTD work. (A) Upward looking LADCP transducer mounted on bow CTD, inset shows broken ADCP pin, (B) downward looking LADCP transducer and UVP mounted on bow CTD, inset shows corroded UVP connector, (C) Adam Ulfsbo (WP10), Alexandra Padilla (WP14), Hans-Jørgen Hansen (SPRS technician) and Julia Muchowski (WP14) preparing the bow CTD for deployment. (D) Alexandra Padilla starts the small additional light sensor mounted on the upper frame of the stern CTD. (A,B,D) ©Julia Muchowski, (C) ©SPRS.

Acoustic data collection

The hull-mounted acoustic equipment was operated from the bridge of *IB Oden* in permitted areas whenever possible during the expedition to collect as much data as possible (*Figure 20.2*). The work stations were operated 24 hours per day in two daily 12-hour shifts by Caroline Bringensparr 20:00-08:00 and Carlos Castro 8:00-20:00. The EK80 wideband transceiver and ES18 split-beam transducer settings were optimized for water-column data collection by Alexandra Padilla, Julia Muchowski and Serdar Sakinan (WP1). The multibeam echosounder (MBES) and the sub-bottom profiler (SBP) settings were optimized for seafloor mapping by Caroline Bringensparr and Carlos Castro. The CTD-mounted acoustic devices were operated during as many CTD casts as possible by Julia Muchowski and Alexandra Padilla in shifts in collaboration with WP1 (Serdar Sakinan and Julek Chawarski) and WP15 (Salar Karam). All raw data from hull-mounted and CTD-mounted equipment were backed up daily to an external hard drive with an additional copy on a redundant array of independent disks (RAID).
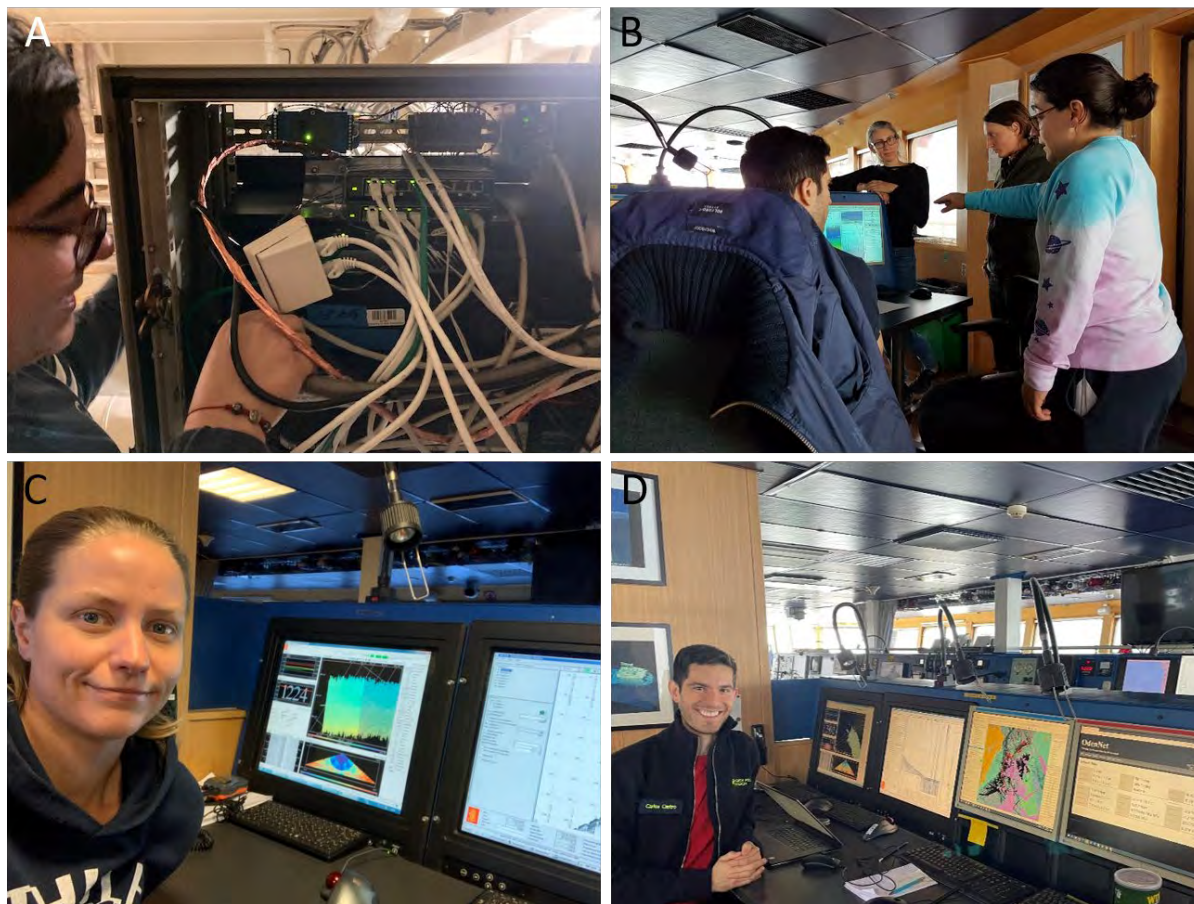
Figure 20.2: Acoustic data collection at the acoustic control centre on the bridge of IB Oden. *(A) Installation of EK80 transceiver. (B) From left to right: Carlos Castro (WP14), Clare Bird (WP9), Julia Muchowski (WP14), and Alexandra Padilla (WP14). (C) Carlos Castro (WP14), (D) Caroline Bringensparr (WP14) (A) ©Julia Muchowski, (B) ©Yannis Arck, (C,D) ©SPRS*

Expedition logbook

An expedition logbook for all Device Operations from the ship (excluding the ice stations), acoustics and other ship events was made in QGIS (version 3.16.7-Hannover) and was operated on the bridge, mainly by Caroline Bringensparr and Carlo Castro. Date, time, current position and modelled IBCAO depths (version 4.1) were generated automatically, while all other information was added manually, through checkboxes and fields, in an attribute form (*Figure 20.3*) and collated into a table with one line per event. This table was exported to an excel file that was placed on the *IB Oden* data server available to all expedition participants.

The final Logbook file underwent quality assurance (QA) and quality control (QC) by the Chief Scientist Pauline Snoeijs-Leijonmalm after the expedition and its final version is the excel file "SO21_Expedition_Logbook" in the SND data repository. For further details regarding the Expedition Logbook, see *Chapter 5*.

Figure 20.3: Screenshot of the Attribute form from QGIS used for logging Device Operations and other ship events during the SAS-Oden 2021 expedition.

EK80 wideband transceiver and ES18 split-beam transducer

Note: This section was written by the whole EK80 team: Serdar Sakinan (WP1), Julek Chawarski (WP1), Julia Muchowski (WP14), and Alexandra Padilla (WP14).

For acoustic studies of the water column a Simrad EK80 wideband transceiver was connected to the ES18 split-beam transducer installed at the hull of *IB Oden* as described in the Ryder 2019 Expedition Report[164] (WP Water Column Imaging, pages 59-60; *Figure 20.2 A*). With this system we continuously collected data whenever in permitted areas. Two major settings were used to optimize for the purposes of the WP1 (EFICA) project (*Chapter 8*) and the WP14 (MWA) project.

As established during Ryder 2019 and previous expeditions[165] with *IB Oden*, the frequency range 15-25 kHz was used for geophysical mapping. The recording range included the seafloor, EK80 synchronized with MBES and SBP, trigger pulse from MBES, varying synch delay to minimize interference between the systems. For WP1 (EFICA) the frequency range was 16-19 kHz, recording range 1200 m, EK80 in standalone mode, MBES and SBP secured.

The newest EK80 software could not be installed during the SAS-Oden 2021 expedition because it required Windows 10, which was not available on the Simrad EK60 computer in the server room on deck 5 of *IB Oden*. We, therefore, used the latest software version compatible with Windows 7 (v1.12.2) and re-entered X,Y,Z offsets and Rotation offset of Motion Unit in comparison to the ES18.

Communication between the transceiver and the EK computer was achieved as follows:

(1) Write down the IP address of ES18 WBT (look up under control panel, devices)
(2) Change the IP address of the EK computer so that it can communicate with WBT, which consists of an IP address of three identical triplets and a sub-mask of 0 (e.g., 255.255.255.0) - this will search for all systems with IP addresses that have the first three triplets the same and the last triplet different, but can be any number

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[164] Jakobsson M, Mayer L, Farrell J, and Ryder 2019 Scientific Party (2019) Expedition Report SWEDARCTIC Ryder 2019. [https://www.diva-portal.org/smash/get/diva2:1458256/FULLTEXT01.pdf]

[165] The SWERUS Scientific Party (2017) The Swedish-Russian-US Arctic Ocean Investigation of Climate-Cryosphere-Carbon Interactions - The SWERUS-C3 2014 Expedition [https://bolin.su.se/data/swerus/reports/leg1/Cruise%20report%20SWERUS-C3%20–%20Leg%201.pdf]

(3) Connect in EK80 Simrad software and talk to WBT

(4) Change the IP address of the WBT to the IP address the EK60 GPT had before replacing it

(5) Change the IP address of the EK computer back to what it was

Procedure for the change of IP address is: Control panel -> Network and Internet -> Network Connections -> Transceiver Unit Teamed, right click, properties, Internet Protocol Version 4 -> properties -> change IP address and sub-mask.

The offsets used for the Motion Reference Unit (MRU) and the Global Positioning System (GPS) sensor are shown in *Table 20.2*. The positions were relative to the null position (0,0,0) at water level (1.3 m above null position), and therefore relative to point (0, 0, 1.3). The MRU and GPS positions were taken from the document "Inmätta punkter på Oden 2007-04, samt omräknade till Odens system med ny nollpunkt, version 07-05-16_1". The EK transducer position was calculated using a cross section drawing of the *IB Oden* keel that shows the position of the MBES, SBP and EK transducers. The rotational angle of the MRU unit can be found in document "Beräkning av roll, pitch och heading", section MRU: roll = 0.02 degree, pitch = -0.06 degree, heading = 0.30 degree.

Table 20.2: Offsets for Motion Reference Unit (MRU) and Global Positioning System (GPS) sensor.

Instrument	L (forward, X) [m]	T (starboard, Y) [m]	H (height, Z) [m]
EK transducer position	26.3	0.6	-9.5
MRU	1.8	0.9	-0.1
GPS	5.4	-0.2	33.4

During the SAS-Oden 2021 expedition the ship's ES-18 transducer connected to the EK80 echosounder was calibrated on 1 August 2021 between 11:44 and 15:41 UTC close to the marginal ice zone. The first CTD of the expedition, CTD EK80 SAS (SO21_00-01), taken between 21:36 and 22:16 UTC on 1 August 2021 at 81.23 °N, 18.49 °E (*Table 5.3*) was used for the calibration of sound speed.

Before arriving at the calibration site, a series of tests was performed. The tests consisted of systematically changing the settings and assessing the noise levels. The test results showed that the cleanest data for observation of biological targets by WP1 would be achieved by using a relatively narrow bandwidth and the following pulse parameters: FrequencyStart 16 kHz, FrequencyEnd 19 kHz, PulseDuration 4.096 milliseconds, SampleInterval 0.256 milliseconds, and TransmitPower 1600 Watt (*Table 20.3*). For WP14 a wider bandwidth setting was more appropriate.

The calibration was coordinated by Julia Muchowski (WP1 and WP14) and Serdar Sakinan (WP1), assisted by other expedition participants. In addition to CTD cast SO21_00-01, the sound velocity profile at the calibration site was measured using an XBT directly after completing the calibration procedure for the different settings for the EFICA (WP1) and MWA (WP14) projects (*Table 20.3*). Established calibration procedures described in Demer et al. (2015)[166] and the SWERUS 2014 Cruise Report[167] were followed. The ES-18 transducer was calibrated with a 63 mm copper sphere on a fishing rod (*Figure 20.4*) that was moved within the acoustic beam, providing the target strength and beam pattern of the ES18 in WP1 and WP14 settings (*Figure 20.5 A-D*). Preliminary results of the correction factor, which considers measurement offsets due to electro-mechanical processes from the EK80 wideband transceiver, were produced (*Figure 20.5 E,F*).

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[166] Demer DA, et al. (2015) Calibration of acoustic instruments. ICES Coop. Res. Report, 326:1-132

[167] SWERUS 2014 Cruise Reports Leg 1 and Leg 2 [https://bolin.su.se/data/swerus/reports.php]

Figure 20.4: Deck work with spheres on fishing rods for calibrating the EK80. (A) Carlos Castro (WP14) and Julek Chawarski (WP1) overseeing the deck work. (B) Lina Holthusen (WP12) assisting with the calibration. (C) Serdar Sakinan holding a sphere. (A,B) ©SPRS, (C) Pauline Snoeijs-Leijonmalm

Table 20.3: ES-18 transducer calibration information. In broadband LFM Up mode the transmitted pulse starts with the lower frequency in the range, and ends with the upper frequency. CW = continuous wave, narrowband.

Transmit signal	WP1 settings (16-19 kHz)	WP14 settings (15-25 kHz)	Continuous Wave mode (18 kHz)
Start Time (UTC)	11:44	13:50	15:07
End Time (UTC)	13:50	15:06	15:42
Pulse Length (ms)	4.096	4.096	1.024
Power (W)	1600	1600	1600
Ramping	Fast	Fast	Fast
Pulse Type	LFM Up	LFM Up	CW

Multibeam echosounder (MBES)

Seafloor mapping was initiated once *IB Oden* left the Norwegian Exclusive Economic Zone (EEZ) off the coast of Norway on 2021-07-29 and continued throughout the cruise until the ship approached the vicinity of the Danish EEZ off the coast of Denmark on 2021-09-19 (*Figure 20.6*). The MBES was running during transits between stations and on occasion while at stations, otherwise secured during most Device Operations to avoid interference with the EK80 and WBAT (CTD-mounted acoustic broadband instrument). During transit, the ship route was arranged to avoid mapping already charted seafloor when possible.
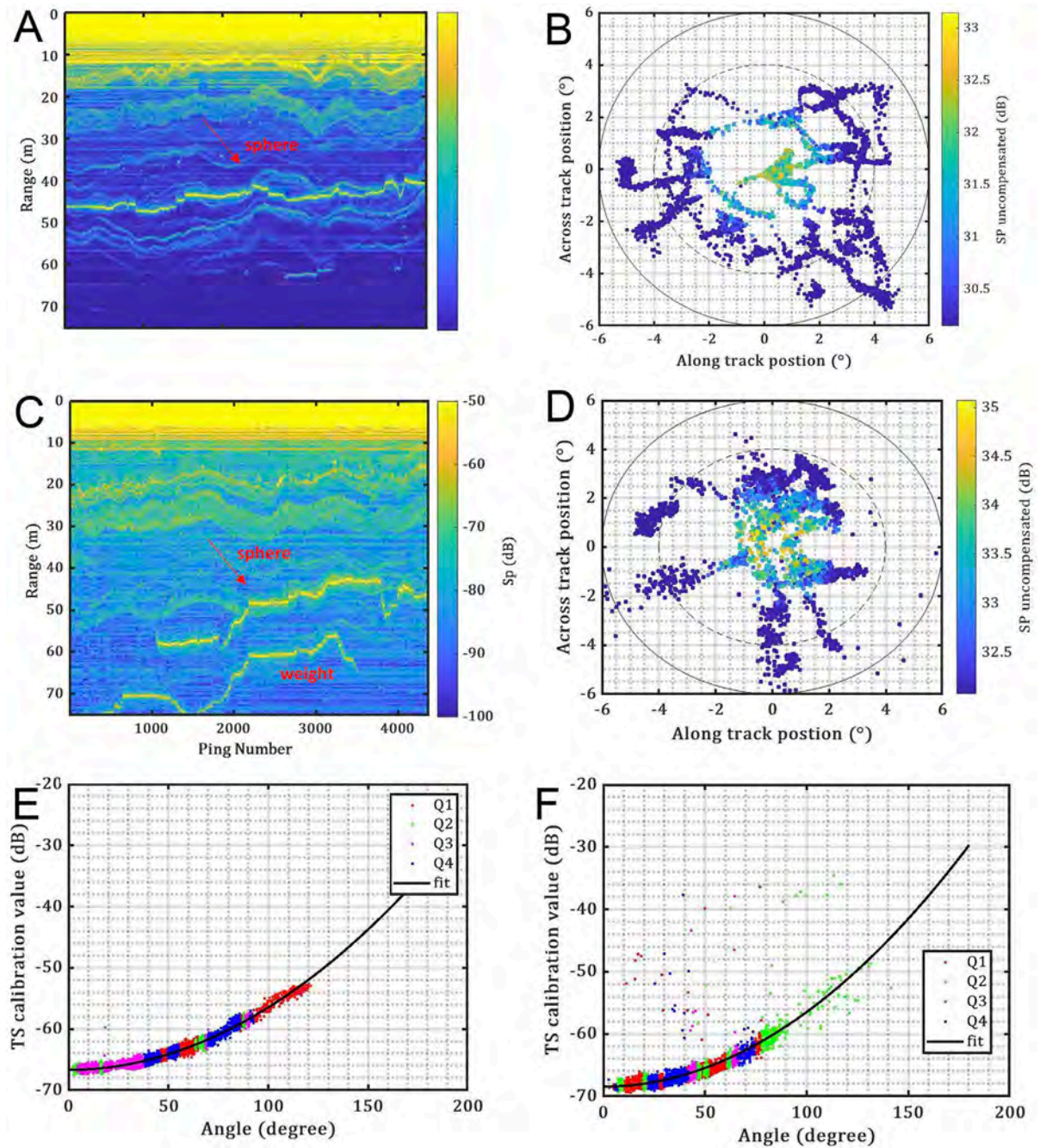
Figure 20.5: EK80 calibration results. (A) WP14 settings - overview echogram of calibration data. (B) WP14 settings - position of sphere in beam. (C) WP1 settings - overview echogram of calibration data. (D) WP1 settings - position of sphere in beam. (E) WP14 settings - calibration offset as a function of electrical phase angle (equivalent to distance from beam centre). (F) WP1 settings - calibration offset as a function of electrical phase angle (equivalent to distance from beam centre). ©Alexandra Padilla

IB Oden is equipped with a Kongsberg EM122 multibeam echosounder (MBES) with serial number 110. The system operates at ca. 12 kHz (range between 11.550 and 12.596 kHz) in a wide swath across-track up to 150° and 1° along-track, using a set of beams that are wide along-track, but approximately 1° across-track. The system is mounted on the lowest part of the hull, on the ice knife, protected by a so-called ice window made from polyurethane and enforced with titanium bars (*Figure 20.7*). The receive array is entirely covered by a titanium plate. Due to the ice protection, the achievable transmit and receive aperture is ca. 140°.
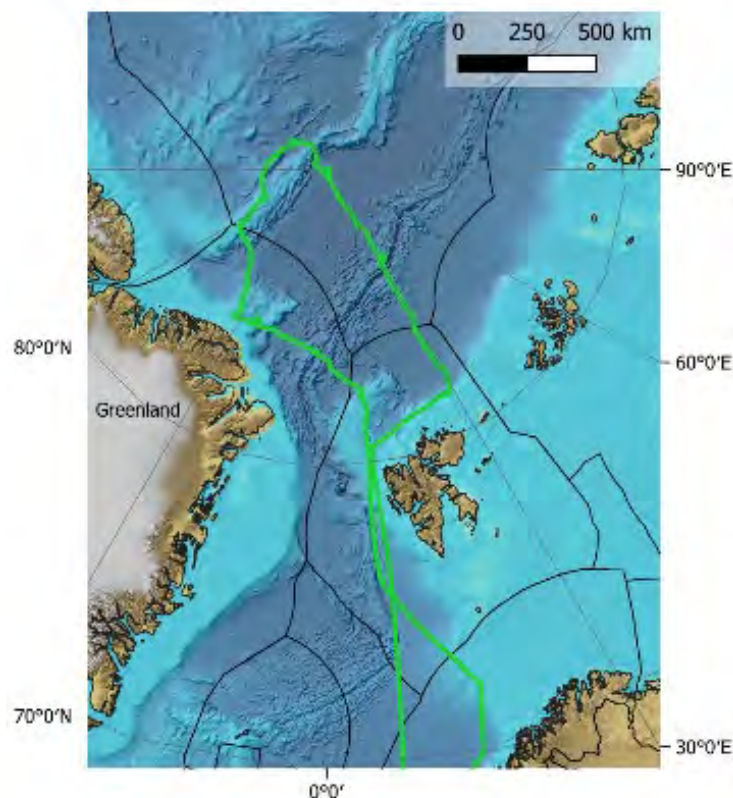
Figure 20.6: Map showing the expedition route of IB Oden (green) along which multibeam, sub-bottom and EK80 data were collected during the SAS-Oden 2021 expedition (including transit), as well as the Exclusive Economic Zones (EEZ) around Greenland, Svalbard and Norway (black). The background map was extracted from IBCAO[168]. ©Julia Muchowski
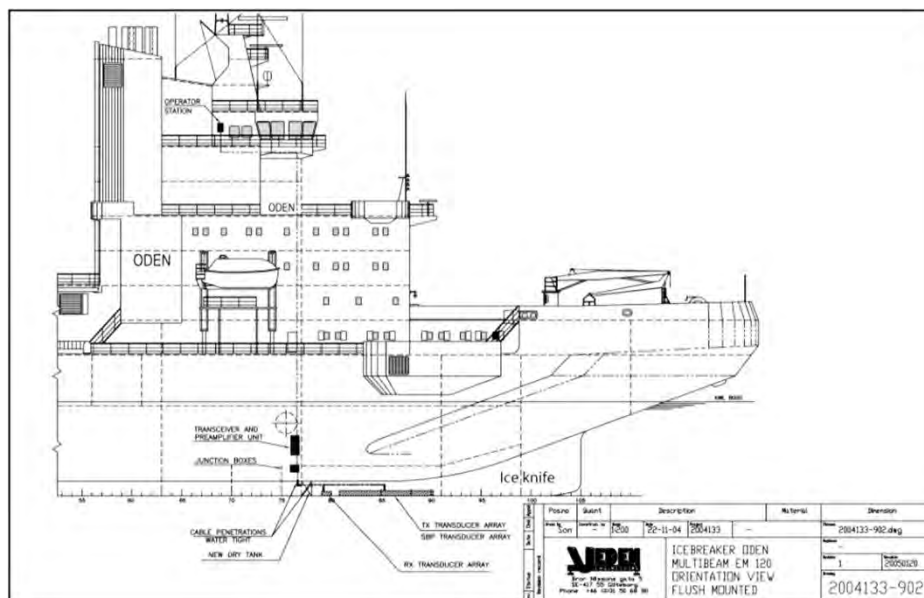

Figure 20.7: Location and configuration of the Kongsberg EM122 multibeam echosounder (MBES), and sub-bottom Profiler (SBP) on the hull of IB Oden.

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[168] Jakobsson M, et al. (2020) The International Bathymetric Chart of the Arctic Ocean, Version 4.0. Scientific Data 7:176 [https://doi.org/10.1038/s41597-020-0520-9]

The MBES collected data with the following settings: Full water column acoustic data were logged and depth and acoustic backscatter calculated for each ping. The maximum angle was adjusted (range 15-65 degrees) to maximize the number of bottom detections for the entire swath and depended on ice/sea condition and depth (ping rate). The ping interval was set to automatic and the most common ping mode setting was DEEP. The min and max depth range was changed to fit the current depth (+/- ca. 500 m, narrower if heavy ice conditions) and the function Force depth (aiding the system to determine which echoes are the correct bottom detection) was used when needed. The sound velocity profile was continuously updated with the latest deep CTD cast data.

A Seatex Seapath 320 GNSS-aided Motion Reference Unit (with an MRU5 motion sensor) is supporting the MB and provides real-time motion data used for beam-forming, and for post-collection correction of motion effects. To collect continuous real-time sound speed at the transducers, *IB Oden* has a Valeport Mini SVS/t sound speed and temperature sensor on the hull next to the MBES transmitter and receiver arrays. This is directly interfaced with the MBES.

Due to time constraints and expedition priorities, the MBES calibration experiment (i.e., a "patch test") was limited to a roll offset test performed on 2021-08-01 and carried out approximately northwest off the coast of Svalbard (geographical coordinates in the SAS-Oden 2021 logbook). The location was selected based on a good coverage of flat seabed and an appropriate depth (ca. 1300 m).

The roll calibration consisted on using two survey lines that ran in opposite directions with the same vessel speed of ca. 4 kt over the same ground. After the last line, an XBT profile was taken to update the sound speed profile of the echosounder. The two lines of data were then uploaded to QPS Qimera for a computer-assisted analysis to identify any angular offsets between the echosounder's reference frame and that of the motion sensor. The resulting offsets of this test (*Figure 20.8*) indicated that the offset calibration was negligible (< 0.01°) and therefore no adjustment to the echosounder's configuration was made. A system diagram for the MBES and the SBP is shown in *Figure 20.9*.

Sub-bottom profiler (SPB)

IB Oden is equipped with a Kongsberg 120 Topas sub-bottom profiler (SBP), which shares a receiver with the MBES, but has separate transmit electronics and transducer (*Figure 20.9*). The sub-bottom profiler was run with EM trigger, unless the EM122 was not pinging. In that case the SBP was set to ping at Fixed rate at a suitable interval for the current depth. The SBP was operating at 2.5-7.0 kHz with a hyperbolic chirp down, pulse length of 2.0 ms and Full array Tx, Rx, and one beam. The acquisition window was set between 500 and 1000 ms depending on the slope. Raw (*.raw) and processed data (*.seg) were collected, and no further processing was made during the expedition to the SBP120 data. A system diagram for the MBES and the SBP is shown in *Figure 20.9*.

Ship noise and interference effects

Previous expeditions have documented that *IB Oden* displays persistent noise in all onboard acoustic systems related to the fuel tank heating system. This so-called "steam hammer" effect was observed on both the multibeam echosounder (*Figure 20.10*) as well as on the EK80. The noise interference was particularly prominent when the tanks were cold and empty. Thanks to the efforts of the *IB Oden* Chief Engineer Jörgen Rundqvist and his staff, this noise was partly ameliorated by increasing the water volume in the bow tanks whenever possible.

Early throughout the cruise, there were persistent ping failures when starting the EM122 echosounder. When pinging failed to start, SIS would typically state: "(DDS) No response from echosounder at start

of pinging, sounder type: EM122, serial number: 110. Try to turn pinging off and on. (524)". After a couple of attempts of waiting and re-pinging, a second error would also appear: "(PU Sensor) 1PPS Clock Synch. on PU port 1PPS is missing (2500)". Running the built-in system test (BIST) for the MBES indicated that the issue could be associated to a shortened transducer on channel 19. The pinging issue could be temporarily resolved by restarting the processing unit, running BIST tests to clear some of the errors, and turning the pinging off and on again a couple of times. This strategy had mixed successes, ranging from a first successful pinging attempt to requiring several hours of rebooting to restart.

The problem mentioned above was communicated offshore with Kongsberg personnel for further advice on how to resolve the issue. After internal discussions, Kongsberg's assessment was that the CPU card/kit (which starts to "drag" over the years) has become more unstable, especially concerning the internal clock synchronization. Thus, the standing suggestion would be to replace the CPU kit with a spare kit for the DVS CPU and the CPU Rio card.

Physical/electrical failures concerning hardware connections for the MBES were also documented in the Ryder 2019 expedition[169] with *IB Oden*. We therefore recommend that this issue is considered for particular attention during the next maintenance period for the MBES.
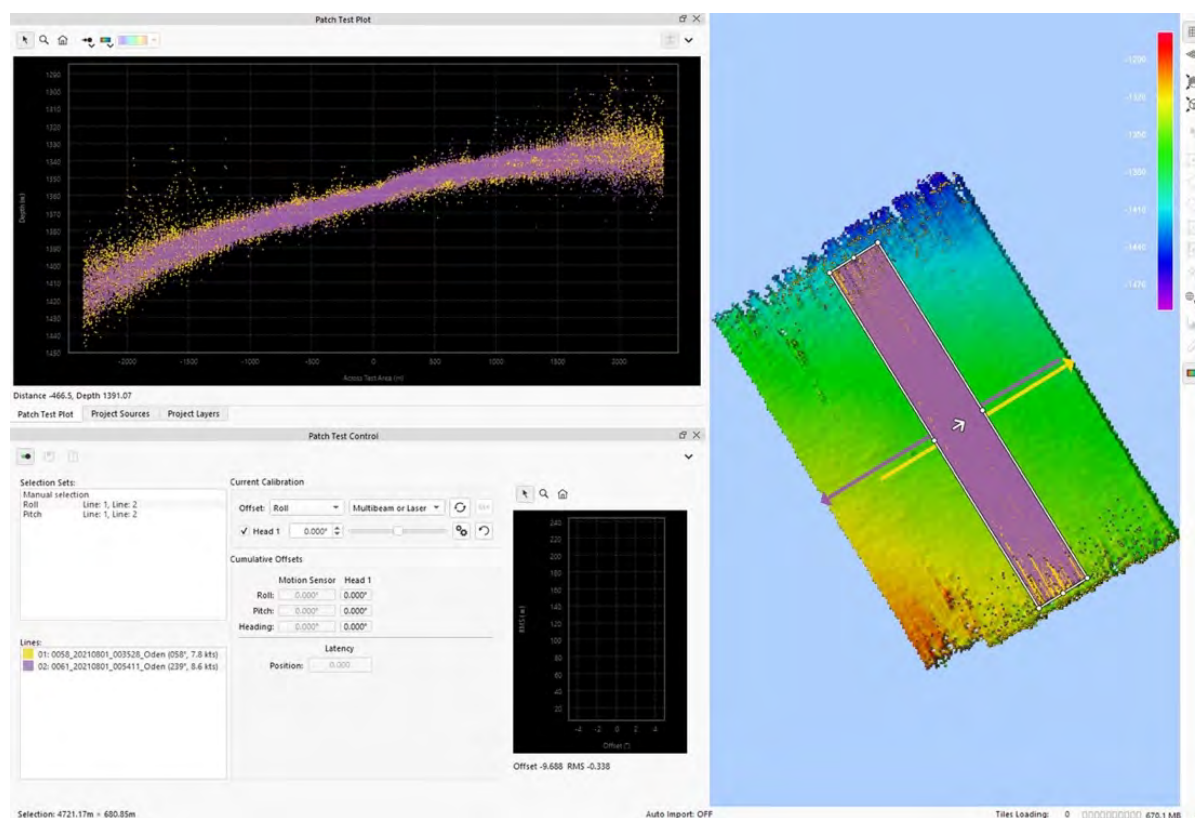
Figure 20.8: Results of the QPS Qimera roll calibration test for the multibeam echosounder (MBES). The resulting offset calibration was <0.01°, which indicated that no adjustment to the calibration was necessary for the SAS-Oden 2021 expedition. ©Carlos Castro

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[169] Jakobsson M, Mayer L, Farrell J, and Ryder 2019 Scientific Party (2019) Expedition Report SWEDARCTIC Ryder 2019. [https://www.diva-portal.org/smash/get/diva2:1458256/FULLTEXT01.pdf]

Figure 20.9: System diagram for the Kongsberg EM122 multibeam echosounder and SBP120 sub-bottom profiler on IB Oden. *The two systems have separate transmitters, but share a receiver array.*

Figure 20.10: Example of the noise caused by the "steam hammer" effect in the fuel heat tanking system onboard IB Oden *during the SAS-Oden 2021 expedition.* ©Caroline Bringensparr

Equipment on the CTD

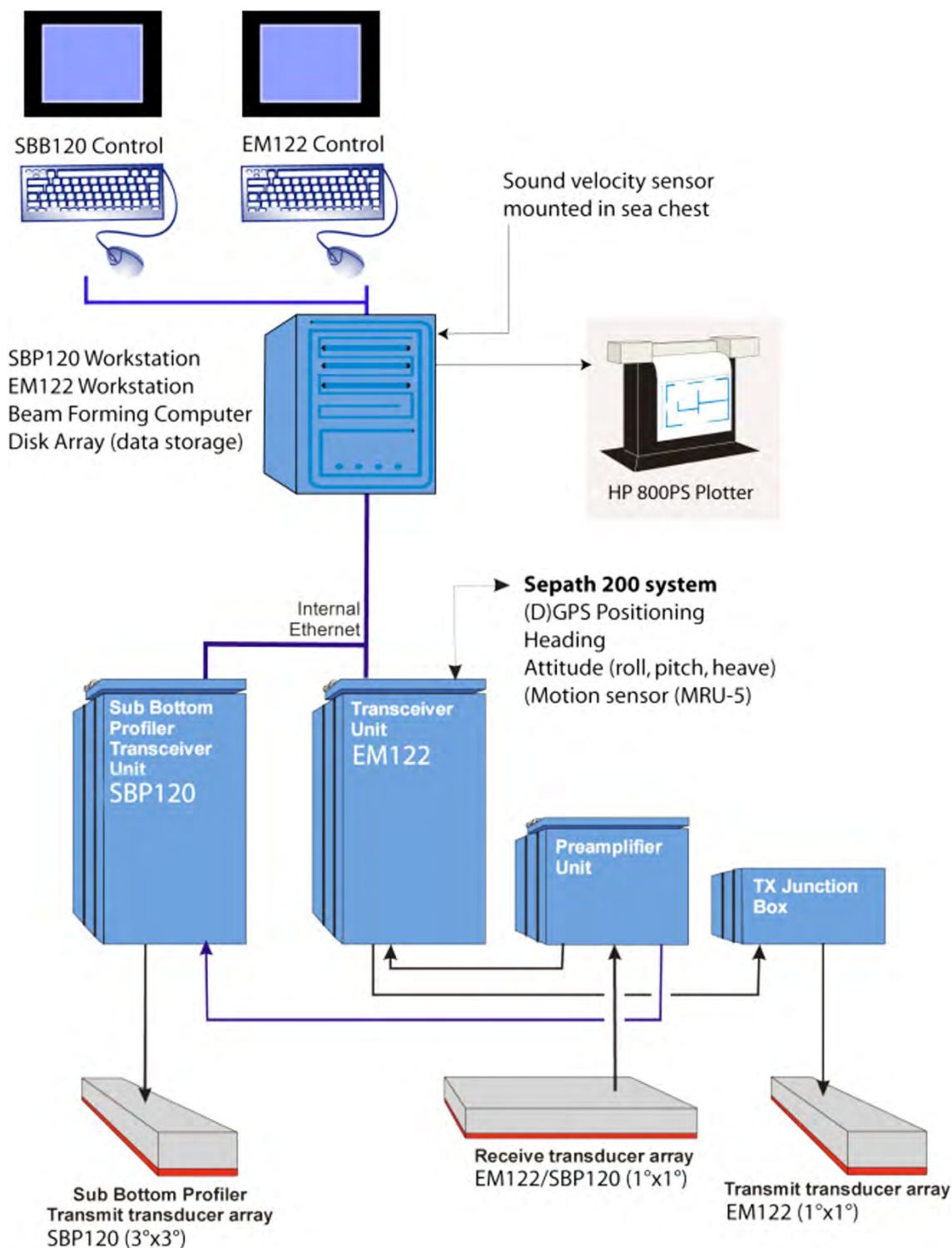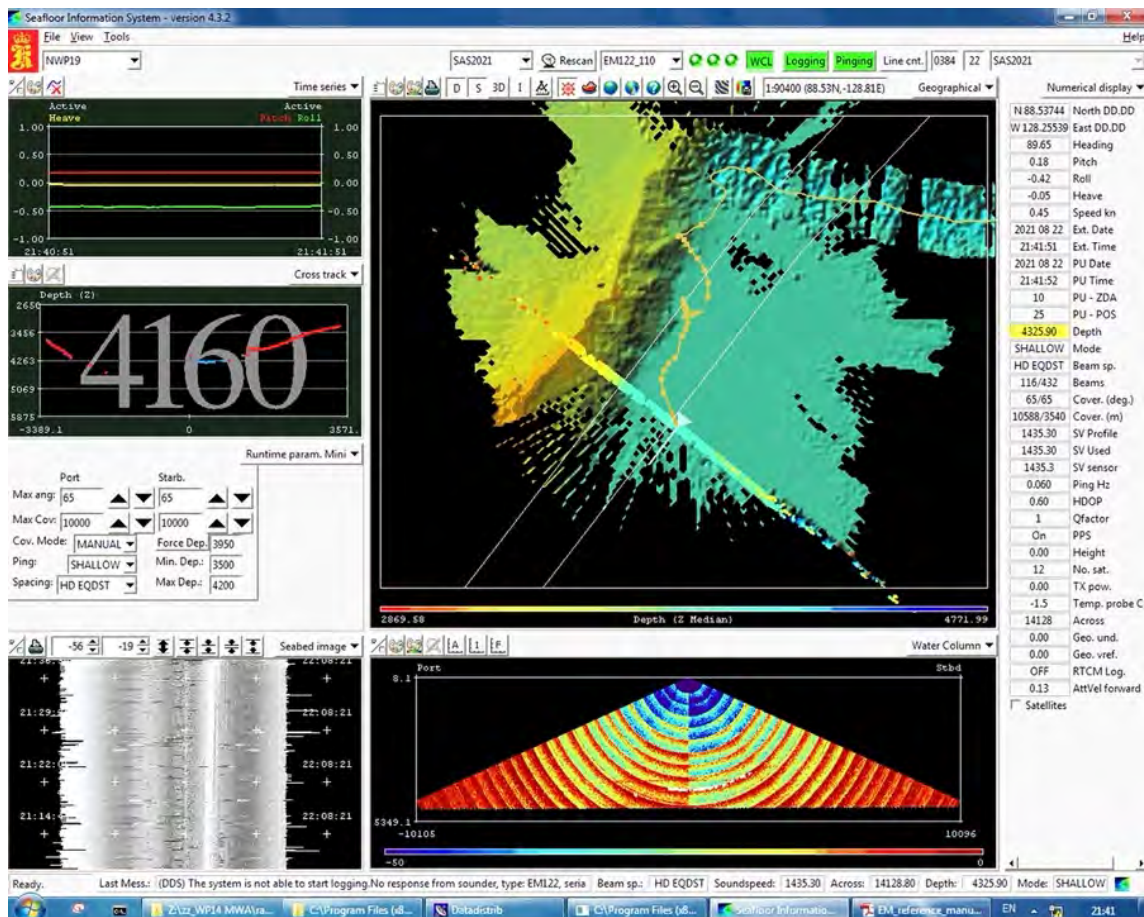**XBT:** Where CTD-based sound speed profiles were not available, but there appeared to be evidence of sound speed variability in the MBES data, an Expendable Bathythermograph (XBT) probe was used to measure the temperature of the water with respect to depth using a Lockheed-Martin Sippican XBT Mk21 launcher.

LADCP: Water velocities were measured with a Teledyne Mariner Workhorse 300 kHz Lowered Acoustic Doppler Current Profiler (LADCP) with two transducers, one upward- and one downward looking that were mounted on the bow-CTD.

WBAT: A Wideband Autonomous Transceiver (WBAT) was installed on the stern CTD by WP1, see *Chapter 8*.

UVP: An Underwater Vision Profiler (UVP, an optical instrument) was installed on the bow-CTD by WP1, see *Chapter 8*.

IMP: The IMP magnetometer and accelerometer was mounted in an opportunistic manner alternating on aft and bow CTD to measure the direction and rotation of the CTD rosette. Mounted on the bow-CTD, the data can be used to improve the in-built compass of the LADCP. Mounted on the aft-CTD, the data can be used to compensate the rotation of the CTD rosette in WBAT data and therefore enable us to track fish and study their behaviour, see *Chapter 8*.

20.4   Summary of data collected

The data collected by WP14 (MWA) during the SAS-Oden 2021 expedition are published in the SND data repository and the Bolin Center Database[170] at Stockholm University. The metadata collected by WP14 during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP14_ MWA" in the SND data repository and summarised in *Table 20.4*.

Table 20.4: Overview of all measurements collected by WP14 (MWA) during the SAS-Oden 2021 expedition.

Parameter	Type of data	Collected by	Data owner
Water-column acoustics (EK18)	Continuous measurements	WP1 + WP14	SPRS
Multibeam (MBES)	Semi-continuous measurements	WP14	SPRS
Sub-bottom profiler (SBP)	Semi-continuous measurements	WP14	SPRS
XBT	17 profiles while steaming	WP14	SPRS
LADCP on bow CTD	53 down-, 6 uplooker profiles	WP14	SPRS
IMP on bow and stern CTD	13 profiles	WP1 + WP14	SPRS
WBAT on CTD (stand-alone)	Continuous CTD profiles	WP1 + WP14	EU Commission (EFICA)
UVP on CTD (stand-alone)	Semi-continuous CTD profiles	WP1 + WP14	EU Commission (EFICA)
CTD pressure (depth)	Continuous CTD profiles	WP14 + WP15	SPRS
CTD salinity	Continuous CTD profiles	WP14 + WP15	SPRS
CTD temperature	Continuous CTD profiles	WP14 + WP15	SPRS

20.5   Summary of preliminary results

During the expedition, we observed thermohaline staircases in the CTD data, which were also observed in the EK80 water column data. Examples of these thermohaline staircases in the CTD data and acoustic water column data are shown in *Figure 20.11*. Thermohaline staircases have been observed in EK80 acoustic data[171] and can provide information about vertical mixing within the water column.

Possible evidence of methane seeps from the seafloor[172] was observed in the EK80 ES-18 echograms on 4 and 5 September 2021 near Station 50 (*Figure 20.12 A*). The acoustic record of these possible seeps needs to be further processed to determine if they are in fact methane gas bubbles. Pockmarks were observed in the multibeam data in the same region where the possible methane seeps were observed in the EK80 ES-18 echogram (*Figure 20.12 B*).

The quality of multibeam data varied significantly, resulting in a gridded horizontal resolution that ranged from ca. 20 m to ca. 200 m. Low data quality was caused primarily by heavy ice breaking and adverse weather conditions, leading to an almost indistinguishable seafloor. High data quality was often related to calm open waters and *IB Oden* drifting or repositioning while on station. High-quality seafloor data revealed structures along the Gakkel Ridge, Lomonosov Ridge, and Morris Jessup Rise (*Figure 20.12 C*).

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[170] https://bolin.su.se/data/

[171] Stranne C, et al. (2017) Acoustic mapping of thermohaline staircases in the Arctic Ocean. Scientific Reports 7:15192

[172] Weidner E, et al. (2019) A wideband acoustic method for direct assessment of bubble-mediated methane flux. Continental Shelf Research 173:104–115
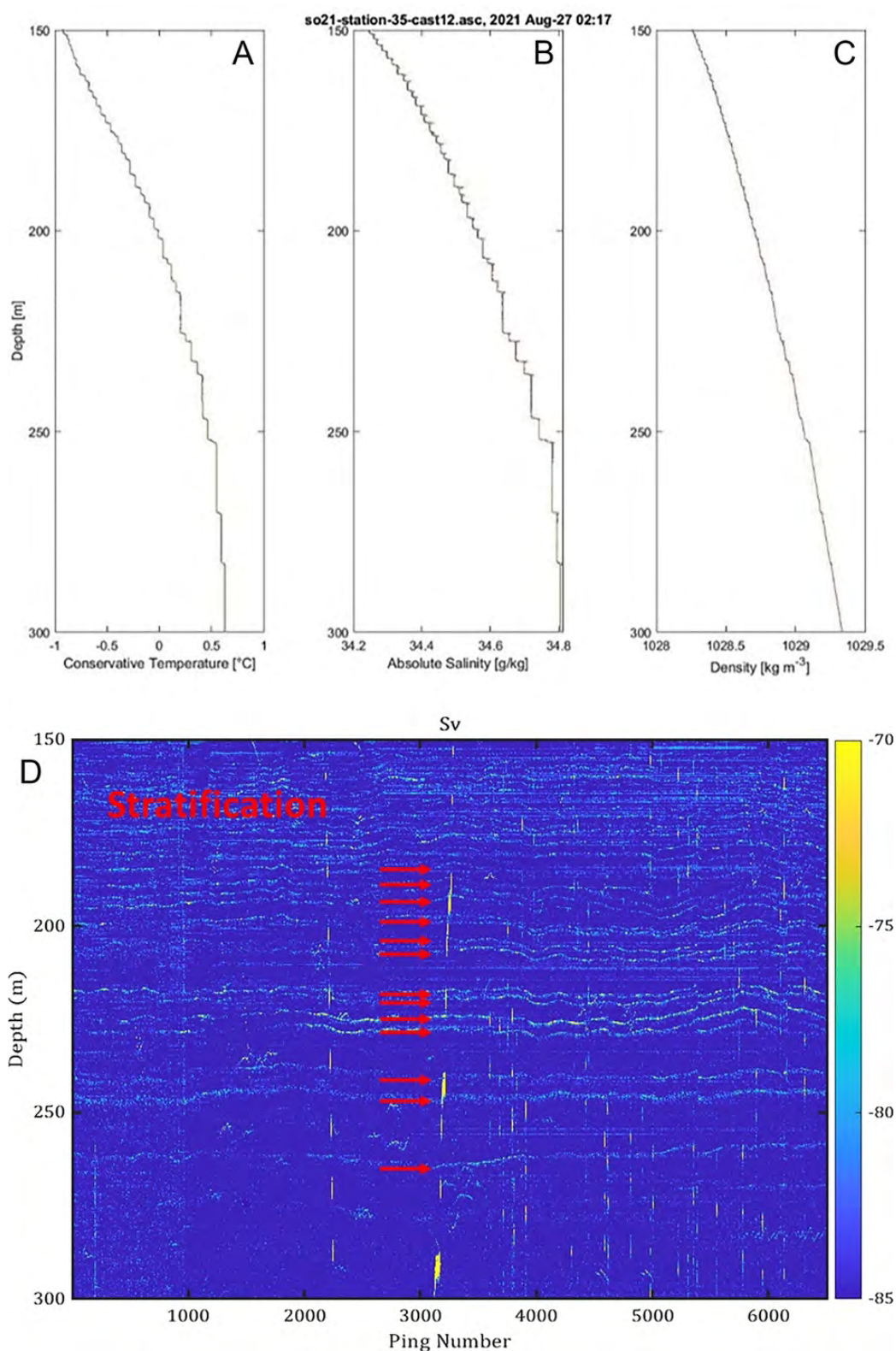
Figure 20.11: Thermohaline staircases observed on 27 August 2021 during the SAS-Oden 2021 expedition. (A-C) Conservative temperature, absolute salinity and density at Station 35 measured by the bow CTD (Device Operation SO21_35-12). (B) The echogram from the EK80 echosounder with ES-19 transducer corresponding to (A). ©Alexandra Padilla & Julia Muchowski
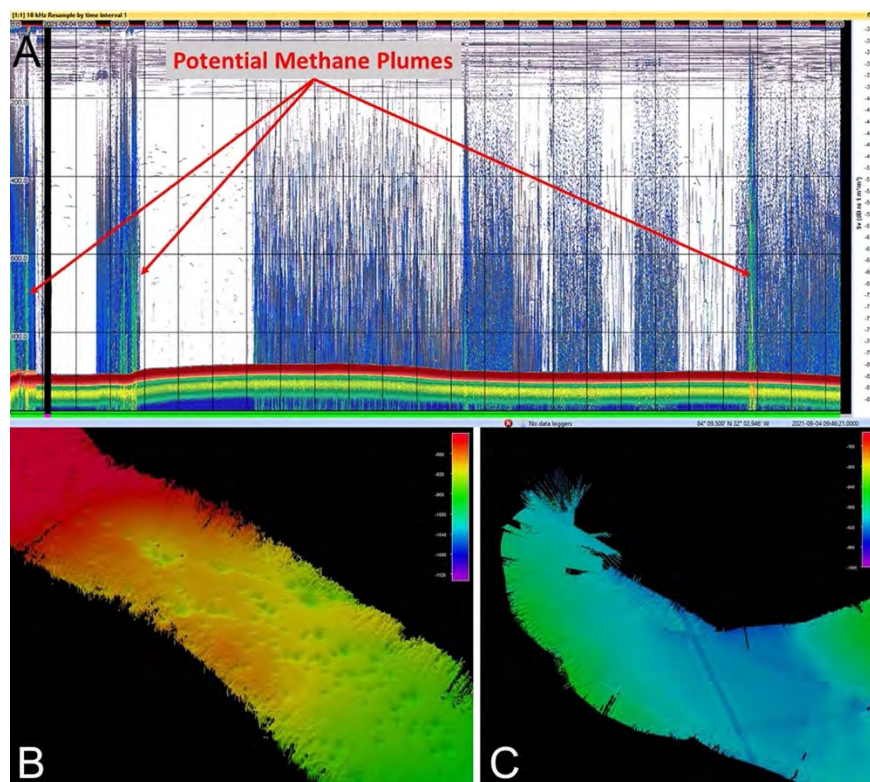
Figure 20.12: Results obtained by WP14 (MWA) during the SAS-Oden 2021 expedition. (A) Possible evidence of methane seeps from the seafloor on 4 September 2021 near Station 50 recorded by the EK80 echosounder with ES-19 transducer, arrows denote possible methane seeps. (B,C) Pockmarks and other seafloor features recorded by the multibeam echosounder near the Morris Jessup Rise. ©Alexandra Padilla & Carlos Castro

20.6 Summary of post-cruise analyses and deliverables

WP14 will manually scrutinize the EK80 and WBAT water-column acoustic data for i.e., thermohaline staircases, vertical mixing and methane gas seeps. If successful, different acoustic inversion techniques could be implemented to convert acoustic measurements into estimates of i.e., vertical mixing and methane gas flux. WP1 (EFICA) will analyse fish and zooplankton abundances in relation to their physical environment from the EK80, WBAT and UVP water-column data.

The metadata information for the bathymetric data are found here:
https://bolin.su.se/data/export.php?n=oden-sas-2021-bathymetry-1&type=datacite&format=xml
All data collected by the multibeam echosounder were cleaned of outliers. Processed multibeam data are available on the Bolin Centre Database at Stockholm University with open access to climate and Earth System. The high-resolution bathymetry of the expedition is available as processed grids with a grid cell size of 100 m in IBCAO Polar Stereographic projection (epsg: 3996):
https://bolin.su.se/data/oden-sas-2021-bathymetry-1. EK80 and sub-bottom data will be made available in the same repository. The CTD and other data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*), see *Chapter 21*.

Deliverables: The data will be made publicly available in the Bolin Centre Database repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*), and as part of the IBCAO (International Bathymetric Chart of the Arctic Ocean) and GEBCO (General Bathymetric Chart of the Oceans) projects. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

21 WP15 (WAOW)

Salar Karam (WP15)

Project title : Why is the deep Arctic Ocean Warming? (WAOW)

21.1 Resources

External project funding: Swedish Research Council (VR, grant number 2018-03859) and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for one berth on the SAS-Oden 2021 expedition to Céline Heuzé. All project participants are listed in *Table 21.1*.

Table 21.1: Overview of all onboard and onshore participants WP15 (WAOW) CTD tea,.

On board	Name	e-mail address	Task in project	Affiliation
No	Céline Heuzé	celine.heuze@gu.se	PI	GU, Sweden
Yes (berth)	Salar Karam	salar.karam@gu.se	WP Leader, CTD data collection	GU, Sweden

21.2 Scientific motivation and specific research questions

Although the discovery of the deep Arctic basins dates back to the Fram drift expedition of 1893, the harshness of this environment means that it is the least observed part of the globe. In total there are fewer than 700 full-depth profiles in the whole deep Arctic[173], i.e., less than one profile per 2500 km², hence the processes responsible for deep water renewal, mixing and transport are still debated. Is deep Arctic water coming from the shallow Russian continental shelf[174] or from the Nordic Seas via the shallow Barents Sea or the 2700-m deep Fram Strait[175]?

The first hydrographic observations of the deep Eurasian Basin in the 1990s revealed that the bottom 1000 m are warmer than expected. This finding was first attributed to geothermal heating from the seafloor[176], until it was shown that the warming signal is four times stronger than expected from geothermal activity alone and hence must be climate-change induced[177]. This further suggests an influence from the Nordic Seas and the Russian shelves, which both are changing in response to climate change[178]. Since warmer waters expand, this observed warming signal in the deep Arctic directly leads to sea level rise. There is therefore an urgent need to determine the sources of this signal and to quantify the spatiotemporal variability of the deep Arctic Ocean and its drivers.
The water properties of the Arctic deeper than 1000 m are still poorly known[179]. The pathways of deep waters are unknown. Their sources are hypothesised, but the mechanism and frequency of their ventilation are unknown. Their exact connections to the rest of the Arctic and even global ocean are unknown. Yet, determining the entire deep Arctic Ocean circulation only requires the hydrographic

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[173] World Ocean Database, total number of casts with deepest measurement at least deeper than 1700 m, north of 82°N (accessed on 20 September 2017)

[174] Aagaard K (1981) On the deep circulation in the Arctic Ocean. Deep Sea Research 28:251–268

[175] Anderson LG, et al. (1994) Water masses and circulation in the Eurasian Basin: Results from the Oden 91 expedition. Journal of Geophysical Research, Oceans 99:3273–3283

[176] Björk G, Winsor P (2006) The deep waters of the Eurasian Basin, Arctic Ocean: Geothermal heat flow, mixing and renewal. Deep Sea Research, Part I 53:1253–1271

[177] Rudels, B, et al. (2013) Observations of water masses and circulation in the Eurasian Basin of the Arctic Ocean from the 1990s to the late 2000s. Ocean Science 9:147–169

[178] Stocker TF, et al. (2013) Climate Change 2013: The Physical Science Basis. Cambridge University Press, 1535 pp.

[179] Bluhm BA, et al. (2015) A tale of two basins: An integrated physical and biological perspective of the deep Arctic Ocean. Progress in Oceanography 139:89–121

properties (mainly temperature and salinity) to identify water masses, with a good spatial resolution throughout the entire Eurasian Basin to quantify regional differences, and a good temporal resolution tracking everything from pulses to the long term effects of climate change.

The overarching aim of WAOW is to determine the complete dynamics of heat transport into the deep Arctic Ocean to project accurately changes in essential climate variables that depend on this deep heat.

The specific research questions of the WP15 (WAOW) project are:

(1) How are, presently, the deep-water masses in the Arctic characterized, and how do their properties vary geographically?
(2) What is the history of these water masses? That is, which route did they take and how fast was their journey?

21.3   Summary of field work performed

Note: This section was written by the whole CTD team: Julia Muchowski (WP14), Alexandra Padilla (WP14), and Salar Karam (WP15). WP15 (WAOW) was together with WP14 (MWA) responsible for the organization of the CTD casts and CTD data retrieval in two daily 12-hour shifts: WP14, 2 persons during day time 8:00-20:00; WP15, 1 person during night time 20:00-8:00. Adam Ulfsbo (WP10) was responsible for the water budgets of the Niskin bottles of the bow CTD. All CTD deck sheets and water budgets were scanned and are available in the SND data repository.

SAS Core Parameters: Together with WP14 (MWA), WP15 (WAOW) was responsible for measuring the SAS Core Parameters pressure (depth), salinity, and temperature in CTD depth profiles from the bow CTD (62 casts) and the stern CTD (50 casts) (*Chapters 6.2 and 6.3*). The CTD team also contributed to acquisition of data from the measuring devices on the CTD that were requested by other WPs (PAR, LADCP, IMP, CDOM fluorescence, chlorophyll fluorescence, UVP, WBAT, light sensor).

General CTD operations

During SAS, two CTD systems with rosettes for water sampling were operated from *IB Oden* due to the large water needs from the various work packages (*Figures 21.1 and 21.2*). One CTD was operated from the bow (*Chapter 6.2*) and another one from the stern (*Chapter 6.3*), with the plan being that casts from the bow and the stern could be deployed in direct succession of each other to save time. However, the ice conditions rarely allowed for this and the ship had to reposition between bow and stern operations. The CTD from the bow was mainly operated to obtain full hydrographic profiles for the oceanographical projects WP14 (MWA) and WP15 (WAOW), and to collect water and associated environmental data for the chemical and biological projects (*Table 6.2*). The CTD from the stern was operated to collect additional water and associated environmental data for the biological projects (*Table 6.3*), and went down to max. 1000 m depth. The CTD operations were carried out by WP14 (MWA) and WP15 (WAOW), while SPRS technicians drove the winch. During deployment and recovery, other WPs helped to roll the CTD to and from the container. During SAS, we planned to carry out full-depth CTD casts (down to 10 m above the seafloor) at 60 ship stations, but the number of stations was reduced to 36 ship stations (*Table 1.1*) for various reasons as described in *Chapter 5.3*. For a map of all CTD stations, see *Figure 1.1*. Altogether, 62 successful CTD casts were made from the bow (*Table 5.3*), i.e., all 20 CTD shallow SAS, all 32 CTD deep SAS, all six CTD deep VACAO, two CTD ChlMax PICO (SO21_08-01, SO21_24-01), one CTD omics SAS (SO21_03-01), and one CTD EK80 SAS (SO21_00-01) for calibration of the EK80 echosounder. Altogether, 50 successful CTD casts were

made from the stern (*Table 5.3*), i.e., all 18 CTD bio SAS, 17 CTD omics SAS, all 14 CTD wbat EFICA, one CTD ChlMax PICO (SO21_56-06).
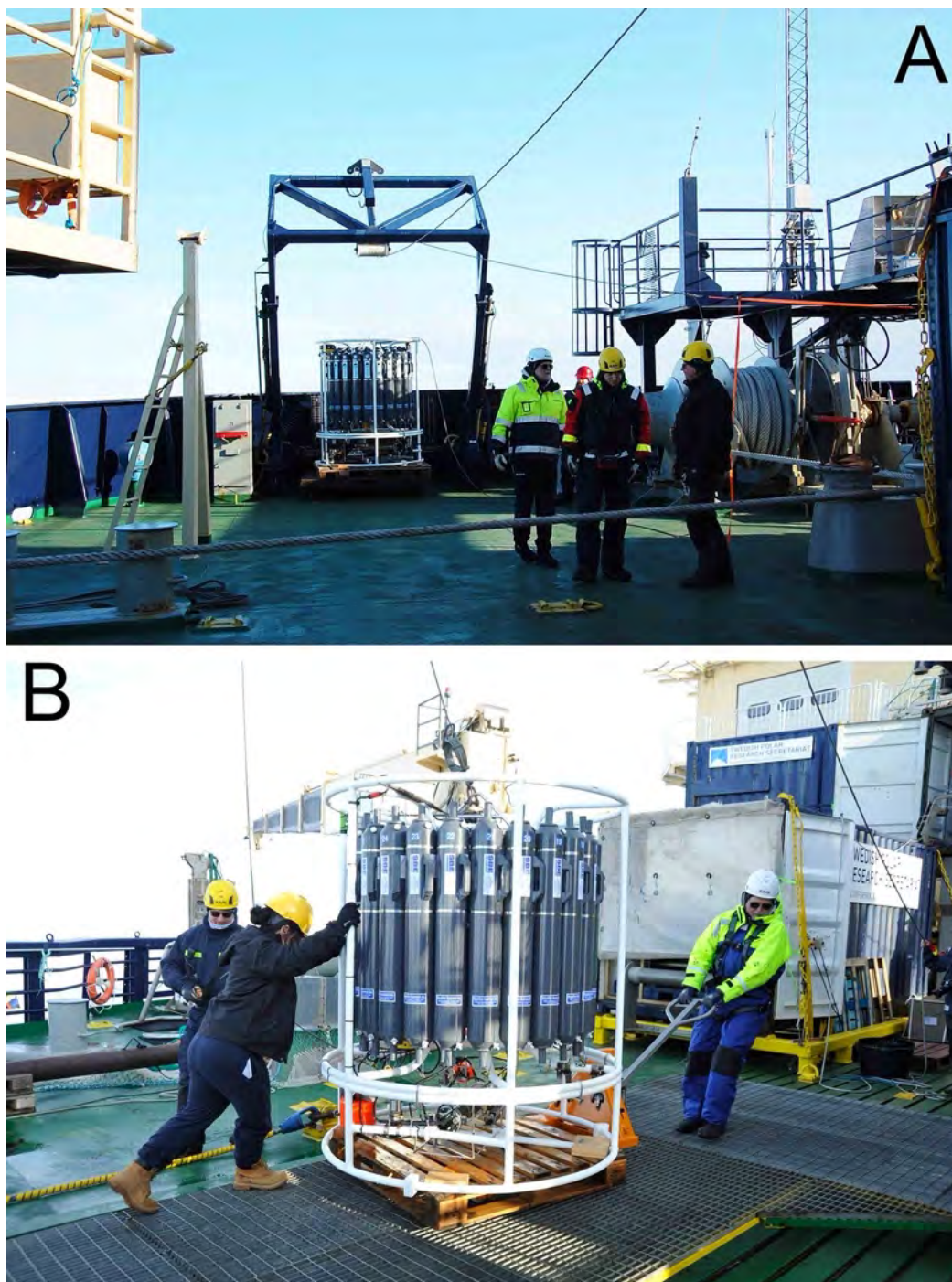
Figure 21.1: Work with the bow CTD during the SAS-Oden 2021 expedition. (A) CTD ready for deployment. (B) CTD retrieval. (A) ©Anna Lunde Hermansson, (B) ©Flor Vermassen

Equipment

Both CTDs had the standard SeaBird SBE911 plus system with dual sensors to measure temperature, salinity and single sensors measuring pressure (depth) and oxygen. Originally an altimeter was also mounted on each CTD to measure the distance to the seafloor, but as the altimeter on the bow CTD did not work, the altimeter from the stern CTD (max. depth 1000 m) was moved over to the bow CTD (full

depth) in order to not risk hitting the seafloor with the latter. The bow CTD also had a CDOM sensor (measuring coloured dissolved organic matter) and a PAR sensor (measuring photosynthetically active radiation) and from Station 50 on there was a turbidity sensor installed on the bow CTD as well. Two LADCPs (Lowered Acoustic Doppler Current Profiler) were also mounted on the frame of bow CTD for WP14 (MWA), reducing the number of Niskin bottles to 22 instead of the standard number of 24. Due to the reduced number of bottles on the bow CTD, a full depth cast was usually preceded by a shallow cast in order to meet water requirements needed by the various work packages and still covering all SAS depths (*Table 6.1*). Additionally, a stand-alone, battery-driven UVP (Underwater Vision Profiler) brought by WP1 (EFICA) was also mounted on the bow CTD. The stern CTD also had a stand-alone deep-sea video camera attached to the frame, as well as stand-alone extra-sensitive light sensor (TDR-Mk9-404A tag) and a WBAT (Wideband Autonomous Transceiver) brought by WP1 (EFICA). There was also a dual fluorometer/turbidity sensor which was at times swapped between the two CTDs as well as a turbidity sensor which was installed on the bow CTD for the final configuration. The sensors being swapped back and forth between the two CTDs resulted in unusually many configurations (*Tables 21.2 and 21.3)*.

Standard CTD procedures

Due to the different instruments on the two CTDs, as well as a slower winch used for the stern CTD, we operated the CTDs slightly differently. Before the CTD was rolled out of the container the rosette was prepared by opening all the bottles carefully to not damage the O-rings and then the external instruments attached to each CTD was turned on. For the bow CTD these instruments were the UVP (WP1, EFICA) and the LADCP (WP14, MWA). For the stern these instruments were the stand-alone WBAT, light sensor (TDR-Mk9-404A tag), and deep-sea camera (WP1, EFICA). The stand-alone UVP was initiated by submerging the instrument into the water; we lowered the CTD down to 15 m of depth and then waited for 1 minute so that the UVP would start collecting data. Then the CTD was lifted back to just below the water surface and then we would start the descent again. Since the stern CTD did not have a UVP attached, we would just wait at the surface for 1-2 minutes until the pump was turned on before starting the descent.

The bow CTD was lowered in the first 500 m with a speed of 0.5 m s^{-1} and below 500 m with a speed of 1 m s^{-1}. The stern CTD was driven by a winch with only two speed settings (0.1 m s^{-1} and 0.5 m s^{-1}), 0.5 m s^{-1} was used for the entire depth interval (surface down to max. 1000 m). For the CTD bio we stopped every 100 m for 3 minutes on the downcast for 3 minutes in order to collect WBAT data. When the bow CTD approached the seafloor we checked the depth reading of the EK80 echosounder or the multibeam. From ca. 100 m above the seafloor the winch speed was gradually reduced so that the altimeter could start reading and ca. 10 m above the seafloor we stopped the CTD to not risk hitting the seafloor. The Niskin bottles were closed during the upcast by slowing down to 0.3-0.5 m s^{-1} ca. 30 m before reaching the target depth and slowed down further to 0.1-0.2 m s^{-1} ca. 10 m before reaching the target depth. At the target depth, the CTD was stopped and we waited for 1 minute before firing the bottles and then continued the cast with a speed of 1.0 m s^{-1}. After the CTD cast it was taken into the container and all WPs took their water samples according to the water budget sheet prepared by Adam Ulfsbo (WP10) before each cast with the bow CTD. After water sampling, all Niskin bottles were emptied and the entire CTD rosette system was thoroughly rinsed with freshwater. All CTDs taken during the expedition are summarized in *Tables 21.4 and 21.5*.

Bow CTD issues during the SAS-Oden 2021 expedition and how they were solved

Conductivity and temperature sensors: During a test cast with the bow CTD on 1 August we noticed that both the primary and secondary temperature and conductivity sensors showed large offsets from their expected ranges with temperature values well below the surface water freezing

temperature and salinity values of 40-45 PSU. We suspected that the sensors had the wrong calibration files, rather than that we had actual sensor issues. Upon inspecting the .xmlcon files, we found that the conductivity and temperature sensors did indeed have the wrong calibration files. After changing them to the correct ones, the sensors showed appropriate values and we performed a full-depth cast on 2 August (Device Operation SO21_01-01).

Altimeter: During Device Operation SO21_01-01 on 2 August we noticed that the altimeter on the bow CTD (serial number 69525) did not give a depth reading when we approached the seafloor. However, we were not at risk of crashing the CTD as we calculated the depth of the seafloor by multiplying the ratio between the mean sound velocity given by the CTD and the set sound velocity of the EK80 with the depth reading of the EK80 as follows:

$$Real\ depth = \frac{Mean\ sound\ velocity_{CTD}}{Sound\ velocity_{EK80}} * Depth_{EK80}$$

When inspecting the altimeter after this cast we found that the connector of the sensor was damaged. We decided to move the altimeter from the stern CTD (serial number 76191) to the bow CTD because the stern CTD was not going near the seafloor in the deep basin (the maximum depth of the stern CTD was 1000 m). By Device Operation SO21_08-01 on 7 August we had repaired the connector for the altimeter with serial number 69525 and reinstalled it on the bow CTD, while moving the altimeter with serial number 76191 back to the stern CTD. After four full-depth casts without receiving any readings from the repaired altimeter (serial number 69525), we reinstalled the altimeter from the stern CTD (serial number 76191) before Device Operation SO21_16-04 on 12 August. After this, we did not use the altimeter with serial number 69525 on any of the CTDs again.

CDOM sensor: The CDOM sensor on the bow CTD had no readily available harness cable, and a properly wired harness cable was built on board *IB Oden*. The CDOM sensor comes with a built-in function to allow for direct output, 10× output or 100× output. After a number of casts with no usable data, we decided to build another harness cable with 100× output. This resolved the issue as we were able to see variations in the readings from the sensor and the data showed appropriate values.

Deck unit: During Device Operation SO21_11-02 on 9 August we lost communication with the bow CTD at ca. 3000 m of depth. As we could not regain communication with the CTD, we brought it back to deck immediately. After initially suspecting a leakage in the underwater power supply unit, we found that the issue was actually due to the power supply of the deck unit. We did not observe any voltage output from the deck unit. A short-circuit caused the fuse on the DC side in the power supply deck unit to blow. After we replaced the blown fuse, we could again communicate with the CTD. The same fuse problem occurred during a few later casts as well. However, at those occasions we already knew what the issue was and we did not bring the CTD back on deck but quickly replaced the fuse while the CTD was still in the water and restarted communication with the CTD and we could continue with the cast. We never succeeded to definitely localize what caused the short-circuits but we did observe small water droplets on the power connector at the subsea junction box, which might have been related to the problem.

Stern CTD issues during the SAS-Oden 2021 expedition and how they were solved

Pressure sensor: A test cast was planned with the stern CTD in the early morning of 3 August. However, when we started data acquisition the pressure sensor showed unreasonable values (-150 m when switched on deck and also during a test in the water). We tried restarting the software and the deck unit several times but this did not solve the problem. We exchanged the plastic components of the pressure sensor tubing and outlet, and refilled the silicone oil in the sensor according to the SBE

9+ manual, but this did not solve the problem either. We then suspected that the SBE 9+ had the wrong calibration file and when we inspected the serial number of the sensor and the configuration file, they did not match. After finding the correct calibration file for the SBE 9+, the sensor showed appropriate values.

Alarm: A test cast was made with the stern CTD in the afternoon of 3 August. The alarm on the SBE 11 deck unit went off several times and we could not acquire any NMEA data. After restarting the deck unit several times, the alarm did not go off and we put the CTD into the water for Device Operation SO21_03-01. At ca. 150 m of depth the alarm went off again and restarting the deck unit while the CTD was in the water did not solve the alarm issue. However, as the data looked reasonable and it was only NMEA data that was not collected, it was decided to complete the cast. Since the deck unit was restarted while the CTD was in the water, two files exist from this cast. After Hans-Jørgen Hansen (SPRS, consultant) discussed the matter with his colleagues at MacArtney Underwater Technology (Denmark), we identified that the error with the SBE11 deck unit could have been caused by several issues, a.o., a shortcut in the bottom switch contact. After inspecting the bottom switch connector, we indeed found that the installed dummy connector was leaking. After appropriate cleaning and greasing of the connector, the dummy connector was re-installed and the issue was resolved.

Niskin bottle leakage: After the first cast with the stern CTD (Device Operation SO21_03-01 on 3 August) we found that most of the Niskin bottles started to leak from the bottom when opening the top air valve. The issue was that the rubber strings attached to the top and bottom lid of the Niskin bottles had been stretched too long and were worn out, and could therefore not form a tight seal when closed. Unfortunately, there were no spare parts to replace the old strings so nothing could be done to solve the issue. Hans-Jørgen Hansen (SPRS, consultant) tied a knot on the strings in order to make them shorter but this did not resolve the issue completely and leaking Niskin bottles remained an issue with the stern CTD throughout the expedition.
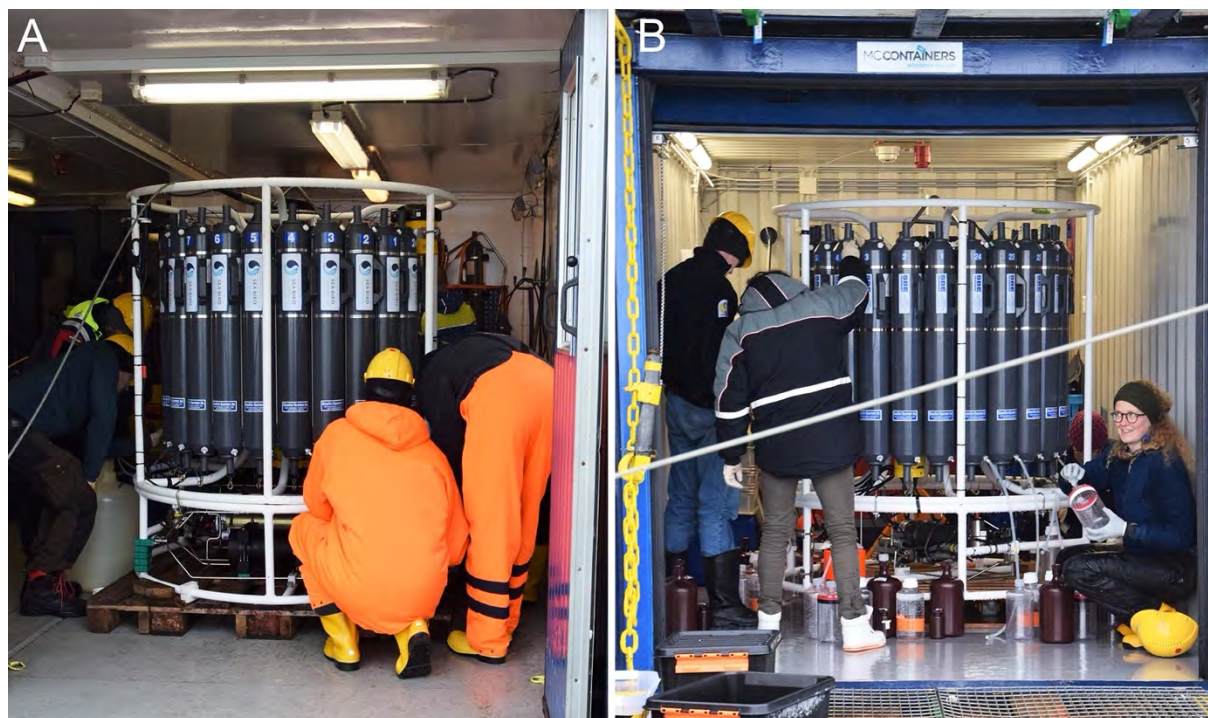

Figure 21.2: Water sampling from the two CTD rosettes during the SAS-Oden expedition 2021. (A) Bow CTD. (B) Stern CTD. © Hans-Jørgen Hansen

Table 21.2: Configurations of the bow CTD during the SAS-Oden 2021 expedition. Changes between configurations are marked in red. Strike-through = wrong number in notes.

Bow CTD - Configuration 1	Primary	Secondary	Notes
SBE3 temperature sensor	sn 5293	sn 6385	- Both primary and secondary temperature sensors (sn 5293 and 6385, respectively) and both primary and secondary conductivity sensors (sn 3653 and 4823, respectively) had the "wrong calibration files and should be swapped" with each other. - There is no turbidity meter' With serial number 2288 in the list of sensors. This is the dual turbidity/fluorometer sensor' With serial number 6255, however the .xmlcon file reads 2288 for some reason. No calibration data has been entered into the .xmlcon file for either the turbidity sensor or the fluorometer sensor.
SBE4 conductivity sensor	sn 3653	sn 4823	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 69525		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	~~sn 2288~~ sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		

Bow CTD - Configuration 2	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	- Primary and secondary temperature and conductivity sensors were given the correct calibration files. - Added SPAR unit, no serial number or calibration data has been entered in the .xmlcon file. - There is no turbidity meter with serial number 2288 in the list of sensors. This is the dual turbidity/fluorometer sensor with serial number 6255, however the .xmlcon file reads 2288 for some reason. No calibration data has been entered into the .xmlcon file for either the turbidity sensor or the fluorometer sensor.
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 69525		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	~~sn 2288~~ sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 3	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	- Altimeter from stern CTD With serial number 76191 was moved to bow CTD as the connector for altimeter with serial number 69525 was damaged (see "Altimeter Issue"). The serial number in the .xmlcon file was not changed however and still reads 69525. It also looks like no calibration data has been added to the altimeter in the .xmlcon file. - There is no turbidity meter with serial number 2288 in the list of sensors. This is the dual turbidity/fluorometer sensor with serial number 6255, however the .xmlcon file reads 2288 for some reason. No calibration data has been entered into the .xmlcon file for either the turbidity sensor or the fluorometer sensor.
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	~~sn 69525~~ sn 76191		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	~~sn 2288~~ sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 4	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	~~sn 69525~~ sn 76191		- Dual turbidity/fluorometer sensor with serial number 6255 was removed from the bow CTD.
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
~~Turbidity meter, WET Labs, ECO-NTU~~	~~sn 2288 (sn 6255)~~		
~~Fluorometer, WET Labs, ECO-AFL/FL~~	~~sn 6255~~		
SPAR, Biospherical/LI_COR			

Bow CTD - Configuration 5	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		- The connector to the altimeter with serial number 69525 was repaired and reinstalled on the bow CTD. It looks like calibration data has been added. - Configuration 5 was never used.
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 69525		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
SPAR, Biospherical/Licor			

Bow CTD - Configuration 6	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		- Dual turbidity/fluorometer sensor with serial number 6255 was reinstalled on the bow CTD. Calibration data was included in the .xmlcon file.
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 69525		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 7	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		- Dual turbidity/fluorometer sensor with serial number 6255 was removed from the bow CTD.
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 69525		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
~~Turbidity meter, WET Labs, ECO-NTU~~	~~sn 6255~~		
~~Fluorometer, WET Labs, ECO-AFL/FL~~	~~sn 6255~~		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 8	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		- The altimeter with serial number 69525 was not working so it was removed and replaced with the altimeter with serial number 76191.
SBE9+ pressure sensor	sn 0859		
Altimeter	~~sn 69525~~ sn 76191		
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 9	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 76191		- Dual turbidity/fluorometer sensor with serial number 6255 was reinstalled on the bow CTD.
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		
SPAR, Biospherical/Licor			

Bow CTD - Configuration 10	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 76191		- No changed sensors, but the CDOM sensor was on the "wrong voltage channel" and was changed to the correct one.
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 11	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 76191		- Dual turbidity/fluorometer sensor with serial number 6255 was removed from the bow CTD.
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
~~Turbidity meter, WET Labs, ECO-NTU~~	~~sn 6255~~		
~~Fluorometer, WET Labs, ECO-AFL/FL~~	~~sn 6255~~		
SPAR, Biospherical/LI-COR			

Bow CTD - Configuration 12	Primary	Secondary	Notes
SBE3 temperature sensor	sn 6385	sn 5293	
SBE4 conductivity sensor	sn 4823	sn 3653	
SBE43 oxygen sensor	sn 3969		
SBE9+ pressure sensor	sn 0859		
Altimeter	sn 76191		- Turbidity sensor with serial number 5796 was installed on the bow CTD.
Fluorometer, Turner Cyclops, CDOM	sn 216K00148		
PAR/irradiance, Biospherical/LI-COR	sn 707782		
Turbidity meter, WET Labs, ECO-NTU	sn 5796		
SPAR, Biospherical/LI-COR			

Table 21.3: Configurations of the stern CTD during the SAS-Oden 2021 expedition. Changes between configurations are marked in red. Strike-through = wrong number in notes.

Stern CTD - Configuration 1	Primary	Secondary	Notes
SBE3 temperature sensor	sn 5986	sn 6388	- Pressure sensor (with actual serial number 1388) had the calibration file of the pressure sensor on the bow CTD (serial number 0859). For some reason the original .xmlcon file (called "sas21-Decktest.xmlcon") has been changed and given the correct calibration data even though we created a new config file ("sas21-ctd-b-conf2.xmlcon").
SBE4 conductivity sensor	sn 4514	sn 4822	
SBE43 oxygen sensor	sn 3835		
SBE9+ pressure sensor	~~sn 0859~~ sn 1388		
Altimeter	sn 76191		

Stern CTD - Configuration 2	Primary	Secondary	Notes
SBE3 temperature sensor	sn 5986	sn 6388	- Pressure sensor with serial number 1388 was given the correct configuration file.
SBE4 conductivity sensor	sn 4514	sn 4822	- Dual turbidity/fluorometer sensor package with serial number 6255 was installed on the stern CTD. data has been entered into the .xmlcon file for either the turbidity sensor or the fluorometer sensor.
SBE43 oxygen sensor	sn 3835		
SBE9+ pressure sensor	sn 1388		
Altimeter	sn 76191		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		

Stern CTD - Configuration 3	Primary	Secondary	Notes
SBE3 temperature sensor	sn 5986	sn 6388	
SBE4 conductivity sensor	sn 4514	sn 4822	
SBE43 oxygen sensor	sn 3835		
SBE9+ pressure sensor	sn 1388		- Removed altimeter with serial number 76191 from the stern CTD.
~~Altimeter~~	~~sn 76191~~		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		

Stern CTD - Configuration 4	Primary	Secondary	Notes
SBE3 temperature sensor	sn 5986	sn 6388	
SBE4 conductivity sensor	sn 4514	sn 4822	
SBE43 oxygen sensor	sn 3835		
SBE9+ pressure sensor	sn 1388		- Installed altimeter with serial number 76191 on the stern CTD.
Altimeter	sn 76191		
Turbidity meter, WET Labs, ECO-NTU	sn 6255		
Fluorometer, WET Labs, ECO-AFL/FL	sn 6255		

Table 21.4: Device Operations (DOs) of the 62 successful CTD casts from the bow (Table 5.3), the configuration (Conf.) used for each DO (Table 21.2), and collection of LADCP data by WP14 (MWA), indicated by "x". The date, longitude and latitude are those recorded by WP14 (MWA) on the bridge at the start and end of each CTD cast. The depth is the maximum CTD depth according to the CTD deck sheet with between brackets the calculated average station depth from acoustic measurements while the ship was drifting with the ice, see the excel file "SO21_Expedition_Logbook" in the SND data repository.

Conf	DO	Date	Long (DM)	Lat (DM)	Depth (m)	DO description	LADCP
1	SO21_00-01	2021-08-01	18°29.175′E	81°13.738′N	-400 (-450)	CTD EK80 SAS	
2	SO21_01-01	2021-08-02	26°36.683′E	81°30.156′N	-1234 (-1190)	CTD deep SAS	
3	SO21_03-01	2021-08-03	30°22.070′E	82°01.289′N	-500 (-3235)	CTD omics SAS	
3	SO21_05-01	2021-08-04	29°25.608′E	83°05.169′N	-100 (-3851)	CTD shallow SAS	x
3	SO21_05-02	2021-08-04	29°21.825′E	83°05.751′N	-3979 (-3892)	CTD deep SAS	x
3	SO21_05-03	2021-08-04	29°28.859′E	83°07.168′N	-3980 (-3926)	CTD deep VACAO	x
4	SO21_07-04	2021-08-05	30°15.270′E	84°18.007′N	-125 (-4001)	CTD shallow SAS	x
4	SO21_07-05	2021-08-05	30°12.708′E	84°18.459′N	-4072 (-4001)	CTD deep SAS	x
6	SO21_08-01	2021-08-07	30°11.400′E	86°30.810′N	-24 (-2674)	CTD ChlMax PICO	
6	SO21_08-02	2021-08-07	30°11.600′E	86°30.810′N	-125 (-2581)	CTD shallow SAS	x
7	SO21_08-03	2021-08-07	30°38.143′E	86°20.470′N	-2078 (-2368)	CTD deep SAS	
7	SO21_08-09	2021-08-08	33°23.954′E	86°26.851′N	-2940 (-3179)	CTD deep VACAO	x
7	SO21_11-01	2021-08-09	30°46.193′E	87°02.982′N	-100 (-3926)	CTD shallow SAS	x
7	SO21_13-04	2021-08-10	29°32.452′E	88°02.715′N	-300 (-4059)	CTD shallow SAS	
7	SO21_13-05	2021-08-10	29°34.992′E	88°02.960′N	-4114 (-4061)	CTD deep SAS	
8	SO21_16-04	2021-08-12	23°53.903′E	88°57.820′N	-300 (-4334)	CTD shallow SAS	x
8	SO21_16-05	2021-08-12	23°26.775′E	88°57.360′N	-4427 (-4335)	CTD deep SAS	x
8	SO21_16-06	2021-08-12	22°43.842′E	88°56.690′N	-4428 (-4336)	CTD deep VACAO	x
8	SO21_18-04	2021-08-13	23°37.915′E	89°09.583′N	-150 (-4309)	CTD shallow SAS	x
8	SO21_18-05	2021-08-13	23°58.442′E	89°09.113′N	-4395 (-4318)	CTD deep SAS	x
8	SO21_20-01	2021-08-14	16°59.618′E	89°36.793′N	-235 (-4249)	CTD shallow SAS	x
8	SO21_20-02	2021-08-14	16°45.809′E	89°36.672′N	-4325 (-4247)	CTD deep SAS	x
8	SO21_20-03	2021-08-14	17°00.896′E	89°36.116′N	-4325 (-4246)	CTD deep VACAO	x
8	SO21_22-08	2021-08-16	60°45.290′E	89°55.227′N	-300 (-4241)	CTD shallow SAS	x
8	SO21_22-09	2021-08-16	62°12.554′E	89°55.173′N	-4318 (-4241)	CTD deep SAS	x
9	SO21_24-01	2021-08-18	145°50.963′W	89°30.877′N	-17.5 (-4156)	CTD ChlMax PICO	
9	SO21_24-02	2021-08-18	145°51.484′W	89°30.861′N	-50 (-4156)	CTD shallow SAS	
9	SO21_24-03	2021-08-18	145°50.960′W	89°30.877′N	-4252 (-4156)	CTD deep SAS	x
10	SO21_25-01	2021-08-18	147°59.402′W	89°19.036′N	-125 (-3318)	CTD shallow SAS	x
11	SO21_25-02	2021-08-18	147°52.294′W	89°19.297′N	-3449 (-3379)	CTD deep SAS	x
11	SO21_26-01	2021-08-19	149°58.376′W	89°06.654′N	-75 (-1313)	CTD shallow SAS	x
11	SO21_26-02	2021-08-19	150°02.487′W	89°06.944′N	-1327 (-1313)	CTD deep SAS	x
11	SO21_28-01	2021-08-21	136°27.121′W	88°44.668′N	-250 (-3949)	CTD shallow SAS	x
11	SO21_28-02	2021-08-21	136°31.071′W	88°44.583′N	-4017 (-3949)	CTD deep SAS	x
11	SO21_28-03	2021-08-21	136°54.079′W	88°44.375′N	-4017 (-3952)	CTD deep VACAO	x
11	SO21_30-01	2021-08-21	129°48.264′W	88°30.435′N	-250 (-3846)	CTD shallow SAS	x
11	SO21_30-02	2021-08-21	129°46.158′W	88°30.640′N	-3979 (-3943)	CTD deep SAS	x
11	SO21_32-02	2021-08-24	115°41.493′W	88°23.195′N	-2920 (-2889)	CTD deep SAS	x
11	SO21_33-01	2021-08-25	100°36.257′W	88°05.883′N	-410 (-2948)	CTD shallow SAS	x
11	SO21_33-02	2021-08-25	100°44.832′W	88°06.014′N	-3004 (-2964)	CTD deep SAS	x
11	SO21_35-11	2021-08-27	86°07.006′W	87°50.423′N	-100 (-1426)	CTD shallow SAS	x

11	SO21_35-12	2021-08-27	86°00.691′W	87°50.269′N	-1401 (-1412)	CTD deep SAS	x
11	SO21_37-01	2021-08-27	76°32.885′W	87°47.376′N	-2593 (-2559)	CTD deep SAS	x
11	SO21_38-13	2021-08-29	66°17.203′W	87°45.516′N	-100 (-1186)	CTD shallow SAS	x
11	SO21_38-14	2021-08-29	66°09.581′W	87°45.876′N	-1198 (-1187)	CTD deep SAS	x
11	SO21_40-01	2021-08-29	63°16.784′W	87°20.559′N	-1173 (-1169)	CTD deep SAS	x
11	SO21_41-01	2021-08-30	59°16.942′W	86°55.934′N	-1142 (-1140)	CTD deep SAS	x
11	SO21_42-01	2021-08-30	57°23.484′W	86°30.876′N	-564 (-562)	CTD deep SAS	x
11	SO21_44-01	2021-09-01	43°09.776′W	86°17.070′N	-3361 (-3310)	CTD deep SAS	x
11	SO21_45-01	2021-09-01	39°58.988′W	86°02.781′N	-3208 (-3161)	CTD deep SAS	x
11	SO21_46-01	2021-09-02	38°28.831′W	85°50.769′N	-3300 (-3253)	CTD deep SAS	x
11	SO21_46-02	2021-09-02	38°25.171′W	85°50.914′N	--3301 (3253)	CTD deep VACAO	x
11	SO21_47-01	2021-09-02	35°40.597′W	85°28.695′N	-2759 (-2716)	CTD deep SAS	x
11	SO21_48-01	2021-09-03	33°27.496′W	84°56.537′N	-1488 (-1496)	CTD deep SAS	x
12	SO21_50-16	2021-09-05	32°23.466′W	84°09.649′N	-891 (-892)	CTD deep SAS	x
12	SO21_52-01	2021-09-05	27°15.576′W	84°16.084′N	-1057 (-1054)	CTD deep SAS	x
11	SO21_53-14	2021-09-06	24°42.356′W	84°31.745′N	-30 (-1364)	CTD shallow SAS	x
11	SO21_53-15	2021-09-06	24°44.026′W	84°31.814′N	-1432 (-1391)	CTD deep SAS	x
11	SO21_56-07	2021-09-08	3°03.916′W	83°49.569′N	-75 (-3028)	CTD shallow SAS	x
11	SO21_56-08	2021-09-09	3°05.429′W	83°49.408′N	-2943 (-3008)	CTD deep SAS	x
11	SO21_58-17	2021-09-11	8°23.950′E	82°22.117′N	-50 (-983)	CTD shallow SAS	x
11	SO21_58-18	2021-09-11	8°19.027′E	82°22.121′N	-1024 (-1015)	CTD deep SAS	x

Table 21.5: Device Operations (DOs) of the 50 successful CTD casts from the stern (Table 5.3), the configuration (Conf.) used for each DO (Table 21.3), and collection of WBAT and FishCam data by WP1 (EFICA), indicated by "x". The date, longitude and latitude are those recorded by WP14 (MWA) on the bridge at the start and end of each CTD cast. The depth is the maximum CTD depth according to the CTD deck sheet with between brackets the calculated average station depth from acoustic measurements while the ship was drifting with the ice, see the excel file "SO21_Expedition_Logbook" in the SND data repository.

Conf.	Device Operation	Date	Longitude (DM)	Latitude (DM)	CTD depth in meters	Device Operation description	WBAT	Fish Cam
1	SO21_03-02	2021-08-03	30°26.788′E	82°03.809′N	-500 (-3235)	CTD bio SAS	x	
2	SO21_07-01	2021-08-05	30°36.847′E	84°16.610′N	-1000 (-4000)	CTD omics SAS	x	
2	SO21_07-03	2021-08-05	30°21.534′E	84°17.308′N	-1000 (-4001)	CTD bio SAS	x	
2	SO21_08-04	2021-08-08	31°46.849′E	86°23.173′N	-1000 (-2992)	CTD omics SAS	x	
2	SO21_08-06	2021-08-08	32°20.597′E	86°23.762′N	-1000 (-2616)	CTD bio SAS	x	
3	SO21_13-01	2021-08-10	29°21.175′E	88°02.003′N	-1000 (-4294)	CTD omics SAS	x	
3	SO21_13-03	2021-08-10	29°31.585′E	88°02.249′N	-1000 (-4252)	CTD bio SAS	x	
3	SO21_14-05	2021-08-11	28°32.608′E	88°29.253′N	-1000 (-4366)	CTD wbat EFICA	x	x
3	SO21_16-01	2021-08-12	25°05.932′E	88°59.796′N	-1000 (-4332)	CTD omics SAS	x	x
3	SO21_16-03	2021-08-12	24°23.936′E	88°58.594′N	-1000 (-4333)	CTD bio SAS	x	x
3	SO21_18-01	2021-08-13	23°14.735′E	89°10.752′N	-1000 (-4301)	CTD omics SAS	x	
3	SO21_18-03	2021-08-13	23°19.136′E	89°10.120′N	-1000 (-4309)	CTD bio SAS	x	x
3	SO21_22-03	2021-08-15	45°29.938′E	89°54.985′N	-1000 (-4241)	CTD wbat EFICA	x	
3	SO21_22-10	2021-08-16	64°48.713′E	89°55.326′N	-1000 (-4241)	CTD omics SAS	x	
3	SO21_22-11	2021-08-16	66°36.549′E	89°55.377′N	-1000 (-4241)	CTD bio SAS	x	x
4	SO21_26-03	2021-08-19	149°28.445′W	89°05.836′N	-1000 (-1319)	CTD omics SAS	x	
4	SO21_26-05	2021-08-19	149°53.656′W	89°06.445′N	-1000 (-1333)	CTD bio SAS	x	x
3	SO21_26-11	2021-08-20	151°04.785′W	89°08.276′N	-1000 (-1355)	CTD wbat EFICA	x	
3	SO21_30-03	2021-08-22	129°25.214′W	88°31.351′N	-1000 (-3936)	CTD wbat EFICA	x	
3	SO21_30-11	2021-08-23	127°36.914′W	88°35.371′N	-1000 (-3942)	CTD omics SAS	x	
3	SO21_30-13	2021-08-23	129°04.905′W	88°37.953′N	-1000 (-3944)	CTD bio SAS	x	x
3	SO21_30-16	2021-08-23	129°27.188′W	88°39.538′N	-1000 (-3944)	CTD wbat EFICA	x	
3	SO21_33-03	2021-08-25	101°48.578′W	88°06.737′N	-1000 (-3049)	CTD omics SAS	x	
3	SO21_33-05	2021-08-25	102°00.896′W	88°08.326′N	-1000 (-2987)	CTD bio SAS	x	
3	SO21_35-08	2021-08-26	86°34.127′W	87°50.843′N	-1000 (-1456)	CTD wbat EFICA	x	
3	SO21_35-13	2021-08-27	85°39.676′W	87°50.043′N	-1000 (-1388)	CTD omics SAS	x	
3	SO21_35-15	2021-08-27	85°29.556′W	87°50.035′N	-1000 (-1389)	CTD bio SAS	x	x
3	SO21_38-08	2021-08-28	66°42.746′W	87°44.601′N	-1108 (-1178)	CTD wbat EFICA		
3	SO21_38-15	2021-08-29	65°50.086′W	87°46.518′N	-1000 (-1201)	CTD omics SAS	x	
3	SO21_38-17	2021-08-29	65°41.240′W	87°46.824′N	-1000 (-1198)	CTD bio SAS	x	x
3	SO21_42-02	2021-08-30	57°20.241′W	86°31.011′N	-500 (-595)	CTD wbat EFICA	x	
3	SO21_42-06	2021-08-30	57°11.410′W	86°31.167′N	-580 (-631)	CTD omics SAS	x	
3	SO21_42-08	2021-08-31	57°06.000′W	86°31.250′N	-500 (-660)	CTD bio SAS	x	x
3	SO21_48-02	2021-09-03	33°29.757′W	84°55.516′N	-1000 (-1547)	CTD omics SAS	x	
3	SO21_48-04	2021-09-03	33°28.724′W	84°55.495′N	-1000 (-1554)	CTD bio SAS	x	x
3	SO21_50-06	2021-09-04	32°15.508′W	84°09.100′N	-800 (-895)	CTD wbat EFICA	x	
3	SO21_50-11	2021-09-04	32°19.139′W	84°09.710′N	-850 (-890)	CTD omics SAS	x	
3	SO21_50-13	2021-09-05	32°21.323′W	84°09.566′N	-850 (-889)	CTD bio SAS	x	x
3	SO21_53-05	2021-09-06	23°59.420′W	84°28.413′N	-1000 (-1250)	CTD wbat EFICA	x	
3	SO21_53-06	2021-09-06	23°59.420′W	84°28.413′N	-1000 (-1250)	CTD wbat EFICA	x	
3	SO21_53-07	2021-09-06	23°59.358′W	84°28.766′N	-1000 (-1250)	CTD wbat EFICA	x	

3	SO21_53-08	2021-09-06	24°17.239′W	84°29.872′N	-1000 (-1351)	CTD omics SAS	x	
3	SO21_53-09	2021-09-06	24°23.569′W	84°30.919′N	-1000 (-1351)	CTD bio SAS	x	x
3	SO21_56-01	2021-09-08	2°30.378′W	83°52.135′N	-1000 (-2650)	CTD omics SAS	x	
3	SO21_56-03	2021-09-08	2°34.176′W	83°51.426′N	-1000 (-2654)	CTD bio SAS	x	x
3	SO21_56-05	2021-09-08	2°53.705′W	83°49.956′N	-1000 (-2654)	CTD wbat EFICA	x	
3	SO21_56-06	2021-09-08	3°01.514′W	83°49.704′N	-17.4 (-3028)	CTD ChlMax PICO	x	
3	SO21_58-09	2021-09-10	8°42.821′E	82°28.438′N	-1000 (-1506)	CTD omics SAS	x	
3	SO21_58-12	2021-09-11	8°42.161′E	82°27.749′N	-1000 (-1274)	CTD bio SAS	x	x
3	SO21_58-15	2021-09-11	8°45.285′E	82°24.272′N	-1000 (-1168)	CTD wbat EFICA	x	

21.4   Summary of metadata collected

The data collected by WP15 (WAOW) during the SAS-Oden 2021 expedition are published in the SND data repository and summarised in *Table 21.6*. The metadata collected by WP15 during the SAS-Oden 2021 expedition are provided in the excel file "SO21_Metadata_WP15_ WAOW" in the SND data repository and summarised in *Table 20.4*.

Table 21.6: Overview of all measurements collected by WP15 (WAOW).

Parameter	Type of data	Collected by	Data owner
CTD pressure (depth)	Continuous CTD profiles	WP14 + WP15	SPRS
CTD salinity	Continuous CTD profiles	WP14 + WP15	SPRS
CTD temperature	Continuous CTD profiles	WP14 + WP15	SPRS

21.5   Summary of preliminary results

Preliminary results of absolute salinity and conservative temperature obtained from the CTD data obtained throughout the expedition are shown in *Figures 21.3 and 21.4*. Below the cold halocline waters (0-100 m), the relatively warm Atlantic water can be observed as a warm core (0.5-2.5°C). The warmer deep waters from the Makarov Basin can also be distinguished from the colder deep waters in the Eurasian Basin.
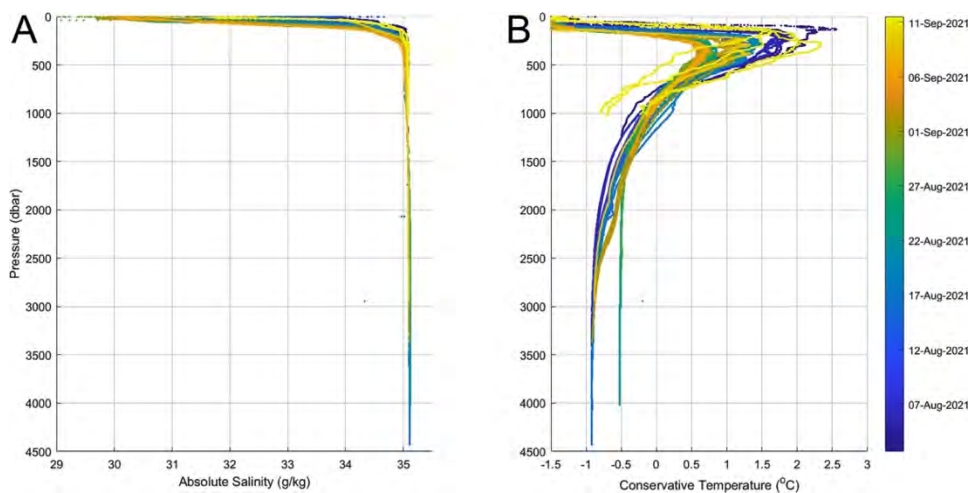
Figure 21.3: Vertical profiles of CTD data from the SAS-Oden 2021 expedition. (A) Absolute salinity. (B) Conservative temperature. ©Salar Karam
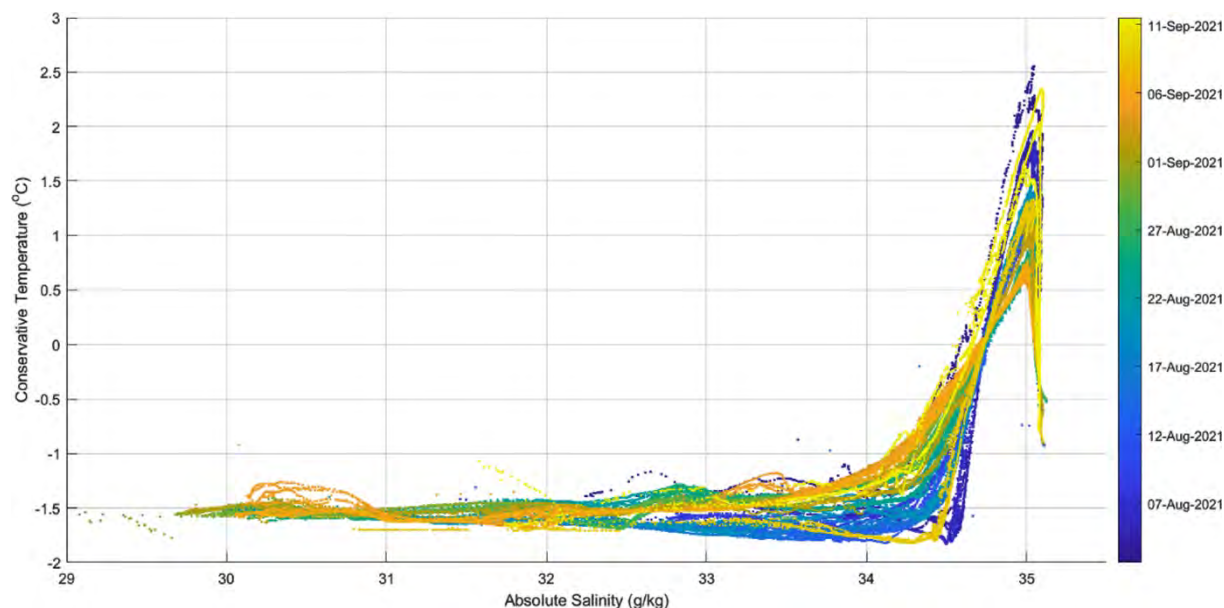
Figure 21.4: T-S plot of CTD data from the SAS-Oden 2021 expedition. ©Salar Karam

21.6   Summary of post-cruise analyses and deliverables

All CTD data that was collected on board will undergo further data processing and will be quality controlled after the cruise together with WP14 (MWA). Furthermore, WP10 (CATCHEM) collected 60 salinity samples from the deep ocean which will be analysed with a salinometer after the cruise (*Chapter 17*), the results of which will be used to calibrate the two SBE4 conductivity sensors on the bow CTD. A final product will be delivered after the sensors on both CTDs have undergone their post-cruise calibrations performed by the SPRS in late 2021 / early 2022, the results of which will be used to account for any possible drift in the CTD sensors.

Deliverables: The data will be made publicly available in the SND data repository according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). The CTD data will also be made publicly available in the PANGAEA[180] data repository. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[180] https://www.pangaea.de

22 WP16 (ACAS)

John Prytherch, Sonja Murto

Project title: Arctic climate across scales (ACAS)

22.1   Resources

External project funding: Knut and Alice Wallenberg Foundation (KAW, grant number 2016-0024) and the Swedish Polar Research Secretariat (SPRS, Implementation Agreement Dnr 2020-119) for two berths on the SAS-Oden 2021 expedition to Michael Tjernström. All project participants are listed in *Table 22.1*.

Table 22.1: Overview of all onboard and onshore participants of the WP16 (ACAS) project.

On board	Name	e-mail address	Task in project	Affiliation
No	Michael Tjernström	michaelt@misu.su.se	PI	SU, Sweden
Yes (berth)	John Prytherch	john.prytherch@misu.su.se	WP Leader, sampling & measuring	SU, Sweden
Yes (berth)	Sonja Murto	sonja.murto@misu.su.se	Sampling & measuring	SU, Sweden
No	Rodrigo Caballero	rodrigo@misu.su.se	Analysis & modelling	SU, Sweden
No	Annica Ekman	annica@misu.su.se	Analysis & modelling	SU, Sweden
No	Radovan Krejci	radovan.krejci@aces.su.se	Analysis & modeling	SU, Sweden
No	Johan Nilsson	nilsson@misu.su.se	Analysis & modeling	SU, Sweden
No	Ilona Riipinen	ilona.riipinen@itm.su.se	Analysis & modeling	SU, Sweden
No	Gunilla Svensson	gunilla@misu.su.se	Analysis & modeling	SU, Sweden
No	Paul Zieger	paul.zieger@aces.su.se	Analysis & modeling	SU, Sweden
No	Rodrigo Caballero	rodrigo@misu.su.se	Analysis & modeling	SU, Sweden

Figure 22.1: John Prytherch (left) and Sonja Murto (right) on their way to measure CO_2 and CH_4 gas fluxes in a lead during the SAS-Oden 2021 expedition. ©SPRS

22.2 Scientific motivation and specific research questions

Environmental change in the Arctic is fast as a consequence of global climate change, faster than elsewhere on Earth. Although the ocean also contributes, the largest part of these changes are mediated through the atmosphere in processes that are active across all scales, from changes in atmospheric temperature and moisture over changes in winds and weather patterns to changes in clouds and the surface energy budget.

All of this contributes to a forcing or modulating of the fluxes of energy and matter at the interface between the ocean/sea ice and the atmosphere, and hence the melt and freeze of sea ice and the conditions in the upper ocean. Many of the manifestations of Arctic atmospheric change are poorly understood and quantified, to a large extent because of a lack of observations. Therefore, observations of the atmosphere must accompany any observationally driven attempt to understand these changes in the sea ice and ocean within the Arctic environment[181].

To better quantify and understand these processes, and because of the very large variability of the atmosphere, there is therefore an urgent need for a larger ensemble of observations, that cannot be met by the very infrequent but detailed observations during Arctic expeditions with an atmospheric focus. Within ACAS we are therefore designing an atmospheric observatory that can be operated with a minimum off staff to enable more frequent deployment, on more expeditions regardless of science focus. An embryo for this observatory was successfully deployed for the first time during Arctic Ocean 2018 expedition with *IB Oden*. During the SAS-Oden 2021 expedition we deployed the next phase: two key remote sensing instruments, a vertically pointing cloud Doppler radar and a scanning profiling microwave radiometer.

Combining these instruments with soundings of the atmosphere and with the already deployed ceilometer lidar and visibility observations allows application of advanced cloud retrieval algorithms, such as CloudNet, while also continuously monitoring the vertical structure of the atmosphere. Eddy covariance observations of surface energy, momentum and gas fluxes deployed in the foredeck mast combined with radiation measurements and surface temperature provide important components of the surface energy budget, while standard meteorological parameters provide context. Soundings transmitted on the World Meteorological Organisations (WMOs) Global Telecommunication System (GTS) in near-real time also contribute to improving the weather forecasts for the expedition.

The project also contributes to carbon budget estimates by examining the magnitude and drivers of ocean-atmosphere and ice-atmosphere CO_2 and CH_4 gas exchange from the foremast and chamber fluxes (*Figure 22.1*). These data will be analysed with the atmospheric CH_4 and CO_2 mean concentration and water-column profile measurements of WP13 (TGB) and with waterside pCH_4 and pCO_2 measurements from the ice stations and the underway line.

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[181] Supporting peer-reviewed publications:

Prytherch J, Yelland MJ (2021) Wind, convection and fetch dependence of gas transfer velocity in an Arctic sea-ice lead determined from eddy covariance CO_2 flux measurements. Global Biogeochemical Cycles 35: e2020GB006633 [http://doi.org/10.1029/2020GB006633]

Tjernström M, et al. (2021) Central Arctic weather forecasting: Confronting the ECMWF IFS with observations from the Arctic Ocean 2018 expedition. Quarterly Journal of the Royal Meteorological Society 147:1278–1299 [http://doi.org/10.1002/qj.3971]

Vüllers J, et al. (2021) Meteorological and cloud conditions during the Arctic Ocean 2018 expedition, Atmospheric Chemistry and Physics 21:289–314 [http://doi.org/10.5194/acp-21-289-2021]

Prytherch J, et al. (2017) Direct determination of the air-sea CO_2 gas transfer velocity in Arctic sea ice regions Geophysical Research Letters 44:3770– 3778 [http://doi.org/10.1002/2017GL073593]

Tjernström M, et al. (2015) Warm-air advection, air mass transformation and fog causes rapid ice melt, Geophysical Research Letters 42:5594–5602 [http://doi.org/10.1002/2015GL064373]

Tjernström M, et al. (2014) The Arctic Summer Cloud Ocean Study (ASCOS): Overview and experimental design. Atmospheric Chemistry and Physics 14:2823–2869 [http://doi.org/10.5194/acp-14-2823-2014]

Tjernström M, et al. (2004) The summertime Arctic atmosphere: Meteorological measurements during the Arctic Ocean Experiment (AOE-2001). Bulletin of the American Meteorological Society 85:1305–1321 [http://doi.org/10.1175/BAMS-85-9-1305]

22.3   Summary of field work performed

ACAS has installed an advanced weather station on the 7th deck of *IB Oden* that, in addition to basic meteorological variables, measures surface temperature from downward-looking infrared thermometers, incoming broad-band solar and thermal radiation, cloud geometry and back-scatter profiles from a lidar ceilometer, and precipitation intensity and visibility from a so-called present-weather sensor (*Figure 22.2*).

Furthermore, a motion-stabilized, vertically pointing, W-band Doppler cloud radar (active), measuring cloud particle backscatter, polarization and vertical velocity up to 10 km height, and a microwave radiometer (passive) deriving profiles of temperature, humidity and liquid water up to 10 km height, are installed on a flat rack, mounted on the roof of a container on the 4th deck of *IB Oden*, close to the ship's centreline alongside the "Triple Lab" (*Figure 22.3 A*). The container itself houses control and data logging systems for the remote sensing instrumentation, and for the other measurement systems listed here.

We also deployed an eddy-covariance system in *IB Oden*'s foredeck mast for measurements of turbulent surface fluxes of momentum heat and water vapor. In collaboration with Patrick Crill and Brett Thornton (SU, WP13), we also sampled their LGR (Los Gatos Research) laser spectrometer system for profiles and eddy-covariance fluxes of CH_4 and CO_2 (*Figure 22.3 B*). A membrane equilibration sensor, measuring continuous pCO_2, was installed and sampled from *IB Oden*'s underway line drawing water from 8 m depth.

Throughout the expedition, we launched 6-hourly radio soundings from *IB Oden*'s helipad (*Figure 22.4*); the data from these (temperature, humidity, pressure and wind speed profiles to ca. 28 km height) were distributed in near-real time across the WMOs GTS and were assimilated in weather-forecast models across the world.
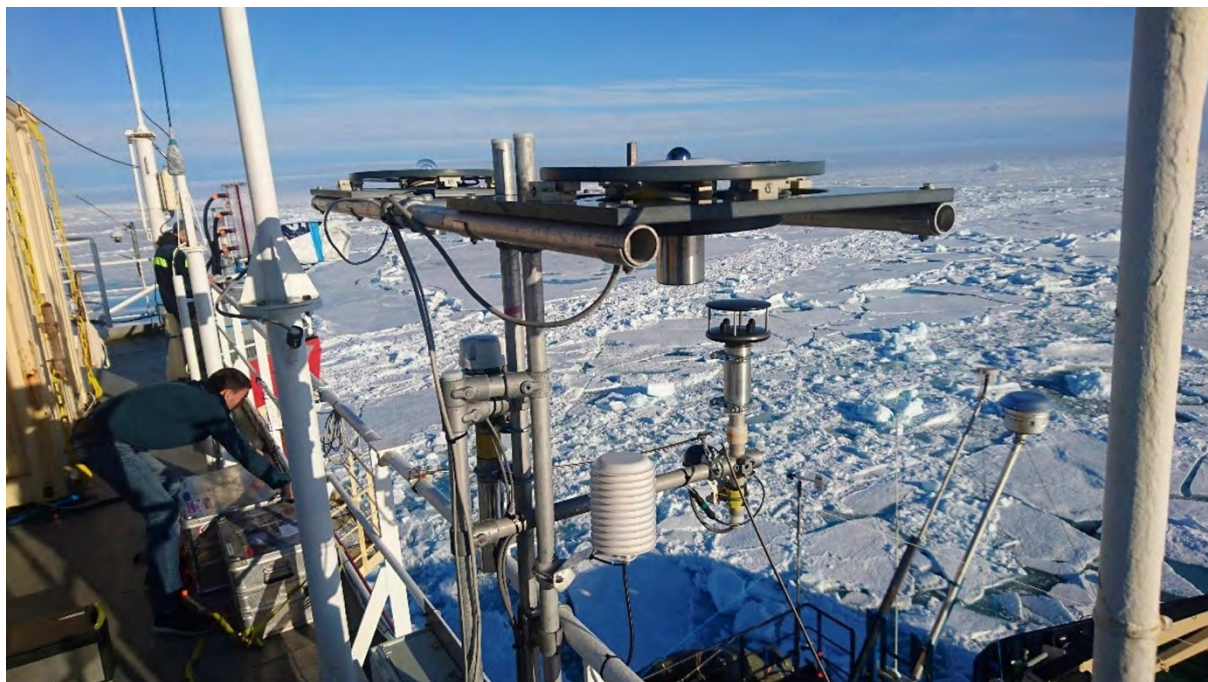
Figure 22.2: Weather station on the 7th deck (above the bridge) photographed during a previous expedition with IB Oden. *©John Prytherch*
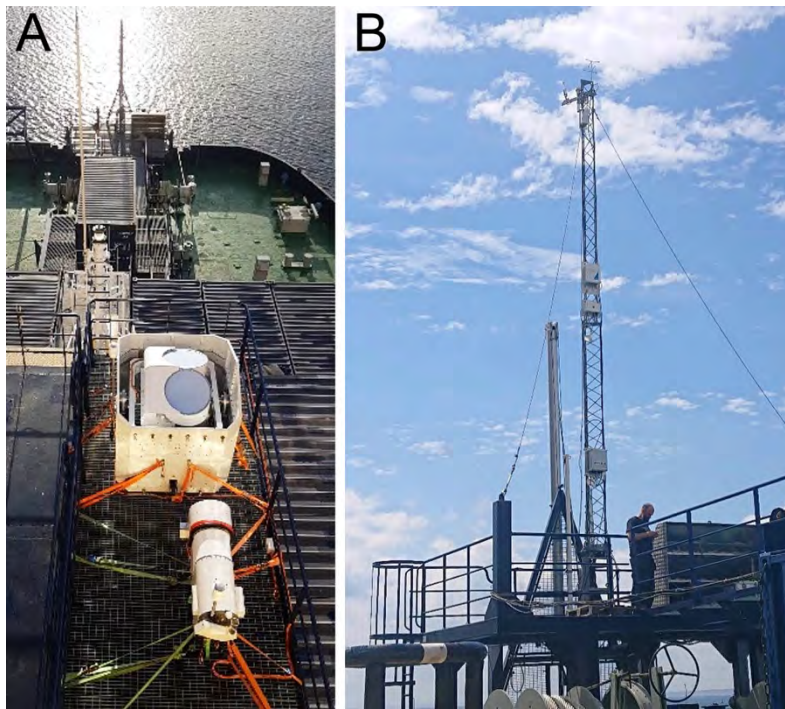
Figure 22.3: Equipment installed on IB Oden. (A) Microwave radiometer (aft) and (at front) the Doppler cloud radar in its cardanic suspension frame, deployed on a container roof on IB Oden's 4th deck-lab. (B) Eddy covariance flux system on foremast. ©John Prytherch

Figure 22.4: Throughout the SAS-Oden 2021 expedition WP16 launched 6-hourly radio soundings from IB Oden's helipad. (A) Weather balloon filling station below helipad. (B) Sonja Murto launching a radio-sounding weather balloon. (A) ©Sonja Murto, (B) ©John Prytherch

Chamber flux measurement systems were deployed during ice stations (ship and helicopter stations) and opportunistically from the side of *IB Oden* during CTD stations to measure surface-atmosphere exchange of CH_4 and CO_2, primarily through lead water surfaces (*Figure 22.5 A,B*), but also ice-atmosphere (*Figure 22.5 C*) and melt pond-atmosphere fluxes. Three separate chamber flux systems were deployed. The primary system being an LGR cavity enhanced laser spectrometer similar to that used at the foremast, mounted in a pulka (*Figure 22.6*). The LGR is powered by 2 12V batteries connected to an inverter, and the chamber, connected to the LGR via plastic tubing, is floating in the water (*Figure 22.6 B*). The chamber measurements were complemented by surface temperature measurements from a thermocouple array, ice depth measurements, and surface water samples from leads and melt ponds (*Figure 22.5 B*) taken for onboard analysis for CH_4 and carbon parameters (collaboration with WP13 and WP10), and for post-cruise analysis for CH_4 and carbon isotopes to enable identification of carbon sources (e.g., biological or fossil-fuel combustion) (collaboration with WP13). Water samples for analysis of CH_4 concentrations were also taken from leads using a Ruttner sampler at depths of 0.5, 1.0, 1.5, 2.0 and 2.5 m depths.
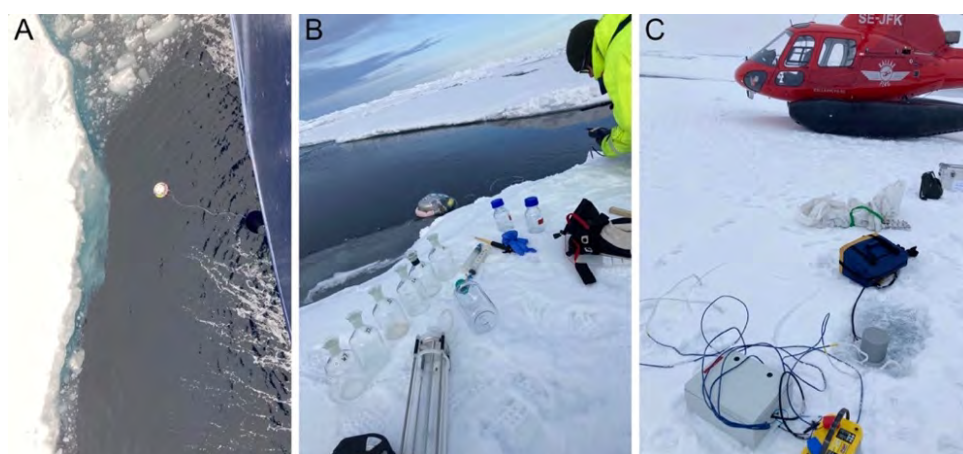
Figure 22.5: Chamber flux measurements during the SAS-Oden 2021 expedition. (A) LGR chamber deployed from the ship. (B) LGR chamber deployed in a lead and glass bottles for water samples for CH_4 and carbon parameters. (C) EGM4 CO_2 chamber flux system measuring ice-atmosphere and snow-atmosphere CO_2 flux, with a thermocouple array (front of picture) deployed alongside measuring snow and ice surface temperature. (A) ©John Prytherch, (B,C) ©Sonja Murto
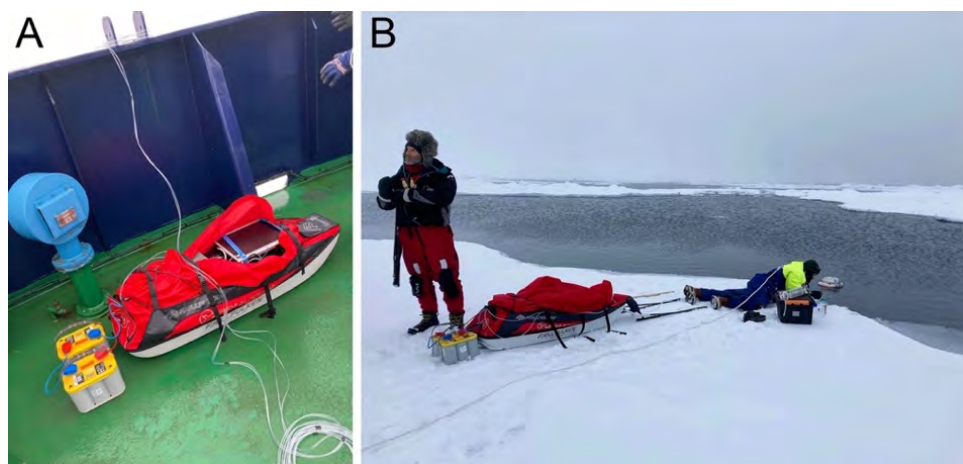

Figure 22.6: The chamber system that was used most during the SAS-Oden 2021 expedition. (A) LGR cavity enhanced spectrometer-based chamber flux system mounted in pulka. (B) John Prytherch deploying the system to measure air-water CH_4 and CO_2 fluxes in a lead with helicopter pilot Ted Juliussen acting as polar bear guard. ©Sonja Murto

22.4   Summary of metadata collected

Meteorological variables were measured continuously throughout the expedition at sampling frequencies between 0.2 and 40 Hz. All systems functioned throughout, with occasional offline periods due to temporary faults or maintenance. Parameters measured include Parameters measured include environmental air temperature, humidity and wind speed and direction, surface temperature (both with infrared sensors and *in situ* during ice stations), incoming solar and thermal radiation, visibility and precipitation, surface fluxes of momentum, heat, water vapour, vertical profiles of air temperature, humidity, wind speed and direction, plus vertical structure and detailed and bulk properties of clouds (geometry, cloud water phase and concentrations).

The processed and quality-controlled meteorological data collected by WP16 (ACAS) during the SAS-Oden 2021 expedition will be published open-access on the Bolin Centre Database[182].

The metadata for the chamber flux measurements and water samples taken by WP16 (ACAS) during the SAS-Oden 2021 are provided in the excel file "SO21_Metadata_WP16_ACAS" in the SND data repository and summarised in *Table 22.2*.

Table 22.2: Overview of all measurements/samples collected by WP16 (ACAS). Nr = number of measurements/samples, GC = gas chromatography

Parameter	Method / instrument	Where	Nr	Analysis
Air-water CH_4 flux	LGR cavity enhanced laser chamber flux system	Leads	153	-
Air-water CH_4 flux	LGR cavity enhanced laser chamber flux system	Melt ponds	5	-
Air-ice CH_4 flux	LGR cavity enhanced laser chamber flux system	Ice & snow surface	25	-
Air-water CO_2 flux	LGR cavity enhanced laser chamber flux system	Leads	153	-
Air-water CO_2 flux	LGR cavity enhanced laser chamber flux system	Melt ponds	5	-
Air-ice CO_2 flux	LGR cavity enhanced laser chamber flux system	Ice & snow surface	25	-
Air-water CO_2 flux	Li-7200 chamber flux system	Leads	41	-
Air-water CO_2 flux	Li-7200 chamber flux system	Melt ponds	12	-
Air-ice CO_2 flux	EGM4 chamber flux system	Ice & snow surface	105	
CH_4 concentration	Water sample	Leads	61	Onboard GC (WP13)
CH_4 concentration	Water sample	Melt ponds	5	Onboard GC (WP13)
DIC & TA	Water sample	Leads	48	Onboard (WP10)
DIC & TA	Water sample	Melt ponds	10	Onboard (WP10)
$d^{13}C$-CH_4 / $d^{13}C$-CO_2	Water sample	Leads	47	Post-cruise GC (WP13)
$d^{13}C$-CH_4 / $d^{13}C$-CO_2	Water sample	Melt ponds	22	Post-cruise GC (WP13)
Ice thickness	Auger/thickness tape	Ice	40	-
Ice freeboard	Auger/thickness tape	Ice	29	-

22.5   Summary of preliminary results

A major task of WP16 on the SAS-Oden 2121 expedition was to launch weather balloons four times daily at 00, 06, 12 and 18 UTC. Initially we tested different balloon volumes in order to reach a favourable ascending rate and based on the test results we agreed upon a volume of 0.8 m³ for the rest of the expedition. Altogether, 163 weather balloons with a volume of 0.8 m³ were launched from the helipad between 31 July 12 UTC and 12 September 18 UTC. We had only five failed balloons, one that

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[182] https://bolin.su.se/data/

crashed on the helipad, one that exploded at launch, and a handful of failed ground checks. In all such failures, the launch was then subsequently accomplished successfully using a replacement sonde and/or balloon. *Figure 22.7* shows the statistical distribution of heights (coloured circles) of the 163 balloons, The average height was 26,657 m (dashed line in (A)), minimum height 13,676 m and maximum height 30,467 m. Twelve balloons (7.4%) exceeded the height of 29 km, whereas only four of 163 balloons (2.5%) reached heights below 20 km. The North-pole group launch at midnight on 17 August 2021 reached the height of 27,354 m.
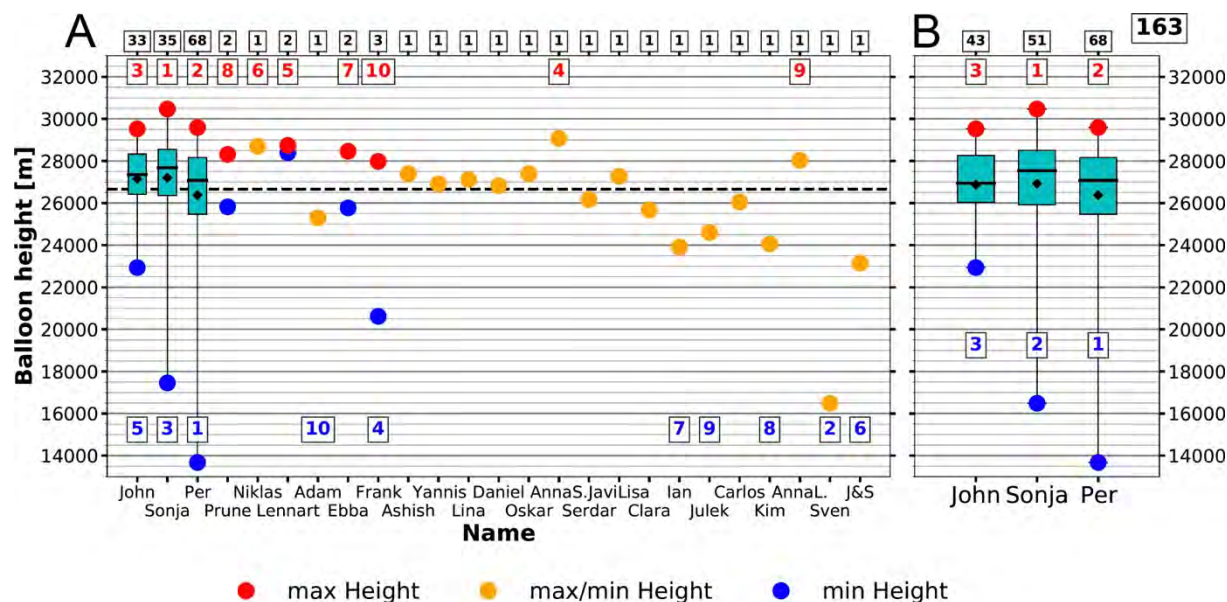
Figure 22.7: Statistics of the 6-hourly balloon launches (163 in total) with radio sonde to record temperature, humidity, pressure and wind speed profiles. The data were distributed in near-real time across the WMO Global Telecommunication System and were assimilated in weather-forecast models across the world. (A) All 25 scientists and crew members who helped with the balloon launches during the expedition. (B) The main three persons responsible for the radio sonde (John Prytherch, Sonja Murto, and the onboard medical doctor Per Arnell). The box for each distribution shows the interquartile range, the whiskers denote the range between the minimum and maximum heights, the median is shown by the horizontal black line and the diamond denotes the mean height. The minimum and maximum heights are shown by the blue and red circles, respectively, and the top ten in (A) and three in (B) of highest and lowest balloons are denoted by the red and blue values in the top and bottom text boxes, respectively. The total number of launches per person is given in the upper text boxes. J&S as the last name in (A) refers to the last launch at 18 UTC by both John and Sonja, which is excluded from the distributions in (B). ©Sonja Murto

Meteorological statistics for the launch of the 163 weather balloons are shown in *Figure 22.8*. All variables except for the true windspeed (ship data) were received from our weather station. The average value is denoted by the text box and the vertical dashed line for each distribution. Relative humidity during the expedition was quite high, ranging from 84 to 100%. For the majority of the time (38%), the relative humidity was 99% or higher, and only a 2.5% of the time the relative humidity was below 90%. The average near surface temperature (taken by our IR sensors) was -1.8°C, but ranged between -8.1 and 5.7°C, staying mainly below 0°C. We had rather calm wind conditions during the expedition (*Figure 22.8 F*), with an average windspeed of 5.4 m s^{-1} and only occasionally exceeding 10 m s^{-1} (6%). There was a slight negative correlation (0.32) between balloon burst height and underlying surface pressure (*Figure 22.8 A*), suggesting that the balloons tended to ascend higher with lower atmospheric surface pressures.
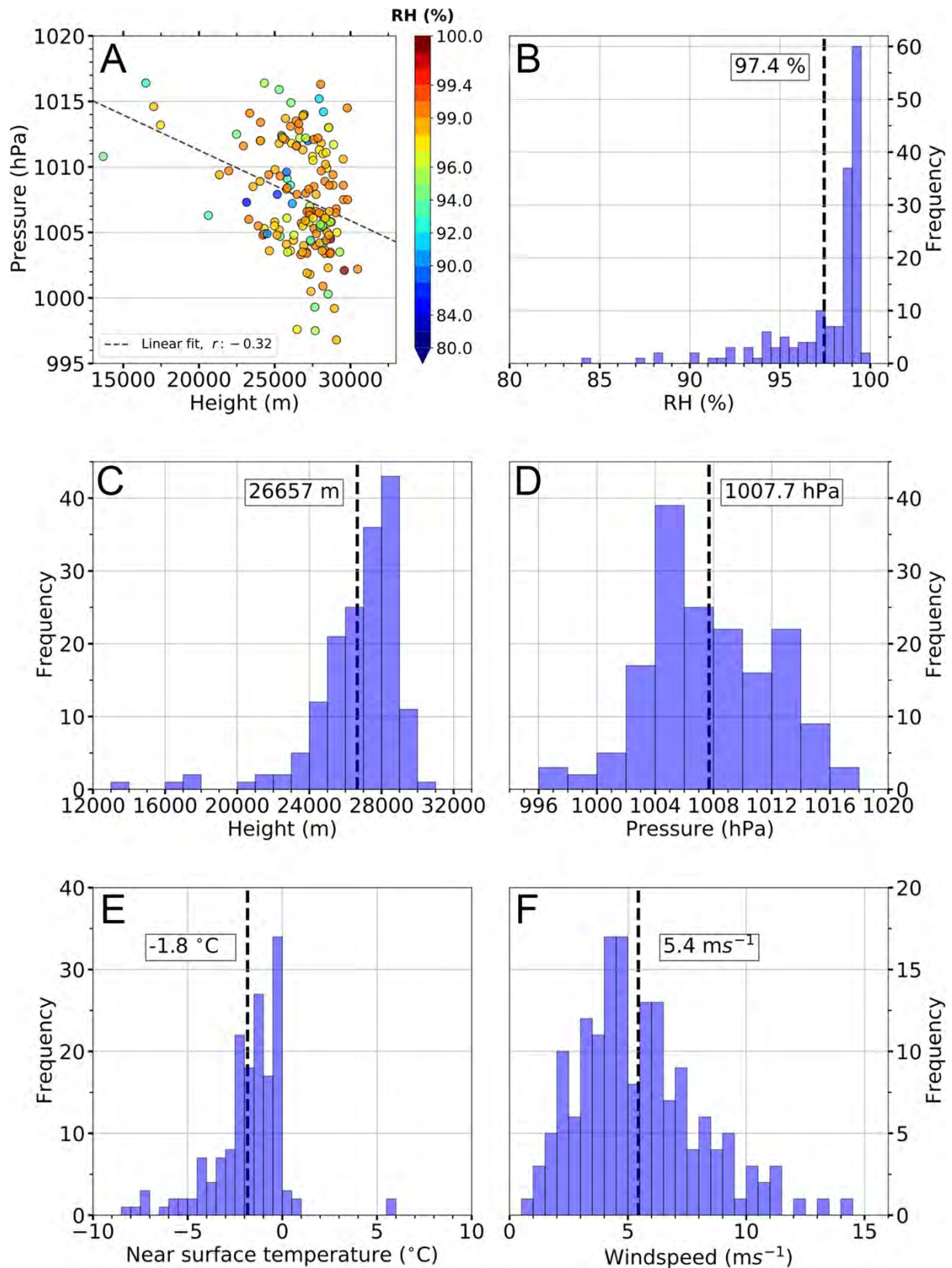
Figure 22.8: Histograms of meteorological variables measured onboard at balloon launch times during the SAS-Oden 2021 expedition. (A) Correlation between balloon height and pressure, coloured by the relative humidity at the balloon launch. (B) Relative humidity (%). (C) Maximum balloon height (m). (D) Surface pressure (hPa). (E) Near-surface temperature (°C). (F) Windspeed (m s⁻¹). ©Sonja Murto
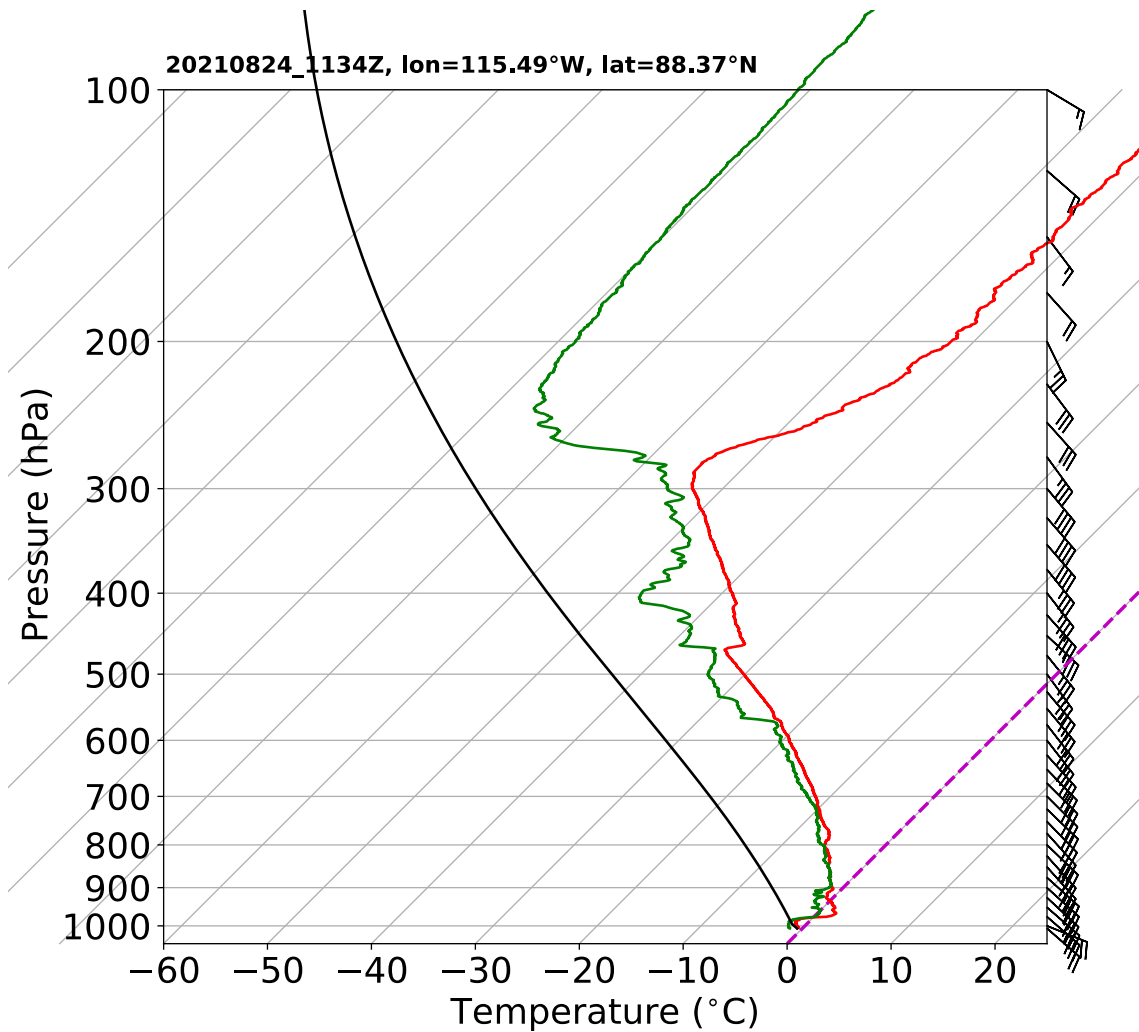
Figure 22.9: Example of a Skew-T plot with vertical profiles of temperature (red) and dew point temperature (green) for the radiosonde launched on 24 August at 11.34 UTC. The black line denotes the track of an air parcel and the zero isothermal is shown by the magenta dashed line. ©Sonja Murto

An example of a skew-T thermodynamic diagram with vertical profiles of temperature and dew point temperature is shown in *Figure 22.9*. We observed a small surface-inversion and above freezing temperatures at ca. 950 hPa. This was a humid and relatively warm day with a moist atmosphere, both at the surface and cloud layers reaching all the way up to ca. 580 hPa. *Figure 22.10* shows the mean equivalent radar reflectivity, mean doppler velocity, liquid water path, and cloud base height around the launch-time for the sounding in *Figure 22.9*. The data is measured by the cloud radar at five second time resolution.

High visibility (20 km visibility or higher) occurred 36% of the time during the SAS-Oden 2021 expedition (*Figure 22.10*). Visibilities below 2 km occurred 24% of the time, of which 16% was classified as fog (visibility below 1 km).
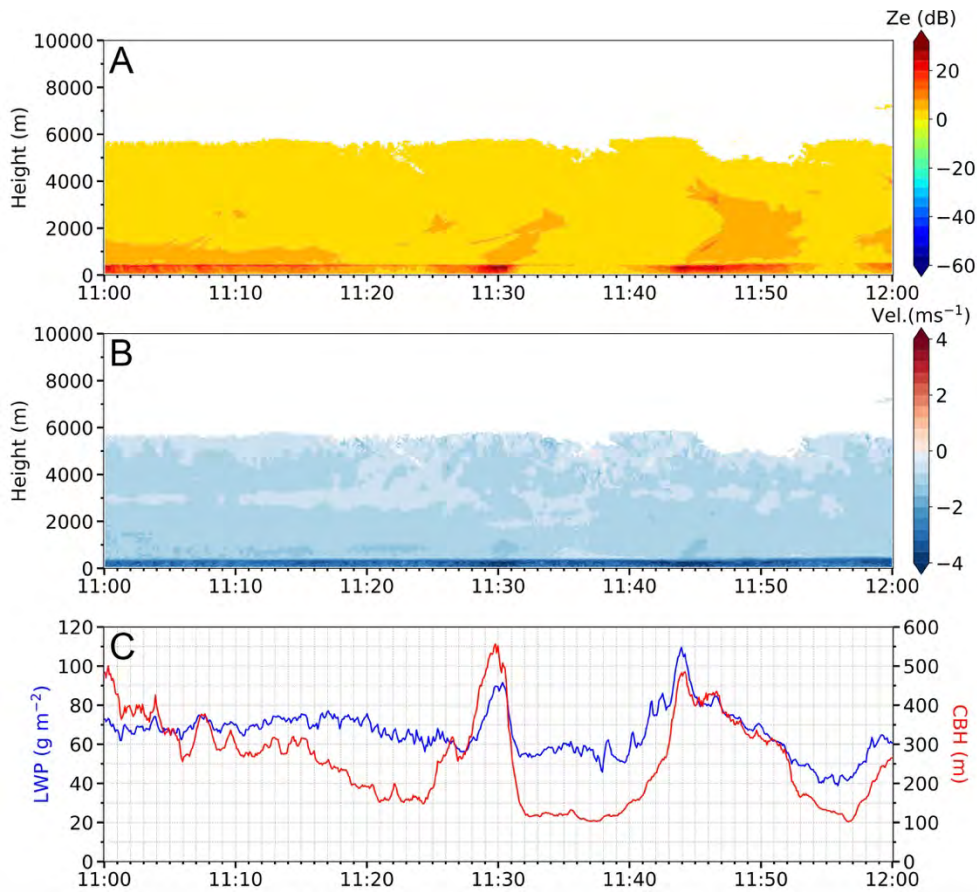
Figure 22.10: Example of cloud radar measurements made by WP16 (ACAS) during the SAS-Oden 2021 expedition for the period 24 August 11-12 UTC (cf. Figure 22.9). (A) Mean equivalent radar reflectivity. (B) Mean doppler velocity. (C) Liquid water path (LWP, left axis, blue) and cloud base height (CBH, right axis, red). ©Sonja Murto
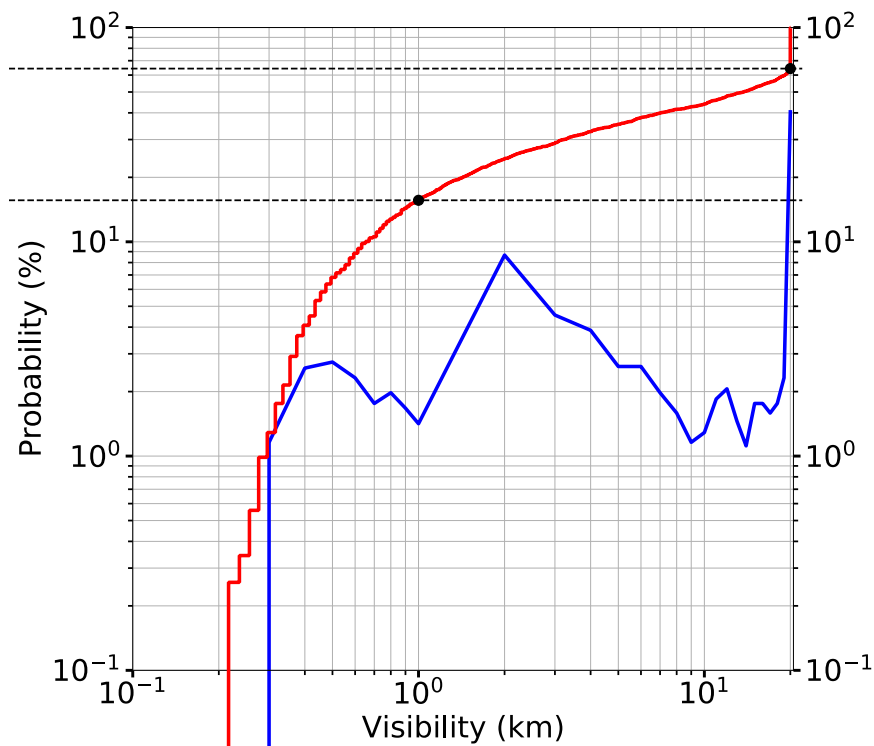

Figure 22.11: Probability (blue) and cumulative probability distribution (red) for the 10-min average visibility at 30 minutes timesteps between 27 July 9.15 UTC and 13 September 23:45 UTC from the ACAS present weather sensor. The black horizontal dashed lines denote the probability of fog (lower line) and clear sky (upper line). Note the logarithmic scales. ©Sonja Murto

22.6 Summary of post-cruise analyses and deliverables

The radio soundings made by WP16 (ACAS) during the SAS-Oden 2021 expedition make an immediate contribution to weather forecast quality via the GTS network.

This expedition was the first with the complete ACAS instrumentation setup. As such it forms a test bed for the ACAS installation and will do much to inform the operations on future expeditions during and after the ACAS project.

Substantial contributions will be delivered to two ongoing analyses, the first one detailing meteorological and cloud conditions in the summertime Arctic, and the second one on carbon budgets and CO_2 exchange in Arctic sea-ice regions. Furthermore, there are plans for a research paper on surface CH_4 concentrations, atmospheric fluxes and biological sources, in collaboration with WP13 and other WPs onboard.

Deliverables: Processed and quality-controlled data will be published open-access on the Bolin Centre Database[183] according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*). It is intended that data publication occurs within one year from the expedition, in special circumstances two, although some data can be delivered almost immediately. The scientific results of the project will be presented at national and international conferences and published in open-access peer-reviewed scientific journals.

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[183] https://bolin.su.se/data/

23 Opportunistic sampling

So-called "opportunistic samples" were taken by some of the expedition participants for seven projects without berths onboard *IB Oden* during the SAS-Oden 2021 expedition, numbered 23.1 – 23.7 (*Table 4.3*). These samples must be an addition to the expedition's research plan and in no way compete with the scientific aims of any of the sixteen SO21 projects on board. As for all other samples taken during the expedition, metadata are available, the final results of the analyses must be submitted to the SND data repository latest by 1 October 2023, and the SPRS and the SAS-Oden 2021 expedition should be acknowledged in any publication of the data as described in *Chapter 7*.

23.1 Aerosol omics

Project title: The role of marine microorganism in the formation of low-level clouds in the high Arctic
PI: Caroline Leck, Stockholm University, Dept. of Meteorology, lina@misu.su.se (not on board)

Air, fog and surface-water samples for aerosol omics analyses were taken by Pauline Snoeijs-Leijonmalm (WP1), Frank Menger, (WP1), John Prytherch (WP16), Sonja Murto (WP16), and Maria Samuelsson (SPRS) for Caroline Leck (SU Sweden) as described below. The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. The results will especially be useful for the SO21 omics collaboration as the aerosol compartment was missing in the original expedition research plan. Since these samples are part of the SO21 omics collaboration, the metadata are provided in the excel file "SO21_Metadata_Omics" in the SND data repository and summarized in *Table 7.7*. The air, water and fog samples will be extracted, sequenced and analysed by bioinformatics at SLU (Uppsala) after the expedition by WP2 (MIME), and will be published in collaboration with WP2. A collaboration with the surface microlayer subproject of WP4 (VIRUS) is also foreseen.

The proposed initiative aims at understanding the connections between microbial processes in the Arctic seas and the overlying low-level clouds that are known controllers of regional climate and therefore the melting and freezing of the ice. In summer, solar radiation is the dominant heat source for the upper ocean and the melting ice. More leads increase illumination of the ocean surface and promote phytoplankton growth taking advantage of the nutrients already present as well as through seeding by ice-bottom algae due to melting. Previous high-Arctic data indicate sufficient organic material in surface waters and surface microlayers (SMLs) of leads to provide biological derived material and exchange with the atmosphere. Bubbles in the water column provide a plausible mechanism for getting the uppermost surface ocean matter airborne. Recent results have also confirmed that the bio-organic material found in near-surface atmospheric aerosol particles as well as low-level cloud and fog droplets behaved in a similar manner to marine polymer gels, which originated from the thin SML film on leads, due to the activity of sea ice microalgae and open water phytoplankton, and perhaps, bacteria. According to these recent results, the link between the sources and roles of marine biogenic polymer gels in the processes of low-level clouds is gaining increased attention but critically needs further clarification and quantification. The approach requires profiling of biomolecules, generated within their marine ecosystem, from the upper ocean through the sea ice into fog/low-level clouds cutting vertically across various reservoirs of the Arctic habitat.

This work will continue our long-term build-up of knowledge of the life cycle of clouds in the high Arctic summer with linkages to the microbial life in ocean and ice and more specifically provide continued processing and evaluation of unique measurements obtained as part of the international

collaboration MOCCHA (Microbiology Ocean Cloud Coupling in the High Arctic) during AO2018 expedition with *IB Oden*[184].

(1) Ship-based collection of atmospheric aerosol microbiome matter

Collection of aerosol particles in air used a set-up identical to previous expeditions with *IB Oden*. At the nine EFICA Master Stations, aerosol particles were sampled through an inlet on a mast that extends at an angle of 45° to ca. 3 m above the "Triple Lab" roof (4th deck of *IB Oden*) so that the height of the inlet is ca. 25 m above sea level. This enables the inlet to be faced into the forward direction to maximize both the distance from the sea and from the ship's superstructure. Extreme care must be taken to prevent contamination of air samples. Therefore, during atmospheric sampling the ship should, if possible, remain facing into the wind to prevent contamination from the ship's exhaust and vents. The inlet has a 50% cut-off diameter of 1-2.5 µm and conducts the sampled air through a 4-cm diameter pipe to the "Triple Lab". The flow was set to ca. 400 L per minute. Inside the laboratory, a particle sampler (Filter Pack, FP) is placed in the sample stream. To collect sufficient material for post-cruise microbial detection, the flow through the filter cassette was set to ca. 250 L per minute. The FP was connected to a Gast pump (2065- V2), P = 110 W, U= 400V, I= 0.3 A, 50Hz) on the roof of the "Triple Lab". The filters were stored at -80°C after sampling.
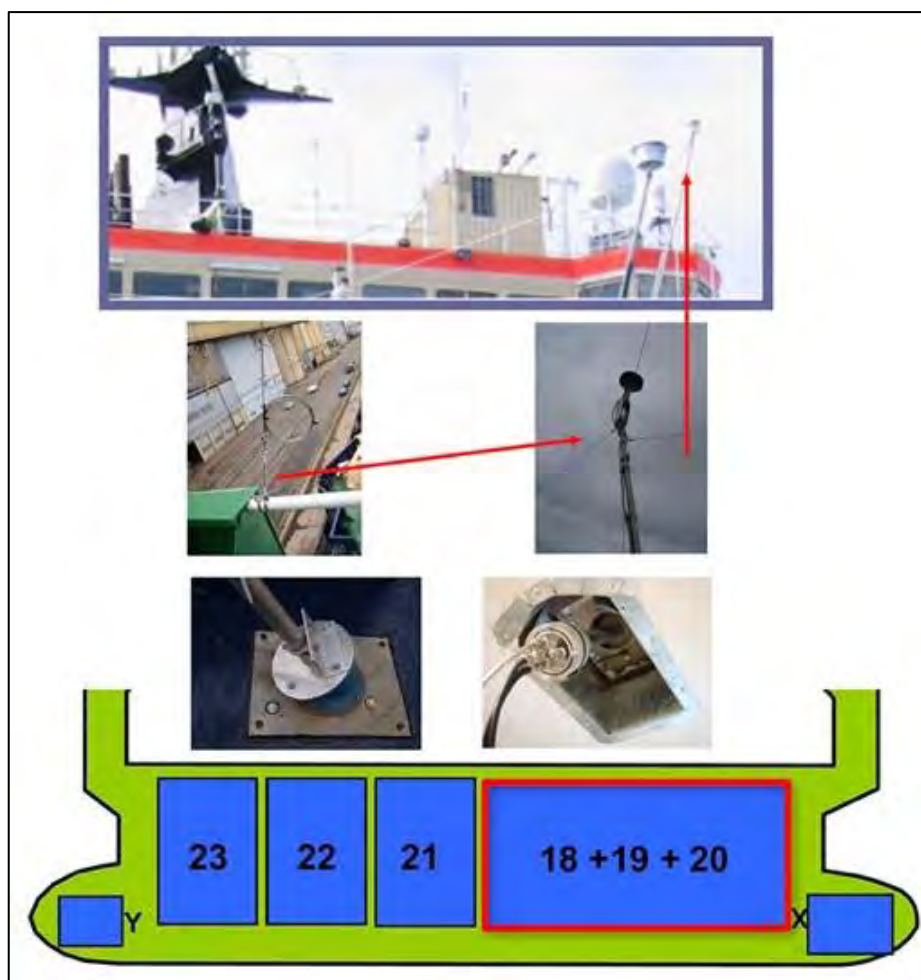

Figure 23.1: Overview of the sampling approach for aerosol sampling at IB Oden. *©Caroline Leck*

[184] Leck C, et al. (2018) Expedition Report SWEDARCTIC Arctic Ocean 2018. Swedish Polar Research Secretariat. [https://www.diva-portal.org/smash/get/diva2:1415744/FULLTEXT01.pdf]

(2) Ship-based sampling of fog and "low-cloud" water

The fog water sampler is installed at the 7th deck of *IB Oden*. The sampler is a cascade impactor, using two jet impaction stages, with cut diameters at 6 μm and 40 μm, respectively. Fog water samples were collected in duplicate brown 250-mL acid-washed plastic bottles connected to the collector. The sampler itself is connected to four blowers with a total volumetric flow rate of ca. 530 m^3 h^{-1}, P = 1100W per blower, using U= 220 V, I= 4×5A = 20A, 50Hz. The sampling was running as often as possible north of 80 °N. Of specific interest was to sample during the nine EFICA Master Stations where the aerosol samples were taken. The bottles were stored at -80°C after sampling.

Figure 23.2: The fog-water sampler on the 7th deck of IB Oden. *©Mario Hoppman*

(3) Collection of upper surface water

A small Ruttner sampler was used to sample water from 1-2 m of depth at four of the nine EFICA Master Stations. The sampler was closed by means of a plumb that runs along the line. Once collected, the water was poured into pre-cleaned 1-L bottles, in total 15 L per station. The bottles were stored at 4°C after sampling.


Figure 23.3: Bulk water sampling from a lead. ©Caroline Leck

23.2 Quantification and isolation of microbes from sediments

Sediment samples for enumeration and isolation of viruses and prokaryotes were collected by Lisa Winberg von Friesen (WP7) and Hanna Farnelid (WP6+7) for Mathias Middelboe, University of Copenhagen (Denmark). The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. The enumeration and isolation data will provide valuable reference data for validating the sediment SO21 metagenomics and metatranscriptomics data that are produced from parallel sediment samples from the same box core samples by the nine onboard biology projects (*Chapter 7.4*). The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

The Middelboe research group at the University of Copenhagen is focusing on virus-host interactions to understand how viral communities impact microbial ecosystem function. To investigate virus-host dynamics we need environmentally relevant model systems. In general, environmentally relevant model systems help us parameterize biologically driven processes and provide valuable reference material for validating sequence-based data. Arctic benthic ecosystems harbour a vast and unknown diversity of microbial and viral life, from which we are interested in isolating novel virus-host systems. Isolated arctic prokaryotes will be used to screen the sediment samples for potential lytic viruses. During SAS-Oden 2021, samples for isolation and flow cytometry were collected from six box cores at three sediment depths per core (*Figure 23.4*). The analyses will be performed in the research group of Mathias Middelboe and by Sachia Jo Traving at the marine biological section of the University of Copenhagen. The results from the isolation efforts on these arctic samples will be compared to marine deep sea sediment samples from the Japan Trench, which will be collected on a research cruise in October 2021.
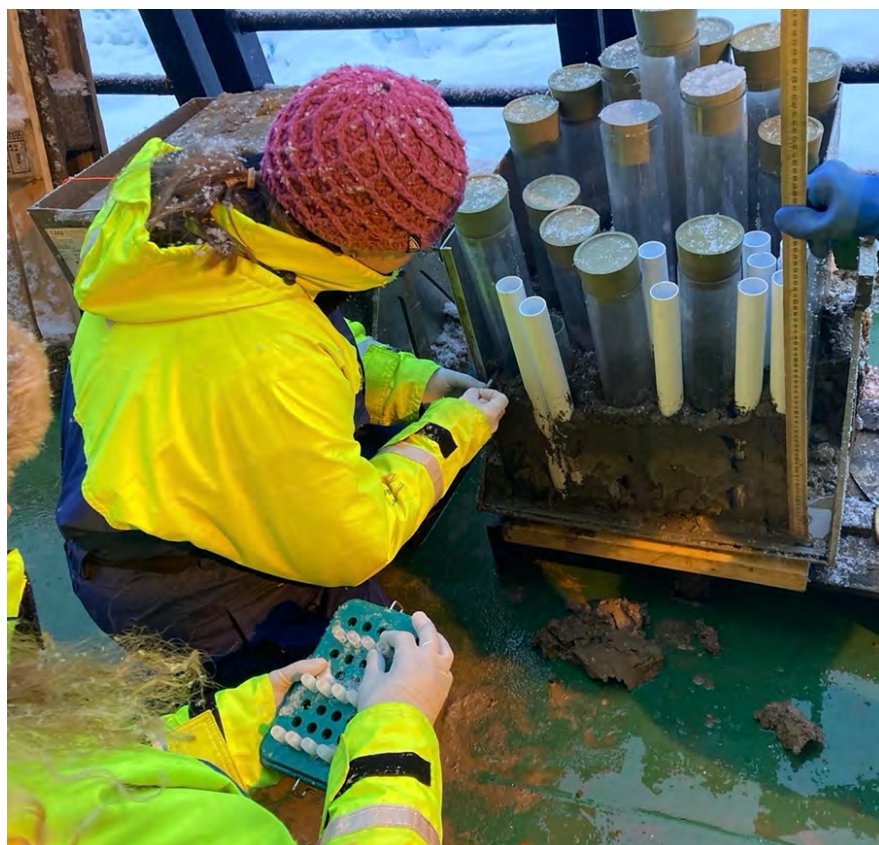
Figure 23.4: Hanna Farnelid (sampling with a 1-mL syringe) and Lisa Winberg von Friesen (holding the tube rack) collecting samples for enumeration and isolation of viruses and prokaryotes. ©SPRS

23.3 Deep-sea benthic nitrogen fixation

Samples for measurement of deep-sea benthic nitrogen fixation were collected by Lisa Winberg von Friesen (WP7). The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. The nifH analyses will provide valuable reference data for validating the SO21 metagenomics and metatranscriptomics data that are produced by the nine onboard biology projects in collaboration (*Chapter 7.4*). The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

Experiments targeting to measure benthic nitrogen fixation were set up at five of the box coring stations during the expedition. Benthic nitrogen fixation has only been measured a couple of times in the Arctic before, and never in the central part. Deep-sea sediments have during the last decade several times turned out to harbour active diazotrophs. During the expedition, five long-term incubations were set up with surface sediment using stable isotope tracers. Harvesting of the incubations will take place later in 2021 at the University of Copenhagen (Denmark) by Lisa Winberg von Friesen (WP7). This is a collaboration with Hanna Farnelid (WP6, LNU Kalmar, Sweden), Pauline Snoeijs-Leijonmalm (WP2, SU, Sweden) and Stefan Bertilsson (WP2, SLU Uppsala, Sweden). The samples will be analysed by Lisa von Friesen (WP7) at the University of Copenhagen.

Figure 23.5: Claudia Morys (WP1,2; left) and Lisa Winberg von Friesen (WP2,6,7; right) taking subsamples from a box core sample at SO21 Station 48 during the SAS-Oden 2021 expedition. ©Pauline Snoeijs-Leijonmalm

23.4   Sea ice algal aggregates

Sea-ice algal aggregates were collected by Lisa Winberg von Friesen (WP7). The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. The sea-ice algal community and nifH analyses will provide valuable reference data for validating the SO21 metagenomics and metatranscriptomics data that are produced by the nine onboard biology projects in collaboration (*Chapter 7.4*). The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

At two occasions during the expedition, it was possible to sample sea-ice algal aggregates (dominated by *Melosira arctica*) directly from the ice or with a hand-held net from a lead. The algal material was put into RNAlater and frozen at -80°C, to later be investigated for the associated microbial communities – especially nitrogen-fixing microorganisms. They have previously been detected based on DNA, but not yet investigated regarding expression (RNA). This is a collaboration with Hanna Farnelid (WP6, LNU Kalmar, Sweden), Pauline Snoeijs-Leijonmalm (WP2, SU, Sweden) and Stefan Bertilsson (WP2, SLU Uppsala, Sweden). The samples will be analysed by Lisa von Friesen (WP7) at the University of Copenhagen. The samples will be analysed by Lisa von Friesen (WP7) at the University of Copenhagen.

23.5   Dissolved TRC

Samples for measuring dissolved thiamine-related compounds (TRC) in the Central Arctic Ocean (CAO) to try to link changes in TRC to specific microbes and their B1 genotypes were collected by Hanna Farnelid (WP6) and Lisa Winberg von Friesen (WP7) for PhD student Meriel Bittner in Lasse Riemann's research group at the University of Copenhagen. The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. No other onboard project is studying TRC levels in the CAO. The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

The samples were collected in connection with the dilution (Dil1, Dil2, Dil3) and uptake (Up2, Up4 and Up6) experiments of WP6. Triplicate samples of 500 mL filtrate (0.2 µm Sterivex-filtered seawater) from the chlorophyll maximum (ChlMax) was collected and frozen at -20°C. Altogether, 18 samples were collected. The samples will be analysed in collaboration with Dr. Ryan Pearl at North Carolina State University.

23.6   TCHO and TAA

Samples for measuring total carbohydrates (TCHO) and total amino acids (TAA) were collected by Birthe Zäncker (WP3) for Anja Engel, GEOMAR (Kiel, Germany). The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. No other onboard project is specifically studying the distribution of TCHO and TAA in the CAO, although TAA is analysed in the experiments of WP2. The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

Water samples taken from the CTD and sea ice habitats during the SAS-Oden 2021 expedition will be analysed in collaboration with WP3 to investigate the predominant carbohydrate and amino acid composition in the CAO. For each of the parameters TCHO and TAA, 186 samples were collected from 15 CTD casts and 10 ice stations during the expedition.

TCHO: CTD water samples (20 mL) for total hydrolysable carbohydrates > 1 kDa were filled into combusted glass vials (8 h, 500°C). At the ice stations, bottom and top parts of the ice cores were sampled with 10 mL due to the limited sample volume and brackish brine, melt pond and ice-seawater interface samples were sampled with 18 mL since the reduced salinity otherwise caused the vials to burst when frozen. The samples were stored at -20°C until analysis. TCHO will be analysed at GEOMAR (Germany), using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) which will be applied on a Dionex ICS 3000 ion chromatography system[185]. The samples will be desalinated using membrane dialysis (1 kDa MWCO, Spectra Por) at 1°C for 5 h. Subsequently, the samples will be hydrolysed for 20 h at 100°C with 0.8 M HCl final concentration and neutralized with acid evaporation (N_2, for 5 hours at 50°C). Two replicates per sample will be analysed.

TAA: Total hydrolysable amino acids samples were taken from 4 mL of seawater and 3 mL of thawed bottom and top ice as well as brackish brine, melt pond and ice-seawater interface samples which were filled into pre-combusted glass vials (8 h, 500°C) and stored at -20°C until analysis. TAA will be analysed by high performance liquid chromatography (HPLC) using an Agilent 1260 HPLC system in the home laboratory at GEOMAR, Germany. The analysis was modified from established methods[186,187]. Samples will be analysed in duplicate. The samples will be hydrolysed at 100°C for 20 h with HCl (Suprapur®, Merck) and neutralized by acid evaporation under vacuum at 60°C in a microwave.

23.7   PFAS

Water, ice, snow and zooplankton samples for later analyses of per- and polyfluoroalkyl substances (PFAS) were collected by Frank Menger (WP1). The intended sample analyses are a welcome addition to the SO21 joint ecosystem study of the SAS-Oden 2021 expedition. No other onboard project is specifically studying the distribution of PFAS in the CAO. The metadata are provided in the excel file "SO21_Metadata_Opportunistic" in the SND data repository. The final data (results of the measurements) will be made publicly available in the SND according to the "SAS-Oden Research Data Management Policy" of the SPRS (*Appendix A*).

Occurrence of man-made chemicals in remote environments like the CAO causes concerns regarding these chemicals as they underwent long-range transport without degradation, which makes them persistent and mobile. Very persistent and very mobile (vPvM) and persistent, mobile and toxic (PMT) substances have lately gained attention, as they pose a threat to, e.g., drinking water resources.

Water, ice, snow and zooplankton samples were opportunistically collected for screening persistent and mobile chemicals in the remote and understudied environment of the CAO. Samples were

[185] Engel A, Händel N (2011) A novel protocol for determining the concentration and composition of sugars in particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater. Marine Chemistry 127:180–191 [http://doi.org/10.1016/j.marchem.2011.09.004]

[186] Lindroth P, Mopper K (1979) High performance liquid chromatographic determination of sub-picomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Analytical Chemistry 51:1667–1674 [http://doi.org/10.1021/ac50047a019]

[187] Dittmar T, et al (2009) The analysis of amino acids in seawater. In: PO Wurl (ed.) Practical guidelines for the analysis of seawater, pp. 67–78. Boca Raton, FL: CRC Press Taylor & Francis Group.

collected opportunistically in connection to sampling for SAS Core Parameters and other research operations, which will put the results from the chemical screening in a broader ecological context.

Water, ice and snow samples were collected in 1-L polycarbonate bottles during ice stations and from the CTDs, with a focus on the EFICA Master Stations (*Table 23.1*). Lead surface water was sampled by hand (n=10), and ice-seawater interface water (n=12), melt pond water (n=12) and brackish brine (n=11) were collected using a handpump with protective net for removal of particles larger than 200 µm. Snow was collected from a pooled sample of ca. 80 L of snow and was sampled for chemical analysis after thawing at room temperature (n=10). Ice samples (n=45) was obtained by pooling melted ice from 10-cm core sections to get a water sample volume of 1 L by using the left-over water from the nutrient analysis performed by WP10 (*Chapter 17*). Water from the stern CTD omics SAS was collected from 100 m (n=9), 1000 m (n=5), chlorophyl maximum (n=8) and temperature maximum (n=8). On two occasions water from deeper than 1000 m was collected from the bow CTD deep SAS, viz. 2000, 2500, 3500 m and bottom at SO21 station 28 (21 August 2021) and 1500, 2500 and 3310 m at SO21 station 44 (1 September 2021). Duplicate 1-L blanks were created from the onboard MilliQ water system and three additional 0.25-L blanks were made from Millipore water that had been brought from the chemistry laboratory in Uppsala to the ship. All samples were stored at -20°C within 24 hours after collection. Zooplankton samples were collected when excess sample material was available, rinsed with MilliQ water from the ship and stored at -20°C.

The samples taken for PFAS analysis will later be prepared and analysed at the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Analysis will be performed by means of liquid chromatography - mass spectrometry (LC-MS). The resulting data will provide insights into the occurrence and distribution of persistent and mobile organic contaminants in the different water compartments of the CAO.

Table 23.1: List of samples taken for the analysis of per- and polyfluoroalkyl substances (PFAS) during the SAS-Oden 2021 expedition. ISI = Ice-seawater interface.

Date	Stat. nr	Snow	Brine	Melt pond	ISI	Lead	Ice core	CTD 100 m	CTD 1000 m	CTD Chl Max	CTD Temp Max	Zoo plankton
210808	8	1	-	1	1	-	5	1	1	1	1	yes
200816	22	1	1	1	1	1	4	-	-	-	-	yes
210820	26	1	1	1	1	1	4	1	1	1	1	yes
210823	30	-	-	-	-	2	4	-	-	-	-	-
210825	33	-	-	-	-	-	-	2	-	2	2	yes
210825	34	2	2	2	2	-	-	-	-	-	-	
210827	35	1	1	1	1	-	4	1	2	1	1	yes
210829	38	-	1	1	1	-	3	1	1	1	1	yes
210831	42	1	1	1	1	1	8	-	-	-	-	yes
210903	48	-	-	-	-	1	-	-	-	-	-	-
210904	50	1	1	1	1	1	4	1	-	-	1	yes
210906	53	-	1	1	1	1	4	1	1	1	1	yes
210908	56	2	1	1	1	1	3	1	-	1	-	yes
210910	58	-	1	1	1	1	2	-	-	-	-	yes
		10	11	12	12	10	45	9	5	8	8	

24 SO21 Standard Operating Procedures (SOPs)

24.1 SO21 SOP: box core

SOP developed by Claudia Morys (SU) and Pauline Snoeijs-Leijonmalm (SU) for the SAS-Oden 2021 expedition

Reviewed by Prune Leroy, Martin Jakobsson, Helen Coxall, Flor Vermassen, Clare Bird, and the multibeam/sub-bottom profiler team: Carlos Castro and Caroline Bringensparr.

Goal

The goal of the box core operations are to sample benthic fauna (WP1, SAS Core Parameter), otoliths (WP1), metagenomics (WP2, SAS Core Parameter), gypsum and TEP particles (WP3), foraminifera and sediment characteristics (WP9). Dedicated people will perform the sampling on board *IB Oden*, but this SOP should make it possible for others to sample if necessary. The dedicated persons are: Claudia Morys (WP1, macro-and meiofauna), Julek Chawarski (WP1, otoliths), Birthe Zäncker (gypsum crystals and TEP particles in a 10-mL syringe from the overlying water), Prune Leroy (WP2, metagenomics RNA), Clare Bird (WP9, metagenomics DNA), Flor Vermassen (WP9, foraminifers and sediment samples SU), Pauline Snoeijs-Leijonmalm (photography). The box corer was borrowed from the AWI (Germany).

Number of stations

Five box core casts are planned at relatively shallow stations (ca. 1000-1500 m). Priority stations are the Morris Jessup Rise, the Lincoln Sea (sea-ice permitting), the Lomonosov Ridge at the Greenland end and close to the North Pole, and the Yermak Plateau. The sampling takes ca. 1 hour for winching the box corer down to 1000-1500 m and up again at a speed of 1 m per second. 1.5 hours should be planned for the entire operation, including clearing the way from ice for the box corer deployment. A procedure to identify good box core deployment sites by using seafloor acoustics was developed by Martin Jakobsson (WP14) in collaboration with Caroline Bringensparr and Carlos Castro, to facilitate selection of appropriate box core stations at the seafloor during the cruise, i.e., for targeting appropriate seafloor topography/terrain at the metre-scale. This will include a description of factors to consider in relation to real-time multibeam and sub-bottom profiler data.

Giant box corer

The giant box corer[188] samples a comparatively large area of the sediment surface (50 × 50 cm) with minimal disturbance and collects a large block of sediment up to maximally 60 cm below the seafloor. This gear is particularly useful for quantitative investigations of the benthic micro- and macrofauna. The square box is fixed to a head with a column that is connected to a frame by a cardanic (gimballed) suspension. This allows vertical penetration of the box into the sediment. A crank with spade including rubber sealed plate is attached to the head of the box. The column is filled with lead weights to aid penetration and in total the box corer weighs 900 kg.

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[188] https://www.awi.de/en/science/geosciences/marine-geology/tools/sea-going-equipment/boxcorer.html

List of equipment - box coring

General		Responsible for bringing on board
1	Giant box corer AWI (Manufacturer: http://www.oktopus-kiel.de/en/), 900 kg	PSL
2	Boxes 50 × 50cm, 60 cm deep with removable front plate, 50 kg each	PSL
1	Camera and cm-scale for photography of the sediments	PSL
1	Go-pro camera on the box corer for studying the sediments before sampling (if possible)	PSL
6	Silicone tubes for taking off the water from the top	PSL
	Syringes for taking water samples from the box corer for gypsum and TEP particles	Birthe Zäncker
Specific for WP1 work		**Responsible for bringing on board**
12	Benthic cores (inner ø 8 cm)	Claudia Morys
1	Benthic cores (inner ø 10 cm)	Claudia Morys
12	Benthic cores (inner ø 3.6 cm)	Claudia Morys
2	Large sieve 300 μm mesh	PSL
3	scoops for scooping sediment	Claudia Morys
	Metal plate for taking cores out	Claudia Morys
	Rack for temporarily placing cores	Claudia Morys
	Other necessary small tools	Claudia Morys
	Ethanol (include safety sheet)	PSL
	Bromide for experiments (include safety sheet)	Claudia Morys
	Syringes for collecting nutrient samples from overlying water	PSL
	Falcon tubes (50-mL) for nutrient samples	PSL
	Eppendorf tubes (2-mL) for experiment	PSL
	10 cm ø plunger for extruding sediment	Flor Vermassen
	3.6 cm ø plunger for extruding sediment	Claudia Morys
	8 cm ø rubber stoppers, plastic lids	Claudia Morys
	3.6 cm ø rubber stoppers, plastic lids	Claudia Morys
	Metal plate for slicing sub-cores	Claudia Morys
100	Sampling jars for collecting macrofauna	PSL
	Plastic bags for sediment samples	Claudia Morys
Specific for OMICs work		**Responsible for bringing on board**
9	Benthic cores (inner ø 3.6 cm) for the OMICs sampling	PSL
18	Lids for benthic cores	PSL
5	Freezer boxes 15 × 8 × 65 cm for -80°C freezer (for omics samples)	PSL
30	Cryovials (for metagenomics test samples)	PSL
Specific for WP9 work		**Responsible for bringing on board**
2	Geo cores (inner ø 8 cm)	Flor Vermassen
2	Geo U-channel cores (square, 2 2 cm)	Flor Vermassen
	Rose Bengal stain for WP9 work (included on chemicals safety sheet)	Flor Vermassen
400	Eppendorf tubes	Flor Vermassen
4	Freezer boxes (plastic or cardboard) for 375 Eppendorf tubes	Flor Vermassen
10	8 × 12 × 75 cm plastic "D-tubes" for storing multiple lithology U-channels	Flor Vermassen
	Plastic bags	Flor Vermassen
	Other necessary small tools	Flor Vermassen
	8 cm ø plunger for extruding sediment	Flor Vermassen
	Metal plate for slicing sub-cores	Flor Vermassen
	8.5 ø cm rubber stoppers	Flor Vermassen

During lowering the box corer to the seafloor and sampling, the flaps at the top of the head remain open to allow a free flow of water. This prevents pressure build-up and following disturbance of the sediment surface. When the box has reached the seafloor, the box is triggered by a trip as the column passes through its frame. While pulling the corer out of the sediment the flaps at the head are closed and the spade is drawn into down into vertical position so that the bottom of the box is closed and the sample is secured. Once the box corer has returned on board the ship and lowered onto the deck, the box can be detached from the frame for subsampling of the sediment.

The square boxes are equipped with a removable front plate to obtain an undisturbed section of the near-surface sediments. The removable front plate allows for access the sediment from the side, to obtain undisturbed sections (subsamples) of the near-surface sediments. This is can be useful for sub-sampling of soft sediments, which can be compressed and disturbed by surface sub-coring.

### Box core station selection and deployment logistics

Once in the desired general area, the specific location of box core stations should be considered through dialogue with WP14, who will identify regions of level, soft sediments from multibeam and sub-bottom profile data: regions of relatively flat topography, or small bathymetric 'highs', characterised by coherent acoustic layering should be targeted where possible. Steep slopes and acoustically chaotic sediment piles should be avoided.

Box corer deployment will always be coupled with CTD (deep) and zooplankton net sampling at the same station. The box corer will be deployed after the CTD and net casts to avoid disturbing the water column with sediments.

All involved in subsampling will be available to assist in the deployment and recovery of the box corer, which is carried out in collaboration with the crew and the SPRS technicians.

All persons responsible for subsampling should have their subsampling devices ready on deck so that subsampling can happen fast. Bring also boxes/crates to put in your samples in immediately after taking them so that they are safe and can be carried efficiently to the laboratories.

Schematic overview of the planned subsampling from a box core sample
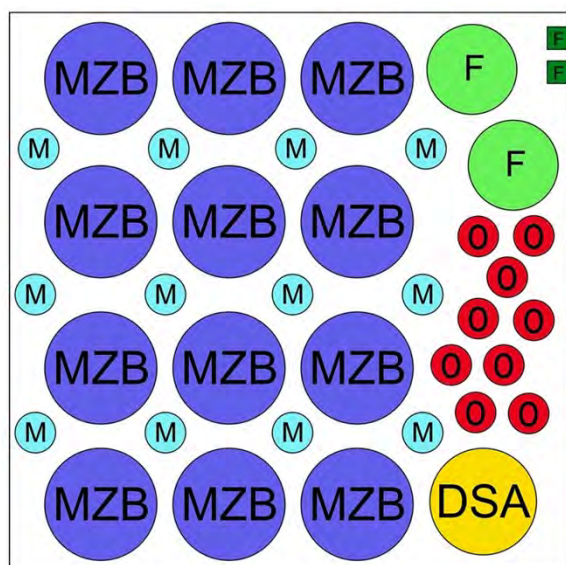
SAS Core Parameters:

MZB = Macrozoobenthos WP1 (inner ø 8 cm),

        including otoliths

DSA = Natural radionuclides WP1 (inner ø 10 cm)

M   = Meiozoobenthos, etc. WP1 (inner ø 3.6 cm)

O   = Metagenomics WP2 (inner ø 3.6 cm)

Project-specific:

F   = Foraminifers WP9 (inner ø 8 cm)

F   = Foraminifers WP9 (2x U-channels. 2x2 cm)

Subsampling procedure

(1)   This work is carried out on deck immediately after getting the box core sample on deck – all subsamplers and their subsampling gear should be ready on-site.

(2)   Water samples from above the sediment are taken with a syringe for gypsum, TEP particle and nutrient analyses. The remaining water is taken away with the silicone tubes.

(3)   Sub-corers are carefully positioned (according to the schematic above (from the top) inside the large sediment block sample, except the FORAM U-channels.

(4)   The front plate is removed and the sediment at the DSA core side is photographed (always with the same camera with the same settings) with a cm-scale and station ID label beside it. Several photographs whole view and close-up photographs of ca. 12 cm sections from top to bottom will be taken.

(5)   WP9 pushes the FORAM U-channels into the side exposed by opening the front plate.

(6)   WP9 takes 2 × surface scoop samples (ca. 50 cm²). Starting from the open side of the box the sub-corers are carefully dug/cut out using a cutting wire/knife or other appropriate tool, to remove the sediment around the tubes. They will be sealed at the bottom and top either with a temporary rubber stopper (to prevent the sediment falling out) or a more permanent lid. Excess sediment will be collected in buckets as far as possible.

(7)   Sub-cores and other samples are immediately labelled with the appropriate core or sample ID and their orientation marked with an up-arrow. Sub-corers are put in racks for transport to the lab. During the sub-core extraction, WP9 takes a sediment scoop from the bottom of the box (ca. 50 cm²).

(8)   WP2 takes some scoops from surface and bottom sediments as well for DNA and RNA extraction testing (freeze in cryotubes).

(9)   Nine metagenomics cores are frozen immediately (DNA, RNA and Archive cores). Priority must be given to fast freezing of the designated OMICS cores at – 80ºC.

(10) The other sub-cores are further sampled or conserved according to the project-specific protocols (see below).

Sub-core sample elaboration plan WP2 (Metagenomics)

From each box core cast, the nine OMICS sub-cores (3× DNA, 3× RNA, 3× archive) will be immediately sealed with lids, packed into plastic bags and frozen at -80°C. This should be carried out as fast as possible after subsampling to optimize preservation of RNA and DNA.

Summary sample elaboration plan WP1 (benthic fauna and otoliths)

From each box core cast, the 11 cores (inner ø 8 cm) and 2 cores (inner ø 10 cm) are sampled through the full depth of the box core sample. Another 12 smaller cores (inner ø 3.6 cm) are sampled down to a depth of 20 cm.

Subsampling Table WP1

Sample type	Purpose	Handling and storage
10 cm inner ø cores (DSA)	1 core for natural radionuclides (used for sedimentation rates)	Remove overlying water, store core upright in freezer Total freezer space at -20°C for 5 casts: 20 dm³
8 cm inner ø cores	11 cores for macrofauna diversity	Sieve over 300 µm mesh, preserve in 96% ethanol solution. Depending on time, cores shall be sliced in 1-cm intervals down to 10 cm, 10-15 cm and down to the bottom for vertical distribution of macrofauna. Depth layers from all 11 cores shall be pooled together. Total storage space at 4°C for 5 casts: 40 dm³
10 cm inner ø cores	1 core for bioturbation and – irrigation, nutrient fluxes	Bring the core to the fridge (set to *in-situ* temperature) and oxygenate core. Acclimatation period min. 24 hours Incubation experiment using luminophores (duration = 21 days). Take water from overlying water (10-mL Falcon tubes) for nutrients once a day. Incubation experiment using bromide starting on day 22 (duration = 3 days). Take pore water and samples from overlying water (2-mL Eppendorf tubes). Slice cores at the end of the experiment (0.5 cm interval down to 5 cm, 1-cm intervals down to 15 cm, put in plastic bags) Total freezer space at -20°C for 5 casts: 60 dm³
3.6 cm inner ø cores	4 cores for micro-and meiofauna diversity	Slice 0-1 cm and 1-3 cm, put in plastic bags Total freezer space at -20°C for 5 casts: 1 dm³
3.6 cm inner ø cores	4 cores for general sediment properties (grain size, porosity, water content, POC)	Slice in 1-cm intervals down to 15 cm, put in plastic bags Total freezer space at -20°C for 5 casts: 3.5 dm³
3.6 cm inner ø cores	4 cores for sediment quality (proteins, lipids, carbohydrates, chlorophyll and phaeopigments)	Slice 0-1, 1-3, 3-5, 5-10 and 10-15 cm, put in plastic bags Total freezer space at -20°C for 5 casts: 3.5 dm³
Budget storage space WP1		**Fridge 4°C = 40 dm³** **Freezer -20°C = 88 dm³**

Sub-core sample elaboration plan WP9 (FORAM)

From each box core, one of the FORAM sub-cores (inner ø 8 cm) will be immediately sealed using a plastic end-cap and tape, placed in a plastic D-tube and stored in a fridge at 4°C. The other FORAM sub-core will be taken directly to the sediment fridge in the Triple Lab for further subsampling.

Summary sub-core sample elaboration plan WP9 (FORAM):
From each of the 5 planned box cores we will take surface samples and bottom samples (50 cm³) and 4 sub-cores: 2 U-channels of 2×2 cm and 2 sub-cores with inner ø 8 cm through the full depth of the box for complementary purposes.

FORAM sub-core subsampling: This will be continuously sliced into 2-cm slices, by extruding the sediment out through the core liner using a custom plunger of ø 10 cm. The 2×10 cm ø discs will be divided into two equal samples using a metal slicer:

(1) 1×50 cm³ surface (top 0-0.5 cm) IP25 sample: Scoop 50 cm³, transfer to a labelled sterile plastic bag and seal. Storage in the -20°C freezer.

(2) Down-core IP25 samples: Cut with a knife, transfer to a labelled sterile plastic bag and seal. Storage in -20°C freezer. Wear protective gloves to avoid contamination.

(3) One each of 100 cm³ surface* (top 0 – 0.5 cm) and bottom (bottom 1 cm) FORAM samples per box core station: Scoop surface sediment, transfer to a labelled plastic bag (no need to be sterile) and seal using the plastic heat sealer. * The surface sample will be halved. One part will be (ideally)

processed as soon as possible for Bengal Staining (see below). The other part will be preserved for radio carbon dating.

(4) Down-core FORAM samples: Cut with a knife, transfer to a labelled plastic bag (no need to be sterile) and seal using the plastic heat sealer. Total fridge space at 4°C for 5 casts: 0.4 dm³.

(5) Rose Bengal staining treatment for the FORAM surface sample scoop: RB-staining will be carried out on the FORAM shallow surface scoop to identify living or recently dead benthic foraminifera specimens for bottom water proxy ground-truthing. The extracted sediment sample will be immediately wet sieved using deionized water over a 63 μm sieve. The >63 μm size fraction will be dosed with Rose Bengal (RB) protein stain and left for a minimum of 14 days in a fume cupboard according to established methods (Schönfeld et al., 2012). The >63 μm size fraction is discarded. After staining the wet sample should be transferred to a labelled jar and fixed with ethanol. If there is not enough time to do the RBS method, the five surface sediment samples should be frozen and this work done post-cruise. The RB solution was prepared using 2 g of RB dissolved in 1 L ethanol (99%).

Subsampling Table WP9

Sample type	Purpose and sampling elaboration	Handling and storage
50 cm³ scoop from box surface (0-0.5 cm)	Present day seafloor sedimented planktonic foraminifera & living/dead benthic foraminifera	Rose Bengal stain for live vs. dead foraminifera scoop/ cut out sample with a knife. Perform the RBS step, including preservation of the wet sample in ethanol, or freeze. Total fridge space at 4°C for 5 casts: 0.25 dm³
50 cm³ scoop box bottom	Present day seafloor sedimented planktonic foraminifera & living/dead benthic foraminifera, large sample for ^{14}C dating	Rose Bengal stain for live vs. dead foraminifera scoop/ cut out sample with a knife. Total fridge space at 4°C for 5 casts: 0.25 dm³
50 cm³ scoop from box surface (0-0.5 cm)	Present day IP25 (diatom -derived) sea-ice biomarker (proxy ground-truthing)	Cut with a knife or plastic plugs. Storage in -20°C freezer Total freezer space at -20°C for 5 casts: 0.25 dm³
Sub-core 1 (inner ø 8 cm)	Down-core planktonic & benthic foraminifera assemblages 3 cm³ samples (large enough for ^{14}C dating), every 2 cm = 25 samples per core	Scoop/ cut out sample with a knife. Storage in 4°C fridge Total fridge space for 5 casts ca. 125 samples of 3 cm³ = 375 cm³ in plastic bags, stored in lager zip lock bags. Total fridge space at 4°C for 5 casts: 0.4 dm³
Sub-core 1 (inner ø 8 cm)	Holocene IP25 down-core sea ice proxy reconstructions 10 cm³ sample volume every 2 cm	10 cm³ samples (extruded slices) sterile plastic bags × 125 samples Total freezer space at -20°C for 5 casts: 36 dm³
Sub-core 2 (inner ø 8 cm)	Holocene, back up IP25	Preserve the whole sub-core (8.5 cm diameter, 50 cm long), + end caps (1 core = ca. 3 dm³) Total fridge space at 4°C for 5 casts: 15 dm³
2 × U- channel sub core (2 × 2 cm)	Sediment characteristics	75 × 12 × 8 cm diameter plastic "D-tubes" for storing multiple lithology U-channels = 2 × 7.2 dm³ per cast Total fridge space at 4°C for 5 casts: 72 dm³
Budget storage space WP9		**Fridge 4°C = 90 dm³** **Freezer -20°C = 40 dm³** **Freezer -80°C = 8.1 dm³**

24.2   SO21 SOP: multinet

SOP developed by Nicole Hildebrandt (AWI) for the SAS-Oden 2021 expedition

(1) Assembling the multinet

Mesozooplankton (WP1/8) and Foraminifera (WP9) are sampled with a multinet "Midi" (five net bags; mesh size: 150 or 55 μm).

Important: There will be two multinets on board. Each multinet has its own specific deck command unit. If you exchange the multinet, you also have to exchange the deck command unit!

Also, there might be two different net bucket arrays, one with a "stirrup system" and one with a "pin system".

- Check the five net bags you intend to attach: are there any holes? Small holes can be fixed with Acrifix glue and maybe a bit of gauze. If there are large holes, use another net.
- Attach the nets to the multinet frame using the zippers. Start with Net 5 (belongs to the lowermost canvas) and work your way up to Net 1. After zipping each net to the frame, close the respective hook-and-pile fastener to secure the zipper.

The multinet cod end (left) and the fixing ring (right) of the "stirrup system"

Cod end and fixing ring of the "pin system"

- Put the lower end of the net bags onto the fixing rings and secure them loosely with a hose clamp (tool: slotted screwdriver)
- Attach the fixing rings to the net bucket array. Mind the numbers: Net 1 is the uppermost, Net 5 is the lowermost one.

Net bag attached to the fixing ring

The fixing rings of the "stirrup system" are put onto the net bucket array and secured with the splint.

In the "pin system", the plastic bolt of the fixing ring is put into the tube of the net bucket array until snap in.

- Attach the cod ends to the fixing rings (again, mind the numbers).

- Stretch the net bags by pulling at the net bucket array. Make sure the net bags are not twisted, otherwise turn the net bags on the fixing rings by hand until the seam of the net bag runs straight from the canvas part to the net bucket.

- Fasten all hose clamps tightly.

- Tie the net bucket array to the multinet frame by using four safety ropes. The ropes shall be slightly shorter than the net bags, so that the ropes hold the weight of the net bucket array completely!

- Install the deck command unit in the CTD container.

- New batteries were inserted before the cruise. If you still have to replace them, please refer to the official Hydro-Bios multinet manual[189]

(2) Multinet speed and depths

- Standard sampling speed: 0.5 m/sec (both, up- and downcast)

- Standard sampling depths:

  Bottom depth >2000 m:
  2000-1000-500-200-50-0 m

  Bottom depth between 1000 and 2000 m:
  Bottom-1000-500-200-50-0 m

  Bottom depth <1000 m :
  Bottom-500-200-100-50-0 m

! Attention !

In case you are sampling close to the seafloor (bottom depth <2000 m), always stay clear of the ground by 20 m! Keep an eye on the ship's display of the bottom depth.

Avoid hitting the ground with the multinet! This might damage the net itself as well as the winch cable! Be especially careful in areas with steep slopes! The ship might drift into deeper waters as the net goes down, whereas the multinet itself is still behind the ship, in shallower waters!
When you notice that the depth on the display of the deck command unit does not change anymore although the winch cable is still going down, immediately notify the winch driver and let him stop the winch!!! Go up until the depth display starts running again, then go up by five more meters. Here, you can stop and open the first net. Proceed as normal, but after the net is back on deck, check if there are damages to the net frame, the net bags or the cod ends.

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[189] https://hydrobios.de/images/datasheets/438%20130%20MultiNet%20Midi%20E_02_20.pdf

(3) Multinet operation

• Attach the cod ends to the net bucket array. Mind the numbers!

Check the net bags and the cod ends for holes from time to time. You can fix small holes using Acrifix glue and (if needed) a small piece of gauze. If there are large hauls which you cannot fix immediately, exchange the net bag.

If possible, provide a hose with running seawater to rinse the net bags when they come out of the water

Bring five plastic buckets for transporting your cod ends to the lab, one bucket for each cod end.

• When working on deck, always wear safety boots, a hard hat and gloves.

• Always have cable ties, the hexagon socket screw key (Allen key) and diagonal pliers in your pocket.

• Place the pallet with the multinet on the working deck.

• Tighten the nets a bit by pulling the net bucket array away from the multinet frame. The nets have to be outside of the multinet frame completely.

• Make sure that the marking pins on the steering cylinder and the gearbox are in coincidence. This should be the normal case if everything went right on the previous cast. If this is not the case, refer to the manual (page 17).

• Stretch the springs of the net closing device by using the tension lever to lift them up until they snap in. Start at Net 5 (the arrow on the frame is pointing to the start; see also picture) and work your way to Net 1.
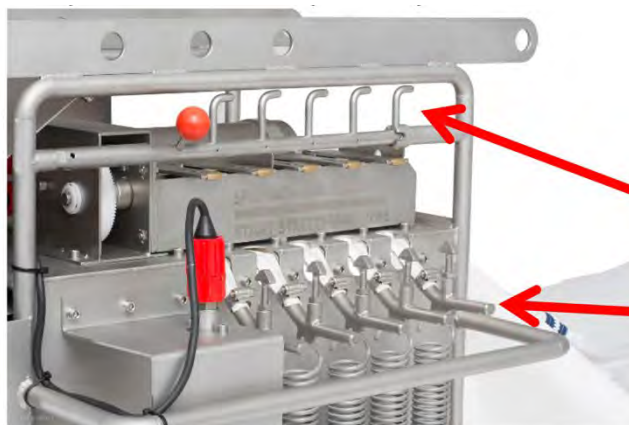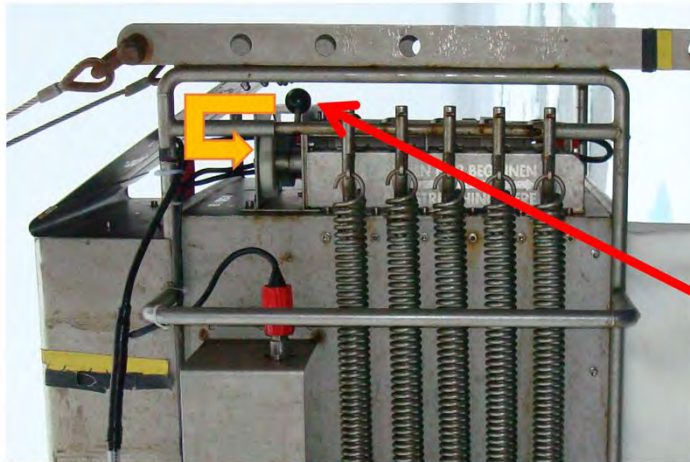

BEFORE stretching the springs, it looks like this.

The safety locking bar is in its "resting position"

Start stretching here (net # 5)

• After stretching is finished, immediately secure the springs by pulling the knob of the safety locking bar down (see picture)! It needs to lock into place properly!

AFTER stretching all the springs, it looks like this.

Pull the knob of the safety locking bar in the direction of the yellow arrow to secure the springs
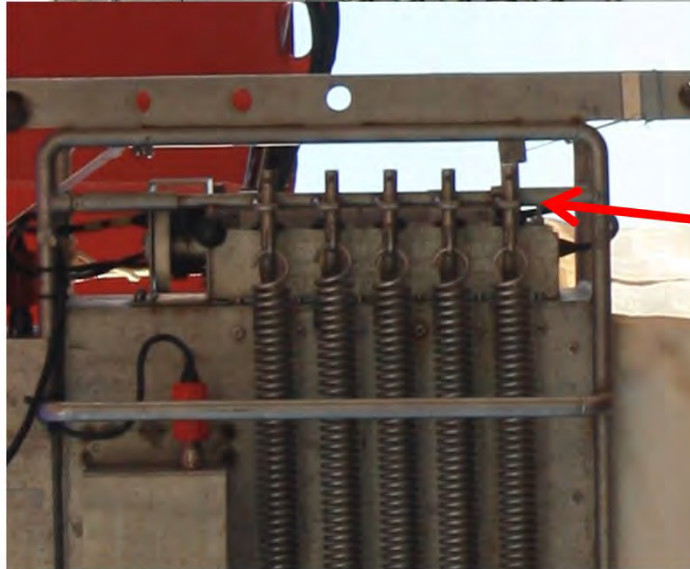
Secured springs: the hooks of the safety locking bar cover the levers of the springs so they cannot snap back

- Open the lid that is covering the motor unit (see picture) by loosening the screws a bit (tool: hexagon socket screw key) and sliding the lid up
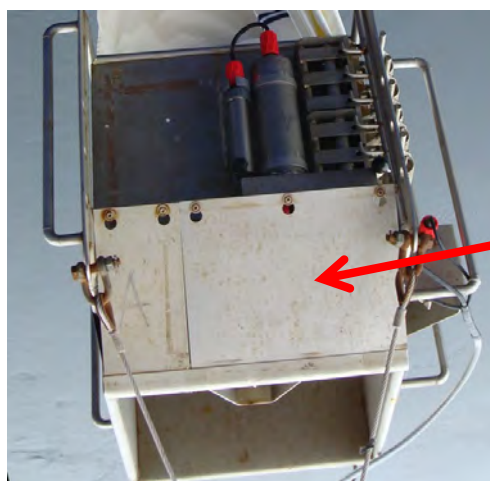
Remove this part of the lid

- Remove the blind plug (left side, 2-pin + location pin) and attach the single-conductor cable of the winch, which the crew will give you. Make sure there is enough grease on the plug. The cable needs to come in from the side (see picture). Secure the cable with cable ties and make sure it is not under tension.
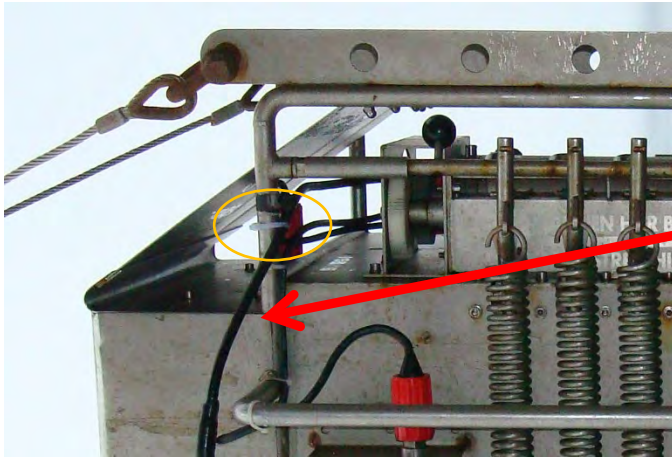
Single-conductor cable, secured with cable tie (circle)

- Put the lid back on and tighten the screws.

- Turn on the multinet by setting the lever switch at the motor unit (on top of the frame) from position O to position I
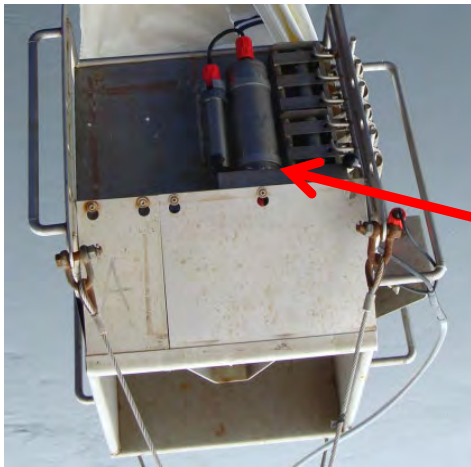
Lever switch

- Turn on the deck command unit; the display will show you the type of instrument incl. the depth range, the current pressure (i.e., the depth), the number of the active net, the battery voltage, the flow (filtered water volume of the active net bag) and the water speed inside and outside of the multinet frame.

- If it says "No data" instead, make sure that the deck command unit is connected to the winch, all the cables are plugged in correctly and the multinet is turned on.

- Make sure the battery voltage is above 7 V (while the motor is NOT running); if not, change the batteries.

- Inform the deck crew that the multinet is ready to be deployed.

- While lifting the multinet with the winch, take care of the nets and the net bucket array! To avoid damage to the nets, hold them up so they will not rub against the pallet or get damaged by the net frame. Once the net is hanging loose, grab the net bucket array and pull it towards the net frame so it will not tip over.

- When the net is hanging, put the safety locking bar back into its "resting position" (see picture); make sure it locks into place properly! If the single-conductor cable is too long and hangs loose, attach it to the crow's foot in a loop using cable ties (see picture).

- Inform the winch driver about the desired depth and winch speed (0.5 m/sec).

- Use the pressure display on the deck command unit instead of the winch length as proxy for the depth of the net. Shortly before reaching the maximum depth (e.g., 10 m before), let the winch driver know that you are almost there. Then, ask him to stop when you reach the desired depth.

- Press the action button on the deck command unit to open the first net. It will state "UNDERWATER UNIT: MOTOR RUNS", and then switch from "Net 0" to "Net 1", if it is finished.

- Now ask the winch driver to go up with 0.5 m/sec and tell him at which depth you plan to stop next. Note the time (UTC) when you start heaving in your station protocol.

- When arriving at the next stop, ask the winch driver to stop (and, again, notify him a couple of meters in advance so he can get ready). Write down the flow, then press the action button to open the next net. The flow will be set to "0" again.

- Repeat the last two steps for the remaining nets.

- The last net (Net 5) will remain open at the end of the haul. This is indicated on the deck command unit by "UNDERWATER UNIT: END OF OPERATION" after opening Net 5.

- When the net opening touches the surface, write down the flow and the time (UTC). Then turn off the deck command unit.

- Rinse all five nets from top to bottom using running seawater while the net frame is still hanging above the deck. Make sure there are no organisms stuck in the nets.

- While the winch driver lowers the net frame, grab the net bucket array and pull it away from the net frame to make sure that the array and the nets do not get damaged. Keep the array in an upright position to prevent the samples from running out of the cod ends.

- Lay the multinet frame down. You need to turn it by 90°, so the lever switch is on top.

- Turn off the multinet by bringing the lever switch to position O.

- If you do not intend to do another haul, open the metal lid and remove the single-conductor cable. Put blind plugs on both ends. Then put the lid back on.

- Detach the cod ends from the array and put them into buckets. Attention: make sure the water level in the cod ends is low enough (i.e., it can be seen through the mesh of the cod end) before the cod ends are removed, otherwise you might spill part of the sample.

- Bring the multinet back into the container.

- Put back the hose for running seawater.

- When finished working up your samples, rinse the cod ends with freshwater and reattach them to the net bucket array. Mind the numbers!

Multinet quick start guide

Things to prepare before (or during) the multinet (MN) deployment

• Attach the cod ends to the net bucket array. Mind the numbers

• Provide a hose with running seawater for rinsing the nets

• Bring five plastic buckets to the working deck for collecting the five cod ends

• Check the nets for holes

Multinet operation

• Bring the MN to the deck

• Pull the net bucket array away from the MN to tighten the nets

• Stretch the springs of the net closing device and secure them with the safety locking bar

• Attach the single-conductor cable and secure it with a cable tie

• Turn on the MN

• Turn on the deck command unit. Check the battery voltage

• While the net is lifted by the winch, take care of the net bucket array

• When the net is hanging, release the safety locking bar

• Lower the MN to the desired depth (standard: 2000 m) with 0.5 m/s

• Write down the times when:

  o MN goes into the water

  o MN is at max. depth

  o Heaving starts (for each net)

  o MN comes out of the water

• At max. depth, open the first net

• Heave with 0.5 m/s until the next desired depth

• Write down the flow

• Open the next net

• Repeat the last three steps for all five nets

• When the net is coming out of the water, write down the flow for the last net

• Rinse the net bags with running seawater while the MN is still hanging

• When back on deck, turn off the MN and remove the single-conductor cable

• Detach the cod ends from the array and put them into buckets

• Bring the MN back to the container

• Put back the hose with seawater

The multinet station protocol used during the SAS-Oden 2021 expedition

Station protocol: Zooplankton sampling　　　　　　　　　　**Cruise:** SAS-Oden 2021

MNs = Multinet Midi　　　B = Bongo net
BC = Bicycle net　　　　　CTD = Seabird CTD
L = LOKI　　　　　　　　G = Gypsum net

no.	date	station	net / mesh size	depth (m)	flow (MN) / flowmeter start - end (LOKI, Bongo)	time (UTC)				sea ice cover (x/8)	PI	remarks
						in the water	max depth/ on ground	profile start (i.e. hoisting)	profile end (i.e. out of water)			
1	01.08.2021	2-5	MNs 150	2000-1000	251	13:16	14:19	14:20		6	NH, JR	for abundance
				1000-500	127			14:52				
				500-200	72			15:07				
				200-50	43			15:13				
				50-0	11			15:15	15:17			
			BC	2000-0	14789 - 16936							for experiments/CN
2	01.08.2021	2-7	L 150 + CTD	1000	12857 - 13829	16:21	16:53	16:54	17:26	7	NH	
3	02.08.2021	3-4	B 55	200	18456 - 18691	12:31	12:43	12:43	12:50	8	NH	for abundance
			B300	200	16384 - 16602							for CN, lipids

24.3   SO21 SOP: surface microlayer

SOP developed by Janina Rahlff (LNU) for the SAS-Oden 2021 expedition

(1) Background and sampling method

The surface microlayer (SML) is the ~1 mm boundary interface between the atmosphere and the ocean with microorganisms and organic matter influencing air-sea exchange processes. We sample the SML from the air-sea interface with the glass plate method[190]. A glass plate is submerged perpendicularly to the ocean surface so that the SML will adhere to the plate, either by deploying it via a rope from the ship or directly from an ice floe. The procedure is repeated until enough sample volume has been collected. Sampling from a reference depth (usually 1 m depth) should be performed in addition. This can be done with a 100-mL syringe connected to a weighted hose.

(2) Parameters

Viral abundance (code: SMLVA)
Samples for measurements by flow cytometry (in duplicates) from five stations. 1 mL of sample is fixed with 20 µL glutardialdehyde (0.5% final concentration), incubated for 2-3 min. and frozen at -80°C.

Bacterial isolation (code: SMLBI)
Samples taken from five stations, 900 µL of 0.2-µm filtered water in 600 µL 50% glycerol (2 vials), freeze at -80°C.

Viral isolation (code: SMLVI)
Samples taken from five stations, 50 mL of 0.2-µm filtered water (2 vials), store in the fridge.

Metagenomes (code: SMLVO)
A maximum three of samples from each SML and underlying water (6 in total) shall be collected. Filtration will be performed sequentially onto 5 µm and 0.2 µm filters (diameter 47 mm) and the flow-through will be flocculated with iron chloride. The protocol of flocculation is slightly different from "SO21 SOP: viromics", and 10 times more $FeCl_3$ will be used (10 mg L^{-1} final concentration) following the recommendation in a recent publication[191].

(3) Equipment

- Glass plates
- Funnels (2)
- Squeegees (2)
- Bottles (brown) to collect SML and 1-m water
- Syringe (100-mL) with a 1-m hose bearing a weight
- Syringes with a 0.2 µm syringe filter for viral isolation
- Falcon tubes (50-mL)
- Flow cytometry tubes

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[190] Harvey GW, Burzell LA (1972) A simple microlayer method for small samples Limnology & Oceanography 17:156-157

[191] Langenfeld K, et al. (2021) Comparison of ultrafiltration and iron chloride flocculation in the preparation of aquatic viromes from contrasting sample types. Peer Journal 10.7717/peerj.11111

- Cryotubes to freeze glycerol stocks
- Glycerol
- FeCl$_3$ stocks (for preparation see "SO21 SOP: viromics")
- Duran glass bottles (1 L and 2 L)
- Membrane filters PC diameter 47 mm, 0.2 and 5 µm pores
- Filters, diameter 142 mm, 1 µm pores
- Ethanol for cleaning
- Anemometer to measure wind speed on ice
- Thermosalinometer
- Gloves
- Parafilm
- Wipes
- Sterile forceps
- DNAaway
- Waterproof pen and notebook
- MilliQ-washed bottles and funnels
- Labelled bottles and tubes

FIG. 1. Glass plate sampler, illustrating removal of microlayer.

(4) Sampling the SML

One or two people perform the dips with the glass plates and another person holds the bottle, does the wiping and takes notes.

- Take photographs of the sampling site
- Measure wind speed, salinity, and temperature of the water at the sampling site
- Record position/coordinates, if not taken from *IB Oden*, and sampling time
- Take notes on sampling site, weather conditions, any specials (sheltered area, slick, foam, blooms...)

- Wear gloves to not contaminate the samples
- The glass plate, funnel and squeegee are cleaned and wiped with ethanol
- The glass plate is completely submerged vertically to the water column and subsequently withdrawn vertically at a rate of ~5-6 cm s^{-1}. At the beginning of sampling, the glass plate should be submerged and withdrawn for a couple of times for rinsing
- After a dip, adhering SML can be scraped off from both sides of the glass plate using the squeegee and collected through a funnel in a sampling bottle that protects the sample from light
- After 2-3 SML collections/dips, the bottle should be rinsed once with the SML sample, and the sample should then be discarded. Now actual SML sample collection starts until a final volume of 500–1000 mL has been collected

(5) Sampling the underlying seawater

- Using the 100-mL syringe connected to a weighted hose, water from the reference depth (usually ~1 m, in our case rather 60 cm) is sucked up
- The syringe and the interior of the tube is rinsed twice with sampling water before the actual sample is collected
- Since underlying water can be depleted in bacterial cell number compared to SML, at least 2 L should be sampled

(6) Filtrations for DNA extraction (using bottle top units)

- The water from the SML and underlying seawater should be filtered as soon as possible after sample collection
- The filter unit must be cleaned at least with MilliQ water before use
- Label 4 falcon tubes with "SAS-Oden 2021_sampleID_running#"
- The running number refers to an excel sheet were the date/time and filtered fraction (0.2 μm or virome) and water type (microlayer, underlying water) must be added
- Screw the filter unit on a Duran glass bottle
- Add the filter membrane using sterile forceps
- Filter sample water at medium pressure through the membrane (high pressure results in cell lysis)
- Freeze the 5-μm prefilter and the 0.2-μm filter at -80°C after filtration
- Add iron-III-chloride (stock 10 g L^{-1}) to a final concentration of 10 mg L^{-1} (1 mL in 1 L, 1:1000) to the flow-through for viral flocculation
- Close and gently shake the bottle
- Incubate the bottle in the fridge (because of Arctic conditions) for 1 hour
- Refilter iron flocks onto one 142 mm 1 μm filter membrane - the filtrate can be discarded
- Filter sample water with peristaltic pump at speed 60-70
- Fold the membrane using sterile forceps with iron to the outside and put it into the labelled 50-mL Falcon tube. Storage of filter at -80°C

24.4 SO21 SOP: acid washing

Safe acid washing on board SAS-Oden expedition

This protocol starts from 37% HCl, but preferably 5% HCl should be brought onboard at the start of the expedition. No higher concentrations of acid are allowed in the laboratory areas.

Acid washing should only take place in the following designated areas:
- Main lab port side
- Triple Lab
- Container 15 (only isotopes)

Acid should be stored in Container 11. All carboys containing acid must be clearly marked. All used acid should be collected in waste containers.

Safety things to consider:
- Use the designated areas only.
- Check that eye rinse is available in close proximity to the washing area.
- Wear a lab coat, safety glasses and gloves.
- When opening the acid container, keep a distance and do not breathe over or close to the container.
- Always carry the acid containers inside a plastic box in case there are spills.
- Wherever you are doing the acid incubation, have always a plastic tray or box in which you are placing your materials to acid wash. That way while you're pouring acid in an out, any splashing or spills land in the plastic container and not directly on the work surface.
- If you don't have rubber or neoprene gloves, nitrile with a minimum thickness of 0.4 mm protects you from full contact for 480 min according to SDS.
- In case of skin or eye contact, rinse thoroughly with only water.

Preparation:
Make space in a fume hood. Place spill tray in hood. Wear laboratory coat, safety glasses and rubber gloves.
For 5 L of 5% HCl: prepare first MilliQ water and then slowly add the right volume of acid on top (4793 mL MQ water and 207 mL of 37% acid). It gets pretty hot while you mix, be slow.
Washing of smaller equipment:
Submerge smaller equipment in box with lid. Box must be clearly labelled with "5% HCl". Plasticware should be submerged for no more than 2 hours. Rinse well with MQ.

Washing of experimental bottles:
Place experimental bottles in a strapped plastic box.
Add approximately 10% of the final volume of the plastic ware of 5% HCl into the bottle. Close lid firmly and inverse bottle so that all surfaces are exposed to the acid. Transfer the HCl to the next bottle. The acid can be re-used ca. 20 times (20 bottles). When done, pour the acid into the acid waste container. Rinse thoroughly 5-6 times with MQ water.

24.5   SO21 SOP: vacuum filtration

SOP developed by Pauline Snoeijs-Leijonmalm (SU) for the SAS-Oden 2021 expedition

The vacuum pump equipments for 25-mm diameter filters in Containers 23 and 14 brought by WP2 can be used by anyone. Beware that Container 14 is contaminated with stable isotopes ^{13}C and ^{15}N while Container 23 is not. Never exchange any equipment between these two containers - to avoid this the two containers are on different sides of the ship. For some measurements, e.g., HPLC, POP, contamination with ^{13}C and ^{15}N is not a problem so Container 14 might be used for these filtrations if absolutely necessary. However, the best strategy is to use Container 23 only for natural samples and Container 14 for samples that are experimentally enriched with ^{13}C and/or ^{15}N. Enriched samples are of course forbidden to enter Container 23.

During the SAS-Oden 2021 expedition we use Advantec® glass fibre filters (pore size ~0.3 μm according to manufacturer) as a standard, but some projects use Whatman® GF/F glass fibre filters (pore size ~0.7 μm according to manufacturer). Beware that you use the correct filter type for your parameters. We will also make relevant filter comparisons between Advantec and Whatman during the expedition. All filters were incinerated overnight at 500°C before the expedition. Foreseen filtrations with the vacuum-pump equipment are:

Field samples (non-enriched) in Container 23:
Non-enriched samples from incubation experiments (WP2, WP6)
Particulate organic carbon and nitrogen (POC, PON) on Advantec (WP7)
Particulate organic phosphorus (POP) on Advantec (WP7)
HPLC pigments on Advantec (WP7)

^{13}C and/or ^{15}N enriched samples in Container 14:
Enriched samples from incubation experiments (WP2, WP6)
Enriched samples from primary production incubations on Advantec (WP7)

Filtration (see also figures below)

(1) In Containers 23 and 14 there is red light to avoid photosynthesis, there are also red head-lamps available if you need more light. **Never** turn on white light while filtering.

(2) Check that the 10-L filtrate vacuum bottle is empty and that all tubing is attached. If you forget to empty this bottle before or while filtering, it gets overfilled - and you have a problem. The guard filter will be full of water and often also the tubing between the guard filter and the vacuum bottle. Pour out the water from the bottle in the sink and replace the guard filter with a dry one – then you can pump again. Never use the equipment without a guard filter - if you get (salt) water in the vacuum pump you destroy the pump. Never throw away a guard filter (expensive!), just let them dry for 24 hours and you can use them again.

(3) Check that the manifold and the lab bench are clean and dry (to detect leakage).

(4) Turn on the vacuum pump system and open the six small handles of the manifold. Always use the vacuum pump at low speed, **never** above 1/3 of the maximum pressure of the pump, otherwise the filter can break and your sample runs through the breaks and you get very little material on the filter.

(5) Load the manifold with six glass fibre filters and attach the plexiglas tubes.

(6) Perform a leakage test by pouring 10-20 mL of MilliQ from the wash bottle (take off the cap) into each of the plexiglas tubes. Leakage is usually caused by a small piece from the previous glass fibre filter - so keep things clean all the time!

(7) Then filter your sample: pour ca. 50 mL water in the plexiglas tube, gently press the cork with injection needle and tubing on the top of the plexiglas tube (no force!) and hang the tubing in the bottle.

(8) Watch the filtering process for leaking or stops, but usually you do not need to do anything until the sample bottle is empty or the filter is clogged.

(9) Sometimes one of the plexiglass tubes empties fast and bubbles appear (but there are no leaks). In such cases it may be necessary to fill the plexiglas tube again from the sample bottle and put back the cork. In rare cases this happens all the time with the same plexiglas tube and then you can try to turn it with slight pressure (but not much). You could also pour this one by hand or exchange the filter completely (and write down how much sample you lost).

(10) When the filtration is ready, look into the tube from the top if the filter is dry – you may need to turn on the light for a second if you cannot judge it in red light. Take off the plexiglas tube and take off the filter with forceps and pack it.

(11) When you are ready, rinse the system with MilliQ, otherwise it will be full of salt the next day. Put the plexiglas tubes, the clams and the corks with the tubing in the red bucket with MilliQ until the next use of the manifold.

The three vacuum-pump set-ups brought by WP2.

24.6 SO21 SOP: spiking with $^{15}N_2$

SOP developed by Pauline Snoeijs-Leijonmalm (SU) and Prune Leroy (SLU) for the SAS-Oden 2021 expedition
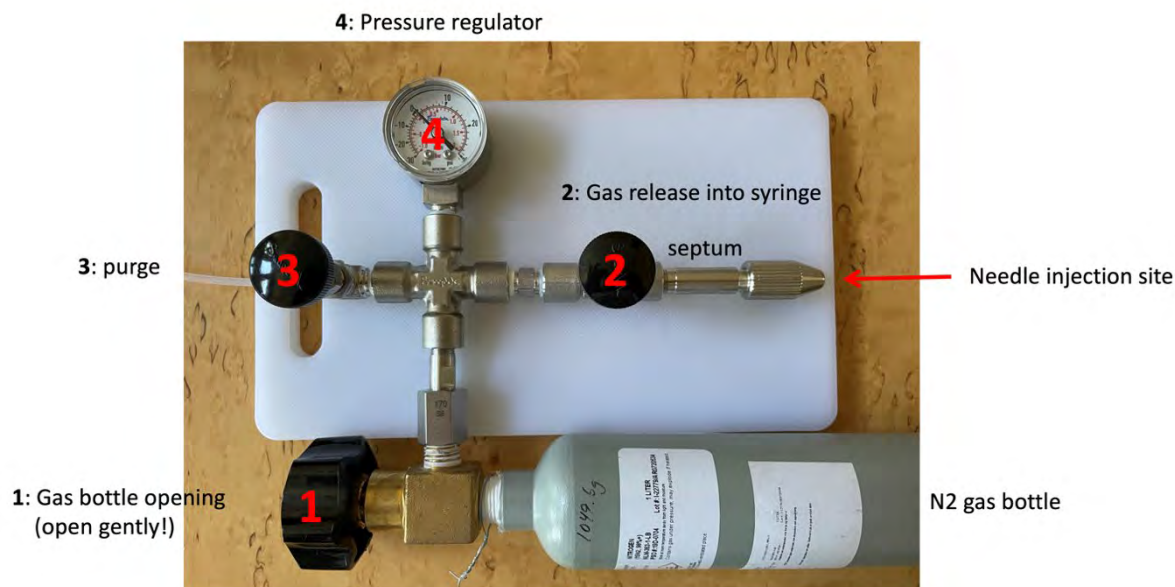
(1) Be sure that all incubation bottles are completely ready for injection
- Note that ^{13}C should already be in the bottles
- Lay the completely filled bottles horizontally
- Check that there are no gas bubbles in any of the bottles
- If you detect a gas bubble: open the bottle, carefully add some more drops of incubation water with a syringe until the very top of the bottle neck is reached, put back the septum lid and check again for air bubbles, repeat if necessary]
- When you are sure that all bottles are OK, proceed with N_2 spiking

(2) Purge the air present in the gas regulator
- Valves 1-3 are closed
- Open valve 1 (gas bottle) very slightly to release N_2 gas until a stable pressure of ~6 psi. If the pressure goes up to ~6 psi there is no leak = OK. Then close 1 [if not OK: tighten valves better]
- Open valve 2 very slightly. The pressure should drop only very little – this means that the septum is tight = OK. Then close 2 [if not OK: replace septum]
- Open valve 3 to release the gas. The pressure should drop back to 0. Then close 3

(3) Take N_2 gas into a 5-mL gas-tight syringe with syringe valve
- All valves of the gas regulator are still closed after purging the air and only N_2 gas is inside
- Take in gas into the regulator by gently opening valve 1 to ~6 psi. Then close valve 1
- Insert the needle of the MilliQ-rinsed syringe with closed syringe valve through the septum at the needle injection site
- Open gently valve 2
- Switch the syringe valve to open position
- Pull out 5 mL of gas, close the syringe valve

(4) Inject N$_2$ gas into the incubation bottles
- stick the gas-tight syringe with 5 mL N$_2$ gas through the septum of the first bottle
- Open the syringe valve and inject the gas, you should see the N$_2$ gas bubbles going in
- Close the syringe and go through procedure (3) once again (add in total 10 mL N$_2$ gas)
- Do this for all bottles that need to be spiked with N$_2$ gas

(5) Mix the N$_2$ gas with the incubation water
- Roll all the bottles for ~15 minutes (gently rolling, no shaking!)

(6) Remove the gas from the incubation bottles
- Place the needle of an ordinary 50-mL plastic syringe just below the septum of the standing incubation bottle
- Pull out the gas into the syringe, remove and empty the syringe
- Check that no gas remains in the bottle (check this in horizontal position)
- Do this for all bottles and incubate them

24.7   SO21 SOP: omics

SOP developed by Pauline Snoeijs-Leijonmalm (SU) for the SAS-Oden 2021 expedition

Note: WP2 organizes the SO21 omics filtrations. Please, ask Prune, Claudia, Javier or Pauline any questions you may have.

Getting water for the filtrations

Get water from the CTD or from ice habitats as described in *Chapter 7.4*. The standard habitats for the SAS-Omics collaboration are:

CTD water-column water	Ice habitat water	Ice habitats after melting*
ChlMax	Melt pond	Snow
100 m	Brackish brine	Top ice (10 cm)
TempMax	Ice-seawater interface	Ice centre-top
1000 m		Ice centre-bottom
2000 m		Bottom ice (10 cm)
3000 m		
4000 m or bottom		

 * = only DNA, no RNA because gene expression has changed after 24-48 hours of melting

The water is collected in the blue 20-L carboys marked for each habitat and "RNA" or "DNA". Do not use other containers because the samples must be kept dark. Take 2 to 4 carboys per habitat so that you will have enough for four replicate filters for DNA and RNA analyses, respectively. In each carboy water from one CTD Niskin bottle (12 L) is taken so that they are still easy to carry. When many carboys need to be lifted up to Deck 4 the crew can lift them up with a crane. Do <u>not</u> leave the carboys outside when the air temperature is below 0°C. Ice forms very quickly, you get difficulties with pouring the water and the organisms in the sample may get damaged.

The blue carboys for sampling the SO21 omics water.

Preparing the Sterivex filter units

All labels for the SO21 omics 0.2 μm Sterivex™ filter units for the whole expedition are printed in sequence so that mixing up filter numbers is avoided. It does not matter in which order you use the numbers because there exists only one label with that number on board.

RNA labels are: "SO21-RNA-0001", "SO21-RNA-0002", "SO21-RNA-0003", etc.
DNA labels are: "SO21-DNA-0001", "SO21-DNA-0002", "SO21-DNA-0003", etc.

To be sure that the gene expression is as close as possible to that in the field, the RNA samples are filtered first, as fast as possible after sampling, and never longer than 30 minutes per filter, all RNA samples at the same time. WP2 brought six peristaltic pumps, each with pump heads for 4-8 tubing places.

Before you get the water, have labelled 0.2 μm Sterivex™ filter units ready, as well as red caps to – after filtration – close the filters on one side, and small squares of parafilm to close the filters on the other side. Tools are available to loosen the filter unit if you cannot loosen it by hand after the filtration.

Have also a 30-mL syringe ready at the sink in the Container to get rid of the water within a filter unit after filtering and before freezing. This is always necessary when a filter gets clogged and needs to be replaced by a new filter. Take out most of the water while keeping the filter in a vertical position, otherwise the water will just move to the other side and not out of the filter.

Preparing the peristaltic pump

The peristaltic pump should be made ready for the next use after each filtration day. This means that the pump heads and all tubing have been rinsed with MilliQ, there is a clean, dry towel under the pump heads, and the installed tubing is known to be not leaking.

To start the filtration, you only need to attach the 0.2 μm Sterivex™ filter units, put the 10-L water bottles in place and press the right button on the steering unit. The steering unit is programmed for 80 rpm – do not increase or decrease; this is the optimal speed for these filtrations while keeping damages to the tubing as low as possible.

Damage to the tubing is the biggest problem of these filtrations. Always be very gentle with the tubing and check carefully that it is in the correct slot. Never throw a piece of intact tubing longer than 40 cm away (very expensive, limited stock!). Use tube fittings to repair tubing. Tube fittings should always be only between the sample bottles and the pump heads (not between the pump heads and the filters)

and the tube must reach all the way to the middle of the tube fitting. The highest pressure is always between the pump head and the filter, keep this distance as short as possible, 30-40 cm at the most. This is the area where the tubing explodes if the pressure gets too high when filters get clogged - and you will have a lot of extra work cleaning lab benches and floor and guessing how much water you lost (you need to write that down if it would happen).

Installing the 10-L bottles

We try to get as much material on one filter as possible. Sometimes the filter gets clogged and pressure gets so high that only single drops pass the filter – **this is the sign to exchange the filter for a new one!**

Metagenomics requires a minimum amount of DNA, and filtration of ca. 20 L of water from the oligotrophic Central Arctic Ocean is around this limit. While filtering time for the RNA samples (gene expression) is limited by time (30 min), a DNA filtration (community composition) can take longer, so working with the DNA filtrations are less stressful than the RNA filtrations. Always do the RNA filtrations first!

We use graded 10-L bottles for measuring the water volume. For making the four replicates, fill four bottles with water from the blue carboy and note the volume before filtration in each bottle with the help of the blue scale on the bottles. When you put the bottles in place in the baskets (this is necessary – even these bottles can fall during filtration when the ship moves!), have the lids on (or you will get wet). Then take off the lids and put in the glass pipette at the end of the tubing. The recommendation is to not work with more than four filters at a time on one pump, this creates stress and you are likely to make mistakes. However, on some pumps it is possible to work with eight filters if this is really necessary timewise (especially for RNA). After a filter is full, note the volume of water that is left in the bottle (you do not need to calculate the volume you filtered, we can do that later).

Filtration and sample harvest

Sometimes you can filter the full 10 L on one 0.2 μm Sterivex™ filter unit. This is great because that means an easier DNA extraction procedure later on. However, in ca. 50% of the filtrations, the filter gets clogged after already 5 L. Try to get as much as possible on each filter, but when it is full (it starts dropping), stop the filtration.

You must watch the filtrations at least every 15 min., preferably stay in the container. Empty the black containers with the filtrate in time – you do not want them to get too heavy or to overflow. WP4 will also have indicated from which samples 20 L of filtrate should be collected in the WP4 carboys for the virus filtrations.

If a filter gets clogged too much you will have a tube explosion = a leakage of an unknown amount of water = not a quantitative sample anymore, damaged tubing and water in your container that you must take up. So: when a filter is approaching to be clogged = only drops of water still pass – be alert to stop the filtration in time! If less than ca. 2 L of water is left you do not need to make a new filter (but note the water volume left!). See the movie on the *IB Oden* server called "A perfect Sterivex filtration" for a good filtration rate. If you have lost water, please try to estimate how much (the volume) and write it down in the lab journal.

When a filter is ready it can be dry or contain water. In the latter case, push out the water with a dry 30-mL syringe (one small drop left in the filter is no problem). Cap the filter on one end with a red cap, a parafilm piece on the other end, and put it into liquid nitrogen . Alter all filtrations are done, transfer all filters to a DNA or RNA box for long-term storage in the -80°C freezer labelled "Omics" in the "Main Lab" of *IB Oden*.

Lab journal

In the lab journal, note the date, station number, sample number on the 0.2 μm Sterivex™ filter unit, water type (see table on the first page of this SOP), the initial water volume in the 10-L bottle, and – after filtration – note the water volume that is left in the 10-L bottle. If all water has passed the filter, you write that all water was filtered. Some milliliters are always left, but for an initial volume of ca. 10 L this is negligible. Do not calculate the volume filtered, only submit your original protocol to WP2 who will scan them and put them in the SO21 omics folder on the *IB Oden* server as soon as possible.

Cleaning up

When all RNA and DNA filtrations are done, take out the tubing and pour ca. 200 mL of MilliQ over the pump heads to get rid of the salt. Use the wet towel under it to clean the bench from salt water. Then put back the tubing and place the glass pipettes in 1 L bottles with MilliQ (ca. 0.5 L per 2 pipettes). Turn on the pump until the water has passed the pump heads. Then release the pump heads, turn off the pump and the water will run off by itself and rinse the tubing. Now the pump is clean and ready for the next use. Rinse the 10-L bottles three times with MilliQ so that they are ready for the next use. You do not need to acid-wash them. Rinse the used towels twice with warm fresh tap water hang them in the Container over-night (**never** use detergent to wash them – think of the MIME project!).

24.8   SO21 SOP: viromics

SOP developed by Karin Holmfeldt and Janina Rahlff (LNU) for the SAS-Oden 2021 expedition

The iron chloride (FeCl$_3$) method described by John SG et al. [192] is used to concentrate viruses from seawater samples. The lab work is performed in Lab Container 22 on *IB Oden*.

We take our samples from the 0.2 µm Sterivex™ filter unit filtrates from the SO21 omics collaboration:

Two CTD depths (ChlMax, TempMax) from approximately every second CTD omics SAS cast. In total we aim at 15 CTD casts, 2 depths = 30 samples. Two samples can be filtered at the same time, using one filter holder per sample.

Three ice habitats (brackish brine, melt pond, ice-seawater interface) from the nine EFICA Master Stations. In total we aim at 9 stations, 3 habitats = 27 samples. Two samples can be filtered at the same time while the third sample needs to be filtered after the other two.

Note that CTD and ice habitat samples cannot be processed on the same day because of the long filtration times.

Make sure to communicate with the OMICS team the day before sampling takes place so that the correct filtrate is collected into the "virus" carboys after the Sterivex filtrations of the SO21 omics collaboration. There is one carboy for each environment (ChlMax, TempMax, Brackish brine, Melt pond, Ice-Seawater interface) + one extra carboy.

Procedure

1.   Always wear gloves when working with the iron chloride precipitation method.

2.   The iron chloride solution is prepared as follows:
     (a) Take a pre-prepared 50-mL Falcon tube containing 1.93 g iron chloride.
     (b) Add 40 mL DNase- and RNase-free water with a 50-mL pipette and a pipette boy. This will give a final concentration of 10 g Fe L^{-1}.
     (c) Dissolve the iron-chloride (shake a bit, it should dissolve fast) and filter through a 0.2 µm syringe filter (a new filter each time) using a 20-mL syringe into another 50-mL Falcon tube.
     (d) Aliquot the 40 mL of iron chloride solution into 2-mL cryovials with 1 mL iron chloride solution each.
     (e) Store the iron chloride aliquots in -20°C.

3.   Thaw one aliquot per sample (2 for CTD, 3 for ice stations).

4.   Mark the Falcon tubes in which the filters containing the iron flocculates will be stored.

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[192] John SG, et al. (2011) A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environmental Microbiology Reports 3:195-202

5. Rinse the 20-L containers that will be used for the iron-chloride precipitation three times with sample water: add ca. 0.5 L water, let it move around on all sides of the container before pouring it out into the sink. Repeat two more times.

6. Use 20 L of the filtrates from the 0.2 µm Sterivex™ filter units used by the SO21 omics collaboration that was collected directly from the filter unit into the 20-L carboys of WP4 up to the 20-L mark on the carboy.

7. Add two 1-mL aloquots 10 gFe L^{-1} stock solution to each 20 L of filtrate using the p1000 iron chloride pipet. Shake the mixture vigorously in any way you can, e.g., put the carboy on one edge and rock it back and forth as fast as possible for one minute after each 1-mL iron chloride addition.

8. Incubate the mixture in Container 22 (dark and cold). Write down how long you incubate. Regularly shake the carboy during this time. The filtration of 20 L iron-flocculated seawater usually takes ca. two hours, but could take longer.

9. Prepare the steel filter holders for filtration (we have 2 filter holders).
   (a) The tubing should be mounted with the thick silicon platinum-cured L/S 36 tubing mounted on the top and fix with cable ties. This can hopefully sit there for the whole cruise. This tubing should then be connected to the silicon platinum-cured L/S 25 tubing that fits to the pump heads. We use the peristaltic pumps WP2 has brought for the SO21 omics filtrations in Container 22. An old L/S 36 tubing should be attached to the outlet underneath the holder and could hopefully sit there for the whole cruise.
   (b) Check the tubing for potential weak spots. If you find weaknesses, please exchange the tubing.
   (c) Add the "grill" to the bottom steel filter holder (see figure below).
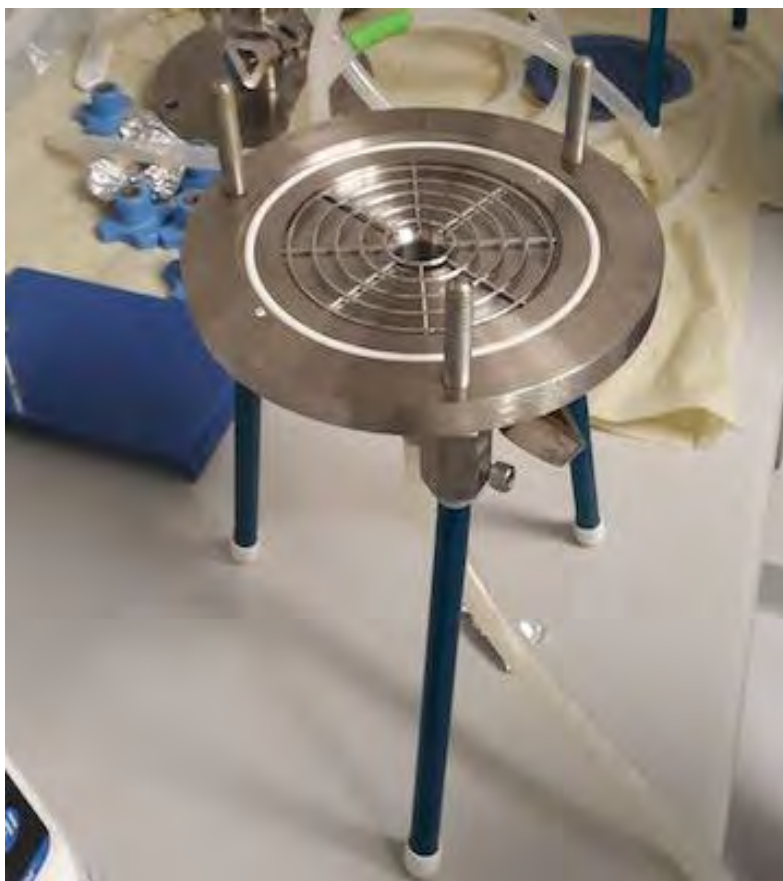
Grill on the filter holder

(d) Add the support disc onto the grill with the blue side up (see figure below).

Support on the filter holder (blue side up)

(e) Add a MF-Millipore membrane filter (0.8 μm pore size) as a support filter (AAWP14250: these are only allowed to be used in Container 22) onto the blue support disc.

(f) On the support filter, add a 1.0 μm, 142-mm, Omnipore PTFE membrane filter (ref nr JAWP14225).

(g) Add the top of the filter holder and tighten the top with the blue screws. Close the screws as tight as you can. If needed, use the whitscrew-tightening tool. See figure below for an overview of the whole setup.

Overview of the setup for iron chloride precipitation filtration.

10. Filtration.

    (a) Make sure the tubing is in the water in the carboy and the air-release valve is standing upwards (see figure below).

Air-release valve in open position (standing upwards).

    (b) Start the pump (in Kalmar we use speed 55, but as this is with other tubing and pump, it might differ). Usually, it takes ca. one hour to filter 7-10 L of water with a flow rate of ca. 150 mL per minute.

    (c) Close the air-release valve as soon as water is coming out. If the water would stop flowing, you might need to let air out during the filtration. Comment from Christien: when I tried this, I barely cracking it open while tipping the unit slightly so the air went towards the release valve.

    (d) Collect the flow-through in 10-liter carboys. The flow-through can be discarded into the sink during transit.

Air-release valve in closed position. If water still squirts out, you can close it by turning the round part.

11. In may be necessary to replace the PTFE filter multiple times during filtration depending on the degree of clogging. Ca. 5-10 L can usually be filtered on one filter, but this may vary between filters. Write how much you filtered on each filter using the marking on the 10-L carboys. Clogging is commonly observed by a lot of water leaking out on the sides of the filter holder. If this happens, try to tighten the blue screws that keep the filter holder together. When a lot of air gets into the filter holder, the filtration stops completely, but you can continue filtering if you let the air out through the air release valve.

12. Once it is time to remove the filter, you turn off the pump. Release the pressure of the pump head while you are removing the top part of the filtration device. Do <u>not</u> remove the pressure from the pump head until you have unscrewed and actually lifted the top of the filter holder slightly from the filter, otherwise water might flow out onto the filter and disturb the sample. You can use the same support filter for several samples but use a new support filter every day.

13. Take off the filter. Clean the tweezers with 70% ethanol before and between handling filters. Fold the filter using two tweezers with the $FeCl_3$ flocculates on the outside and place the filter in a pre-labelled 50-mL Falcon tube - all filters from the same sample in the same tube. To fold a filter, you hold the filter with the two tweezers on the opposite side to each other so "half circles" of the filter fall down and attach to each other when they touch each other. Then it is the trick to try to fold the filter in squares and then in eights without touching the iron. Sometimes it just does not work so well and then you do the best you can. Be careful not to scrape off any of the $FeCl_3$ on the edge of the tube. Parafilm the cap and store at 4°C in the fridge marked "Microbial Fridge" in the "Triple lab").

14. In the lab journal, note: (1) speed of the pump, (2) how long time the filtration took, (3) how much water went through each filter (measure the left-over volume in the 10-L carboys).

15. Wash the tubing that goes into the filter holder with 70% ethanol and rinse with MQ.

(a) Dismantle the top of the filter holder so you have the smaller part that the tubing is attached to on its own (see figure below).
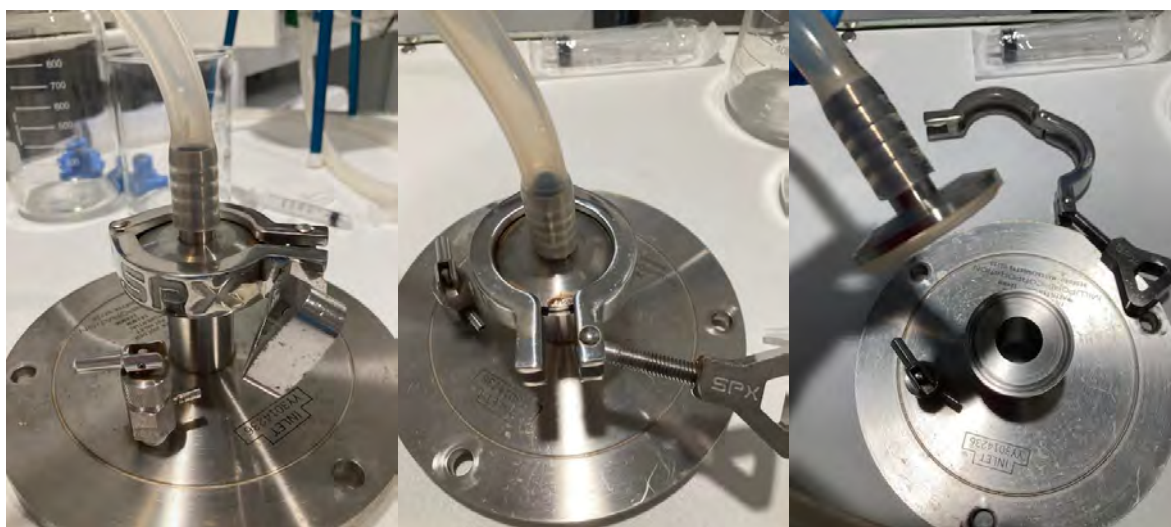
The steps to remove the top part of the filter holder to clean with 70% ethanol.

(b) Fill the tubing with 70% ethanol and seal the ends with a 1.5-mL Eppendorf tube (the "tube" end) and a 3-mL syringe in the steel end (see figure below). Let the ethanol sit for at least 1 hour.

(c) After removing the 70% ethanol, rinse the tubing with MQ.
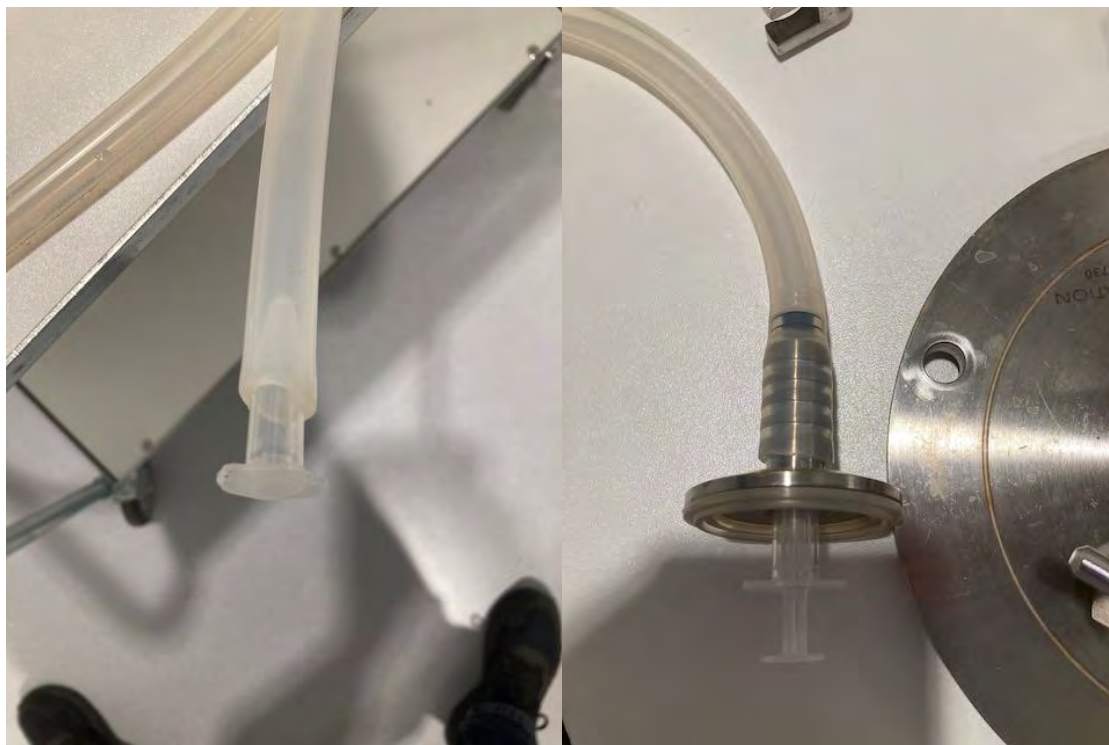
Seal the ends of the tubing with a 1.5-mL Eppendorf tube and a 3-mL syringe. Save and re-use Eppendorf tube, syringe and ethanol.

16. Rinse the steel part of the filter holder with 70% ethanol and then MQ again.

17. Before the next sampling, wash the carboys with 0-5 to 1 L of 5% HCl at the acid-wash station (main lab port side). Shake the first carboy with acid, then pour the acid into the second carboy (and third if needed). Rinse containers three times with MQ. Follow "SO21 SOP: acid washing" (*Chapter 24*).

Materials brought by WP4:

PTFE filters: 200 filters.
MF-Millipore membrane filters (support filters): 100 filters
Boxes for 16 Falcon tubes: 4 boxes

For one sample 2 mL of iron chloride is needed. We brought six tubes with 1.93 gram iron chloride in each and 6 tubes with 40 mL DNase- and RNase-free water. This makes 240 mL, enough for 120 samples.

24.9 SO21 SOP: viral isolation

SOP developed by Janina Rahlff (LNU) for the SAS-Oden 2021 expedition

Coordinate with sampling for isolation of picoplankton and heterotrophic bacteria by WP6.

Sample filtrate from the SO21 omics filtrations (0.2 μm Sterivex™ filter units) from all CTD omics depths from six stations (ChlMax, 100 m, TempMax, 1000 m) and from the ice habitats melt ponds, brackish brine, ice-seawater interface, and melted ice-samples.

Sample filtrate from the tangential flow filtrations from the WP6 experiments.

Use the label "SAS-Oden 2021-VIRIS"

(1) Mark a 50-mL Falcon tube with a VIRIS label

(2) Fill the 50-mL Falcon tube with ca. 50 mL 0.2 μm filtered water

(3) Store the tube in a fridge in 4°C. The samples should be kept dark (in a box in the fridge)

24.10   SO21 SOP: prokaryotes

SOP developed by Johan Wikner (UMU) and Ashish Verma (UMU) for the SAS-Oden 2021 expedition

Note: The term "bacteria" encompass in the following text prokaryotes covering both the taxonomic domains Bacteria and Archaea. Heterotrophic Bacteria is a dominating functional group detected by the applied methodology.

(1) Prokaryotic abundance

Measured parameter
Bacterial abundance (cells dm^{-3}), bacterial biomass (µg dm^{-3}), bacterial biovolume (µm^3).

Description of parameter
Epifluorescence microscopy on Acridine orange-stained cells analysed by image analysis by neural network (i.e., artificial intelligence) technology[193],[194]. Bacterial samples are filtered down on a blackened filter paper and stained with acridine orange in a filter funnel. The filter is mounted with immersion oil between slides and coverslips to create minimal reflection of light. Image Analysis software is used to estimate the morphology, abundance and the volume of the bacterial cells. The carbon content per cell is calculated from the biovolume estimate, providing biomass values. Bacterial abundance is measured on the whole water samples and on the seawater prefiltered through < 1.2 µm filtration (Isopore polycarbonate filters, 1.2 µm pore size, hydrophilic polycarbonate membrane, 142 mm diameter).

Methodological Description
Special requirements/gear: Fume-hood, filter funnel assembly with towers, air pressure. Laboratory coat and protective gloves to allow work with formaldehyde and acridine orange or 4-6-diamidino-2-phenylindole (DAPI).

Sampling depths
Eight standard depths [10 m, ChlMax, 30 m, 50 m,100m, 200 m, TempMax, 500 m] in two environments (open-waters and ice-covered). At some other stations a sample from the ice-seawater interface is taken in addition.

Glassware/chemicals/instruments
Sterile syringes, Acrodisc™ syringe filters (Supor membrane, sterile, 0.2µm, 25mm), GF/C 25 mm filters, 0.2 µm black-stained polycarbonate filters (25 mm, DHI), immersion oil, glass slides, coverslips, epifluorescence microscope (Zeiss Axioscope 5, Zeiss GmBH Germany), Image Analysis software, Acridine orange or 4-6-diamidino-2-phenylindole (DAPI), 37% formaldehyde.

Steps performed on board
1. Collect the seawater sample from a particular depth using a Niskin bottle. Subsample required amount of seawater into an acid-washed polycarbonate (PC) bottle for measurement of different variables.

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[193] Hobbie JE, et al. (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology 35:1225–1228

[194] Blackburn N, et al. (1998) Rapid determination of bacterial abundance, biovolume, morphology, and growth by neural network-based image analysis. Applied and Environmental Microbiology 64:3246–3255

2. Rinse a 50-mL Falcon tube with the sample water and collect 50 mL volume. Take duplicate technical replicates in 10% of the cases. Add 2 mL 37% formaldehyde (1.4% final concentration) for preservation of bacterial cells in a fume hood. Vortex the tubes briefly and store in dark at 2-4°C until processing.

3. Rinse the filter funnel assembly with Milli-Q water using a vacuum setup.

4. Put a 25 mm GF/C filter on the holder using a sterile tweezer and add 1 mL of Milli-Q. Open the suction and let it flow.

5. Place a 0.2 μm black-stained polycarbonate filter over the 25 mm GF/C filter with the shiny side up. Cover the filters with the stainless-steel towers.

6. Add 10 cm^3 (0-50 m) or 20 cm^3 (50-500 m) of preserved water sample onto the filters and filter with -13 kPa (-100 mm Hg, 0.87 bar, 14 psi) vacuum applied.

7. Add 15 drops of acridine orange (10 mmol dm^{-3}) using sterile syringe connected to 0.2 μm filters. Incubate 10 minutes at room temperature. Filter dry as above. Close the valve and release the pressure.

8. Wash the filter with 1 mL of the Milli-Q. Filter dry as above.

9. Remove the stainless-steel tower and the black polycarbonate filter with the help of tweezer. Dry the filter at the room temperature while holding the same for 45 seconds.

10. Label a glass slide with sample ID (must be traceable to CTD-cast, sampling depth and any treatment) and filtered volume with a permanent ink pen on one end of the glass slide. Usually, two filters per glass slide are made.

11. Drop the immersion oil on the glass slide using immersion oil dropper. Streak out the drop with the downward side of the filter.

12. Place the dried, black-stained PC filter with sample on top of the oil, without any air bubble formation. Add one drop of immersion oil on the filter. Be careful not to touch the filter.

13. Put a cover skip on top of the filter and oil drop. Press the cover slip hard enough using filter papers to flat the filters and remove any traces of air bubbles and immersion oil.

Sample storage

Store the slides at -20°C in a slide box until processing.

Steps performed in the home laboratory

1. After the samples are shipped to the home laboratory, analyse the microscope slides in epifluorescence microscope at 63X (Zeiss Axioscope 5, Plan-Apochromat 63 x/1.4, oil, ∞/0,17), and photograph 5 fields for each filter, semi-randomly distributed over the filtered area (Zeiss AxioCam 502 Mono).

2. Analyse the abundance, morphology and volume using Image Analysis software (Blackburn et al. 1998, LabMicrobe, Bioras™, Denmark).

3. Convert the cell volume to cell carbon density according to function described by Norland[195] and Simon & Azam[196].

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[195] Norland S (1993) The relationship between biomass and volume of bacteria. In: Kemp PF, et al. (eds) Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton

[196] Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series 51:201–213

(2) Prokaryotic biomass growth

Measured parameter
Bacterial community growth in cell (cells dm^{-3} d^{-1}) or carbon biomass units (μg C dm^{-3} d^{-1}). For budget calculations amount of substance unit may be used (μmol C dm^{-3} d^{-1}). Dividing with bacterial abundance give the bacterial specific growth rate in per day unit (fraction of increase d^{-1}).

Description of parameter
The method is based on the uptake of DNA base thymidine by pelagic bacteria[197]. For the original description of method, see Fuhrman and Azam[198]. The sensitivity is increased for Arctic waters by double sample volume and three times longer incubation time (in total six times more sensitive). Bacterial production can be measured in the whole water and on the seawater prefiltered through < 1.2 μm filtration (Isopore polycarbonate filters, 1.2 μm pore size, hydrophilic polycarbonate membrane, 142 mm diameter).

Methodological description
Special requirements/gear: Specific area designated to work with the 3H or the isotope lab. The operator must have undergone the radiation safety course beforehand.
Sampling depths: Eight standard depths [10 m, ChlMax (12-45 m), 30 m, 50 m,100m, 200 m, TempMax (200-411 m), 500 m] at two environments (open-waters and ice-covered). In ice-covered areas a sample at the ice-seawater interface (1 m) is taken in addition.

Glassware/Chemicals/Instruments
Tritiated [methyl-3H] thymidine with specific activity of 80 Ci/mmol and concentration 12.5 μM (1 mCi mL^{-1}), 2-mL microcentrifuge tubes, 50-mL polypropylene tubes, plastic floaters for microcentrifuge tubes, vortex, cold centrifuge at 4°C providing 16 000 g, Pasteur pipette connected to water vacuum device in a ventilated hood, thermostat (Eppendorf ThermoStat plus) set at 2°C, Pipettes (0.2-10 μL, 100-1000 μL), Trichloroacetic acid (TCA, ice cold), Optiphase HiSafe 3 Scintillation liquid.

Steps performed on board

Preparations
1. Collect the seawater sample from a particular depth using a Niskin bottle. Subsample required amount of seawater into an acid washed polycarbonate (PC) bottle for measurement of different variables. Rinse a 50-mL Falcon tube with the sample water and collect 50 mL volume. Collect ca. 1 L of sample water into a thermos (wide opening) from each depth for incubation at approximate *in situ* temperature.
2. Measure (e.g., CTD) the temperature of seawater from the different depths. Use water resistant marker pen for labelling the 2-mL Eppendorf tubes. The sample ID (must be traceable to the CTD-cast), sampling depth and any treatment need to be put on the lid of the tube.
3. Start the cold centrifuge at 4°C and the Eppendorf thermostat at 2°C before starting the experiment.
4. Prepare 50% (w/v) and 5% (w/v) TCA. Store TCA in a 50-mL polypropylene tube in ice-bath while processing the samples and in refrigerator during storage.

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[197] Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Marine Microbial Foodwebs 6:107–114

[198] Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Marine Biology 66:109–120

Labelling with thymidine

5. For every treatment or sampling depth, 4 microcentrifuge tubes are filled with 2 mL sample each (3 sample and 1 control). The same tip can be used for different treatments or sampling depth, but rinse once with 2 mL of sample water in between those.

6. Add 100 μL of 50% (w/v) TCA to the controls and close the lid. Flip the tubes, vortex for 3 seconds and incubate controls for 5 min at 2°C.

7. Add 4 μL thymidine to the samples and then to the controls (gives 23 nM 3H-thymidine in tube). Vortex for 3 sec. Note the start time, batch number and the specific activity of the thymidine lot.

8. Incubate the microcentrifuge tubes at the relevant temperature in the thermos from the corresponding depth by letting the tubes to float in the floating rack for 3 hours.

9. Stop the incubation by putting the tubes at 2°C or in cooling block for 5 min. Note the stop time.

TCA Precipitation

10. Add 200 μL of the freezing cold 50% (w/v) TCA solution to the samples. Vortex for 3 seconds and flip upside down to mix completely. If further processing cannot be done, the samples can be stored at < 2°C for one week.

11. Place the microcentrifuge tubes in the cold centrifuge with the necks facing outwards. Centrifuge at 16 000 g (13 000 rpm) for 20 minutes at 4°C. Store the extra samples in the refrigerator in the meantime.

12. Carefully remove the supernatant including the condensation below the lid and around the rim of the microcentrifuge tubes without suspending the pellet. Remove the supernatant preferably by Pasteur pipette with water vacuum device connected to a tap in the ventilated hood.

13. Add 1 mL of ice-cold 5% (w/v) TCA in the microcentrifuge tubes. Flip and turn the tube upside down to mix the TCA properly. Be careful so that no air bubble is trapped inside the tube. Vortex for 5 seconds.

14. Centrifuge the microcentrifuge tubes in the cold centrifuge at 16 000 g (13 000 rpm) for 10 minutes at 4°C with the necks facing outward. Remove the supernatant as mentioned above.

15. Add 1 mL of Optiphase HiSafe 3, close the lid and vortex for 5 seconds and place in storage boxes.

16. Clean the working area and perform wipe test.

Sample storage

Store below 10°C for up to 2 months in storage boxes until scintillation counting

Shipment address: Follow the radioactive safety guidelines to ship the samples to the following

Steps performed in the home laboratory

Analyse the samples regarding incorporation of thymidine in a calibrated scintillation counter with a standard tritium setting. A thymidine conversion factor of 1.5×10^{18} cells [mol thymidine]$^{-1}$ is assumed to calculate cell production from thymidine incorporation[199,200]

[199] Kirchman DL (1992) Incorporation of thymidine and leucine in the sub-arctic Pacific-Application to estimating bacterial production. Marine Ecology Progress Series 82:301–309 [http://doi.org/10.3354/meps082301]

[200] Wikner J, Hagström Å (1999) Bacterioplankton intra-annual variability at various allochthonous loading: Importance of hydrography and competition. Aquatic Microbial Ecology 20:45–260 [http://doi.org/10.3354/ame020245]

(3) Plankton respiration

Measured parameter

Plankton respiration (RESP) is measured in µmol O_2 dm^{-3} d^{-1}. In more productive waters and seasons this may encompass mesozooplankton respiration in a representative manner.

Description of parameter

Oxygen consumption measured in 1 dm^3 water samples. Measurement is performed by dynamic chemiluminescent quenching using Aanderaa model 4330 oxygen optodesTM with a titanium housing[201,202]. Bacterial respiration can be measured in samples prefiltered by a 1.2 µm filter (Isopore polycarbonate filters, hydrophilic polycarbonate membrane, 142 mm diameter).

Special requirements/gear

Oxygen optodes with titanium housing and internal temperature sensor. Incubator-box based on peltier-element technique developed by Umea Marine Sciences Centre (UMF) and Dept. of Applied Physics and Electronics (TFE) at Umea University, Sweden. USB- and RS232-hubs connected to a computer with Aanderaa Real-Time software.

Sampling depths

Measurements of plankton respiration (RESP) are done at the ice seawater interface (ISI), 10 m, ChlMax, 30 m, 50 m, 100 m, 200 m, TempMax, and 500 m depth. An estimate of the temperature sensitivity of plankton respiration is done at one depth (10 m). Bacterial respiration is performed in true duplicates at 4 depths (10 m, ChlMax, 100 m, and TempMax) in two different environments (open waters and ice-covered).

Glassware/chemicals/instruments

Aanderaa model 4330 oxygen optodesTM (Aanderaa Data instruments AS, Norway), sample bottles with magnetic beads (40 mm long, 160 rpm), stoppers with attachment for the optode, incubator box with access to 8 sample bottles with optodes, 0.3 M HCl, Milli-Q water, Isopore polycarbonate filters (1.2 µm pore size, hydrophilic polycarbonate membrane, 142 mm diameter), optode cables, RS232-serial hub, USB-hub, portable computer with data collection software (Aanderaa Realt-Time, v. 6.0.57).

Steps performed on board

1. Start the incubator in a cold room (within +10°C from *in situ* temperature) 1 hour before running the measurements. Run the fan at the maximum speed. Make sure the correct temperature has been attained in each slot.
2. Clean the sample bottle with magnetic bead (40 mm long), stopper and optodes with 1 dm^3 tap water, rinse the inside with 0.3 M HCl [2.5% (v/v)] and finally two times with one 10th column of Milli-Q water.
3. Collect the seawater sample from a particular depth using Niskin bottles. Wash the acid-washed glass bottle (wide neck, NC60) with one-tenth of sample water. Subsample 1.2 dm^3 of seawater into the bottle (i.e., full bottle).
4. Take any subsamples for e.g., prokaryotic abundance or growth from the bottle with clean tips (max. 100 mL).

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[201] Wikner J, et al. (2013) Precise continuous measurements of pelagic respiration in coastal waters with oxygen optodes. Limnology & Oceanography: Methods 11:1–15

[202] Vikström K, et al. (2018) Improved accuracy of optode-based oxygen consumption measurements by removal of system drift and nonlinear derivation. Limnology & Oceanography: Methods 17:179-189

5. Note temperature (1 decimal) and salinity (3 decimals) of the sample water either through CTD cast or by manual measurement.

6. The lid of the incubator needs to be open during operation to supply the fan with the cool air.

7. With the sample water to the bottom of the neck, mount the optode stopper carefully in the sample bottle so as to avoid any formation of air bubble below the o-ring. Let the stopper fall by itself onto the solution for the last few cm.

8. Tighten the optode carefully by gently pressing it into the bottle neck. If there appears to be any air bubble, then again fill the sample bottle and repeat the optode mounting. Note: Check that the O-ring of the optode stopper are of correct dimension and in good condition. O-rings should be replaced annually and more often at high usage.

9. The sample bottles attached to optode stopper are then mounted in the incubator slot for the respiration measurement. Note optode number per slot (i.e., depth, treatment) in the station protocol

10. Start the stirring (approx. 160 rpm) and check that the magnetic beads (40mm long) are turning freely around the centre of the sample bottle.

11. Attach the cable with the same serial number as labelled on the optode. Connect cables to the computer and power cable to the power outlet.

12. Start logging the measurements in the Aandera Real-Time software according to the instructions. Note the start and stop times in the station protocol. Choose a folder for the files and use file names identifying the samples.

13. The optodes measure the oxygen concentration every minute and the values were logged using Real-time Collector Software (Aanderaa Data instruments AS, Norway). Check that the logfiles are created and increase in bit-size in the designated folder.

14. Run the measurements for 48 hours and analyse the results using SensorDataFlow software. Stop the measurement according to the software instructions.

15. Respiration rates are derived by linear or a quadratic polynomial function for best accuracy and precision of oxygen consumption[203].

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[203] Vikström K, et al. (2018) Improved accuracy of optode-based oxygen consumption measurements by removal of system drift and nonlinear derivation. Limnology & Oceanography: Methods 17:179-189

24.11 SO21 SOP: chlorophyll-*a*

SOP developed by Christien Laber (LNU) for the SAS-Oden 2021 expedition

Reviewed by Hanna Farnelid (LNU), Pauline Snoeijs-Leijonmalm (SU), Lisa Winberg von Friesen (UCPH)

Goal

To measure chlorophyll-*a* (Chl*a*) concentrations in two size fractions: 0.3-2 µm (picoplankton) and 2-200 µm. The sum of these fractions will provide the concentration in the bulk phytoplankton. The samples are taken from the CTD at 12 depths and from eight ice habitats. The bulk Chl*a* concentrations in seawater are expected to be very low: in the order of 20-200 ng L^{-1} in seawater and 5-50 ng L^{-1} in ice habitats. The amount of water filtered should be high enough to allow for adequate measurements. The detection limit for this method is 25 ng Chl*a* L^{-1} (per fraction). Dedicated people will perform the sampling, filtrations and analyses on board *IB Oden*, but this SOP should make it possible for others to perform these tasks if necessary.

Stations and samples

Chl*a* concentrations are measured from each of the CTD bio SAS casts. The sampling depths are the 12 standard depths: 10, ChlMax, 30, 40, 50, 75, 100, 125, 150, 200, TempMax, 500 m. Note that the 500 m sample is and extra "zero", no Chl*a* is expected here, but phaeopigments could be there.

Chl*a* concentrations are measured at each SAS Ice Station from: ice cores (4 depth intervals), ice-seawater interface (ISI), brackish brine (BB), melt pond (MP), and nine times snow (only at the EFICA Master Stations). Chl*a* in the lower 10 cm of the ice cores can be very high, much higher than in seawater, and in this case less water should be filtered. Chl*a* in snow can be extremely low. The CTD and field sampling and melting of ice and snow for the SAS Core Parameters is further described in *Chapter 6*.

Preparations

• Make sure the 10-L FSW reservoir used for rinsing after each sample has filtered through is full.

• Place a 2 µm polycarbonate (PC) filter in the first green banded Swinnex. Make sure they are well-centred on the base of the holders and that the o-rings lay on top of the filter. Carefully screw the top of the Swinnex on and until there is a light tension. Attach a filter-loaded Swinnex to the luer lock fitting at the end of the peristaltic tubing for each of the 12 depths.

• Place a 0.3 µm Advantec® glass fibre (GF) filter into the second red banded Swinnex. Close as described above, for all 12 depths.

• Attach the second Swinnex to the first Swinnex by simply pushing the top riveted ends of the second Swinnex into the bottom smooth ends of the first Swinnex. If any fitting feels loose as if they will not hold, wrap a small bit of silicon tape around the end of the first Swinnex (but do not block the flow) so that it has a tighter fit.

List of equipment and consumables (for one CTD station with sampling from 12 depths)

Equipment		Consumables	
Sampling		**Sampling**	
12	Sample collection tubing for CTD		
12	200 µm mesh square		
12	Rubber Bands		
12	Amber Chl*a* sampling bottles (4.5 L) - CTD		
8	Amber Chl*a* sampling bottles (4.5 L) - ICE		
1	Tubing Tray		
Filtration		**Filtration (Water/Ice)**	
1	Carboy with tap (10 L) for filtered seawater	10 L	0.2 µm (Sterivex) filtered seawater
12	Amber Chl*a* subsampling bottles (2.25 L)	24+1	2.0 µm polycarbonate (PC) filters
12	Sippers (plastic pipette)	24+1	0.3 µm Advantec® glass fibre (GF) filters
1	Masterflex peristaltic pump for 4 heads		Silicone tape
4	Double pump heads (8 places)	200 mL	MilliQ for blanks
12	Peristaltic tubing (LS 25 only!) w/ luer fitting		
50	Swinnex filter holders (25 mm)		
Many	Zip Ties		
3	Measuring cylinders (100-200-500 mL)		
1	MQ Bottle + squirt bottle		
1	Forceps / Funnel / Filtrate waste container 1 stopwatch Syringes with correct fitting for pushing water out of clogged filters		
Extraction		**Extraction (Water/Ice)**	
1	Dark box with labelled rack for glass tubes	48+2	Glass test tubes with caps
1	Container for EtOH waste		EtOH 95% in flask with dispenser
1	EtOH dispenser/flask		EtOH 95% in squeeze bottle
Fluorometry		**Fluorometry**	
1	Turner Trilogy Fluorometer		Chl*a* standards
1	Crochet needle		0.25 M HCl
1	Micropipette (for 72 µL 0.25 M HCl)		EtOH 95% in squeeze bottle
1	Box with Kimwipes disposable wipers		Pipette tips + waste
1	Waste container for EtOH + HCl		
1	Waste container for glass		
Cleaning up		**Cleaning up**	
1	Sallad spinner (for fast drying Swinnex)	MilliQ	

Water sampling

• Sample the seawater from the 12 L Niskin bottles in the amber 4.7 L bottles by attaching sampling tubes with 200 µm mesh to the Niskin bottle nipples and opening the flow. The depth should be clearly written on the amber flasks (e.g., on a piece of permanent yellow tape with a permanent marker, both on the lid and on the flask) alongside the numbers 1-12. Ice sampling bottles have the ice environment alongside numbers 1-8. Wear latex gloves while sampling and processing the samples.

• First rinse the bottle 3x with a small amount of the 200 μm filtered sample water (ca. 100 mL). Then fill the bottle completely and put it in a rack. When the samples are retrieved bottles are transported to the filtration lab in the racks so that they cannot fall. The lab should preferably be cold (~ 40 °C) and dark, or with red LED light.

• Water from three of the eight ice habitats is available immediately after sampling (ice-seawater interface, brackish brine, melt pond) while ice cores and snow need to be melted first (see SoW). Filtering should happen as soon as possible. It is possible to keep filters at -80°C before analysis if direct treatment cannot take place.

• Sample the ice-habitat water from the mixing tank taps) and the melting tanks (make sure to mix tanks well before sampling) in the amber 4.5 L bottles. The sample type should be clearly written on the amber flasks.

Filtration

• The filtration setup is a multi-head peristaltic pump system with the capacity to filter eight samples simultaneously on 16 filters (2 filter pore sizes per sample). The peristaltic pump pushes seawater through the series of two filters inside the Swinnex filter holders.

• Each part of the filtering system can be numbered 1-12 and B (for blank), so there is no confusion which sample bottle connects to which tube.

• Water column filtrations are performed in duplicate (2.25 L per filtration). If biomass is very low in the ice waters, cores and snow, one can filter more than 2.25 L (up to 4.5 L).

• Subsequently, the lower five depths and MilliQ blank filters are filtered, followed by their duplicates. Only one blank (one PC and one GF filter) is required per sampling occasion.

• Blanks are prepared with 200 mL MilliQ.

• Invert the 4.5 L bottle several times to mix and pour a 2.25 L subsample into a 2.25 L amber bottle.

• Insert the peristaltic tubing into pump head and rest between the teeth.

• The 2-L bottles are placed to the left of the pump in a rack and the filters and the container for the filtrate is placed to the right. Make sure the tubes are positioned so there is enough tubing on both sides to reach the respective containers.

• Attach the sippers (plastic pipette) to the tubing.

• Close all the pump heads and turn the pump on. Make sure the rotation rate is set at 30 rpm and press the play button to start the filtration.

• The water will slowly start pumped through the system. Once it has reached the Swinnex filters, carefully check that there are no leaks at the seams and that water is only leaving the system through the end of the second Swinnex.

• Approximately every 5 minutes, the crate containing the sampling bottles should be rocked to keep the seawater mixed in the bottles while filtering.

• Let the 2.25 L of seawater flow through the system until you start to see bubbles of air enter the system through the sipper. At this point, add ca. 100 mL of filtered seawater to the sample bottle and give it a small whirl. This will help to rinse the tubing from cells that might otherwise remain in the tubing and not on the filters. If the filters would get blocked with biomass before the 2.25 L bottle is empty, detach the Swinnex and gently push the remaining water out with a syringe without over drying the filter. Push just enough air in to purge the capsule of water. Write down the volume of water left in the bottle (use a measuring cylinder).

• Once the 100 mL of filtered seawater has gone through both Swinnex filter holders, allow the pump to pump air through the system until you stop seeing drips coming out at the end of the tubing. All water needs to be completely purged from the system before you can open up the Swinnex.

• Press the pause button on the peristaltic pump to stop the motor and let it sit for several seconds before opening the pump heads.

• Remove the Swinnex and gently open and remove the o-rings. If water is still present for some reason, carefully close and purge more air through the system to remove the water.

• Using clean forceps, place the filters into labelled glass test tubes at least half way down the tube. Try to not to fully block the tube with the filter at this point, as it will make adding ethanol more difficult. Fold the filter once and insert to the tube, then allow the filter to open and circle to wall of the tube. Handle the Advantec filters extra carefully to not break them. Clean the forceps in 95% EtOH and wipe of residual EtOH with Kimwhipe disposable wipers, between filters.

• Using the auto-distributor add 2.25 mL of 95% EtOH to each of the tubes, make sure the filter is far enough down to be covered in ethanol and close the tube with a plastic stopper. Do not invert the tubes. Place tubes in the dark box in labelled tube racks. Note the time that the extraction starts. NOTE: The low EtOH volume is due to the low expected Chl*a* values.

• Repeat this process for the other samples

• Store the dark box at room temperature for 16-20 hours.

Cleaning up

• All bottles, tubing and filter holders and must be cleaned well because the samples may have very different Chl*a* concentrations.
• Rinse the 4.5 L bottles 3× with MQ before storing them. Can be stored damp.
• Rinse the 2.25 L bottles 3× with MQ.
• Rinse the Swinnex filter holders carefully with MQ. Then use the salad spinner to speed up the drying process.
• Pump MQ through all the used peristaltic pump tubing to remove residual seawater.
• Refresh the FSW container with 0.2 FSW from the Omics waste
• Leave all pump heads in the open position so tubing is not being clamped while not in operation.

Calibration of the Turner Trilogy Fluorometer

Chlorophyll-*a* standard is prepared from a 1 mg *Anacystis nidulans* chlorophyll-*a* ampule with a stock concentration of 10,000 µg L⁻¹ in 95% EtOH. This stock is kept in a glass amber bottle wrapped in aluminium foil at 4°C and AWAY FROM ACIDS. The fluorometer is initially calibrated at LNU before being taken to sea. It is generally very stable, but we will be checking the calibration regularly (bi weekly) throughout the cruise and updating the calibration if values deviate from the initial calibration. At the beginning of the cruise, we will also conduct multiple initial calibration checks to insure stability of the signal.

Creation of Working standards is conducted through serial dilution of the stock Chl a in 95% EtOH using 100-mL volumetric flasks. The stock should be diluted by 90% at each step of dilution (10 mL stock to 90 mL 95% EtOH) down to 10 µg L⁻¹ (10000 ng L⁻¹) before creating working standards. These will be at 10, 100, 1000, 10000 ng L⁻¹ and optionally higher.

• Turn on the fluorometer and let it warm up for 20 minutes before measurement
• Use Kimwhipe disposable wipers to remove any moisture and salt from the glass tubes before measuring.
• Select the "Chl-A" option and press "OK" to confirm.
• Press "Calibrate" and "Run New Calibration".
• Press "ug L⁻¹"
• Insert a blank EtOH sample into the instrument and press "OK".
• Write down the blank fluorescence value that appears on the screen.
• Insert the 1 µg L⁻¹ standard, enter the concentration and press "OK".
• Press "OK" to measure the fluorescence before acidification (Fb)
• Write down the fluorescence value that appears on the screen.
• Remove and acidify the standard with 64 µL 0.25M HCl. Close the cap and gently invert and wait for 1 minute before removing the cap again. (this is 3.2% of 2 mL)
• Put the standard back into the instrument and press "OK" to measure the fluorescence after acidification (Fa)
• Write down the fluorescence value that appears on the screen.
• Press "Enter More Standards", enter the concentration and press "OK".
• Insert the 2 µg L⁻¹ standard, enter the concentration and press "OK". Etc.
• Repeat for each additional standard to be included.
• Press "Proceed with Current Calibration" to accept these values and "Yes" to save the calibration.
• Enter the Calibration name as A-SAS-YYMMDD and press "Save".

Fluorescence values should also be recorded in the chlor-acidification-calculation-spreadsheet.xls spreadsheet provided by Turner Instruments. This spreadsheet will be used to calculate the chlorophyll and phaeophytin concentrations rather than the calibrations saved in the instrument. This is because more values can be used for the calibration, covering a larger range, additional acidified values can be used for an average ratio value, and lower concentrations of chlorophyll can be measured.

Sample measurements with the Turner Trilogy Fluorometer

- Turn on the fluorometer and let it warm up for 20 minutes before measurement and remove samples from the 4°C to equilibrate to room temperature.
- Use Kimwhipe disposable wipers to remove any moisture/finger prints from the glass tubes before measuring.
- Select the "Chl-A" option and press "OK" to confirm.
- Keep the instrument in raw fluorescence mode.
- First measure the blank sample. This sample should measure similar to the RFU value of the blank in current calibration. Refer to either the calibration excel spreadsheet or the calibration file in the fluorometer for this value.
- Take a sample (glass tube + filter) out of the dark sample box and gently invert several times to mix.
- Remove the filter from the tube with clean forceps or knitting needle and discard. Rinse the tips of the forceps over a EtOH waste container with 95% ethanol to remove any residual chlorophyll before using them with the next sample.
- Place the tube into the fluorometer and close the lid.
- Press "Measure Raw Fluorescence" and record the RFU value. If the output fluorescence value is greater than the highest value in our standard curve (see excel measurement spreadsheet), dilute the sample by a factor that will put it within the range of our calibration curve and measure again. If the value is now in the correct range, enter the value in the excel spreadsheet under (Fb) along with the sample name, volume (mL of water filtered, volume of ethanol for the extraction, and the dilution factor. Dilute samples by pipetting required volume into a new test tube and raising to 2 mL with diluent EtOH.
- Acidify the sample with 64 μL 0.25M HCl, replace test tube cap and gently shake to mix
- Proceed to next sample similarly.
- Once all samples have been measured for Fb and acidified, wait 5 minutes for acidification reactions to complete.
- Starting from the first sample measured, remeasure all samples raw fluorescence again. This will give the Fa value of the degraded chlorophyll. For each sample, record the Fa value in the excel spreadsheet and write down all information for the sample in the log book, including the calculated Chl*a* and Phaeophytin-*a* concentrations.
- Once finished, discard samples into the EtOH + HCl waste container. Discard the test tube into the glass waste container. Stoppers can be rinsed and dried in the salad spinner.

Note that the data in the Chl*a* log sheet should be transferred to an excel file preferably on the same day so that you can make notes of unexpected events or observations when you still remember them.

24.12   SO21 SOP: flow cytometry

SOP developed by Hanna Farnelid (LNU) and Christien Laber (LNU) for the SAS-Oden 2021 expedition

Reviewed by Pauline Snoeijs-Leijonmalm

Goal

To collect samples for enumeration of microalgae, bacteria and virus, as well as discrimination of phytoplankton populations (picocyanobacteria, picoeukaryotes, heterotrophic nanoflagellates), based on cell size and fluorescence properties using flow cytometry (FCM).

Stations and samples

CTD samples: FCM samples for microalgae, bacteria and viruses are taken from each of the CTD Bio SAS casts. The sampling depths are the 12 standard depths: 10 m, ChlMax, 30 m, 40 m, 50 m, 75 m, 100 m, 125 m, 150 m, 200 m, TempMax, 500 m. Additional FCM samples for bacteria and viruses are taken from the CTD deep SAS (always from the cast made immediately after the CTD Bio SAS from which the first 12 samples were taken). The sampling depths are the 10 standard depths of CTD deep SAS below 500 m: 700, 1000, 1500, 2000, 2500, 3000, 3500, 4000 m, bottom -50 m and bottom.

Ice station samples: FCM samples are taken from seven sea-ice habitats at the SAS Ice Stations: ice (4 depth intervals), brackish brine (BB), ice-seawater interface (ISI), melt pond water (MP). At the nine EFICA Master Stations also snow samples will be taken. The CTD and field sampling and melting of ice and snow for the SAS Core Parameters is further described in *Chapter 6*.

From the CTD Bio SAS, five replicates will be taken with the purpose of measuring: virus abundance (FCM-virus, analysed by WP4), prokaryote abundance (FCM-prok, analysed by WP2), autotrophic phytoplankton and heterotrophic nanoflagellates (analysed by WP7), + one extra sample. From the CTD deep SAS only three replicates will be taken with the purpose of measuring FCM-virus and FCM-prok.

List of equipment and consumables (for five replicate samples)

Equipment	Consumables
	Gloves (nitrile or latex)
Fume hood	25% glutaraldehyde solution 10% Pluronic F-68
Liquid nitrogen dewar (table model) Tool for taking out samples (nylon stocking) Safety glasses Lab coat	Liquid nitrogen
Micropipette P5000 Micropipette P200 Micropipette P100 Micropipette P10	Pipette tips P5000 Pipette tips P200 Pipette tips P100 Pipette tips P10
Rack for cryotubes	5× 4-mL cryovial Cryobox for -80°C freezer (for 81× 4-mL vials)
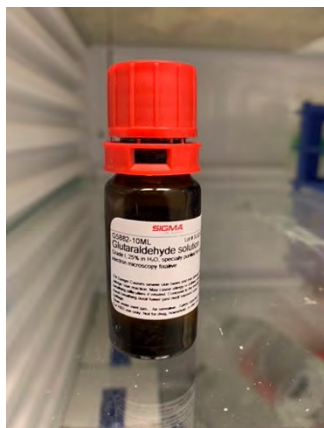
Preparations

• Remove one bottle of 25% glutaraldehyde solution (Glu stock) from the -20°C freezer and place in the fume hood to thaw.
• Aliquot volumes of 4 mL into Eppendorf tubes. Store the aliquots in the -20°C freezer. If there are only few aliquots in the freezer, make new ones from the bottles that are stored in the -20°C freezer.
• Before each sampling, remove one or two aliquots (see below) and place in the fume hood to thaw.
• Each CTD cast will use 5×12 = 60 cryovials from the CTD bio SAS and 3×10 = 30 cryovials from the CTD deep SAS = 90 cryovials, for which 90 × 0.076 mL = 6.84 mL of Glu stock is necessary (= 2 aliquots).
• Each SAS Ice Station will use 5×7 = 35 cryovials on the helicopter ice stations and 5×8=40 on the EFICA Master Stations (including snow samples), for which 40 cryovials, 40 × 0.076 mL = 3.04 mL of Glu stock is necessary (= 1 aliquot).

Note: always use FRESH Glu stock = always store it at -20°C and never let it sit at room temp for more than a few hours.

Water sampling

Seawater is retrieved from the CTDs in 250-mL HDPE amber sampling bottles and for the ice habitats in the melting- and mixing-tanks in Lab Container 22 (keep track of when you can get the samples).

FCM sampling[204]

• With a 5-mL pipette, bring 3.8 mL of the sample water into a 4 mL cryovial.
• Add 76 µL 25% EM grade Glu stock (final concentration 0.5%) to each replicate. Close and invert the cryovials several times to mix.
• To the replicates for autotrophic picoplankton and heterotrophic nanoflagellates, +1 extra, add 3.8 µL 10% Pluronic F-68 (final concentration, 0.01% see Marie et al. 2014). Pluronic F-68 is a

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[204] Marie D, et al. (2014) An improved protocol for flow cytometry analysis of phytoplankton cultures and natural samples. Cytometry Part A 85:962–968

surfactant that has a positive effect for preserving in particular eukaryote cells for longer term storage.

• Close and invert the cryovials several times to mix. Let the samples incubate at room temperature for 5 minutes before flash freezing in liquid N and then placing in the -80°C freezer in cryoboxes (SAS freezer in "Main Lab").

• Place the samples in the most recently started cryobox in order of depth. When getting a new cryobox, label it with SAS-Oden 2021, FCM, and write the ID number of the first sample on the outside of the box.

• Regularly transfer list of samples to the TCM electronic spreadsheet.

Labels

Each series of replicates has a running number for the whole expedition

PP = primary producers = with Glut and Plur
BV = bacteria & viruses = with only Glut

CTD bio: 5 replicates per depth (3×PP, 2×BV) - 1 small bag per depth, numbering from 0001

CTD deep: 3 replicates per depth (3×BV) - 1 small bag per depth, numbering from 1001

Ice station: 5 replicates per habitat (3×PP, 2×BV) - 1 small bag per habitat, numbering from 9001
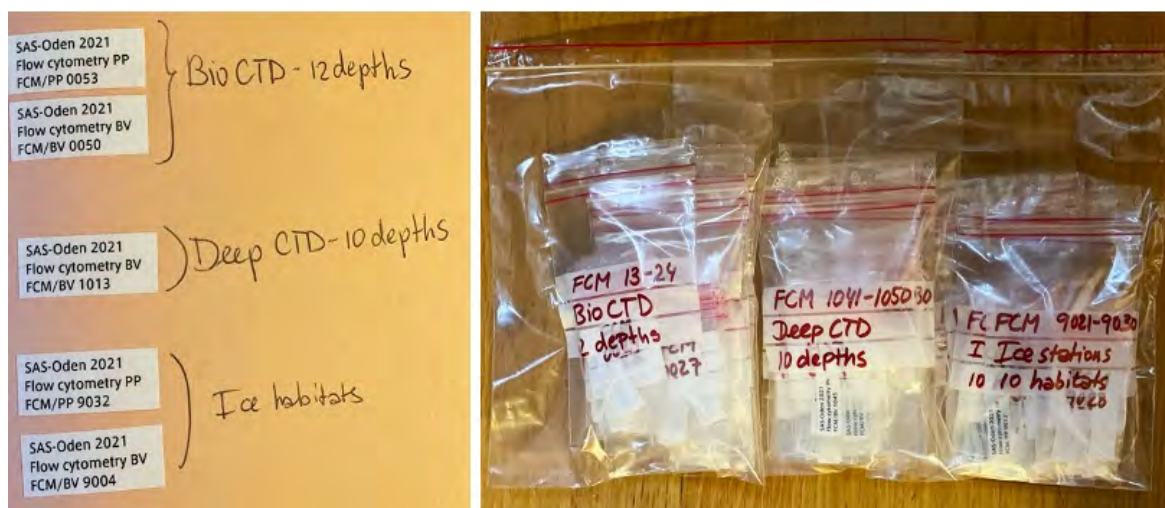
24.13   SO21 SOP: primary production

SOP developed by Clara Pérez Martínez (SU) for the SAS-Oden 2021 expedition

Reviewed by Pauline Snoeijs-Leijonmalm (SU) and Hanna Farnelid (LNU)

Goal
To measure primary production (PP), i.e., assimilation of inorganic carbon via photosynthesis by phytoplankton communities by performing simulated *in-situ* (SIS) incubations from natural seawater and ice habitats.

List of equipment and consumables (for one CTD station with sampling from 12 depths)

Equipment	Consumables
Sampling 12 wide-neck 2.3 bottles (t=0) 12 narrow-neck 2.3L transparent bottles (light) 12 narrow-neck 2.3L dark bottles (dark) 6 transport baskets 12 Sample collection tubing for CTD 12 200-μm mesh square glued to tubes	Sampling Latex gloves
Spiking and incubations Shading mesh bags to create light gradient Temperature loggers Li-Cor light meter 4 black containers for transportation	Spiking and incubations Latex gloves 1 1-mL stable isotopes pipette 36 1-mL pipette tips 33 mL MilliQ water
Filtration Filtration pumps Tubing Forceps Thin permanent marker	Filtration Latex gloves 36 precombusted Advantec® 0.3 μm filters MilliQ water Aluminium foil

All equipment is brought by WP2 (Pauline Snoeijs-Leijonmalm, SU)

Working places

You will be mostly working and performing the spiking in Lab Container 14. For the t=0 samples, use Lab Container 23 (the t=0 samples should never enter Container 14 to avoid contamination with stable isotopes). You should always work in dim red light and use latex laboratory gloves.

For the incubations, use the two fridges in the Chemical Storage Container 11 (one light and one dark fridge). Since this is a chemical storage you cannot work here.

Important to keep in mind all the time:
There are contamination risks with the use of stable isotopes onboard that can adventure your own and other people's research. Be mindful! Only Lab Container 14 is for isotope-handling.

Preparations

Always: in the evening before, make the necessary preparations (protocol, labels, etc.)

At the start of the expedition: create a light extinction gradient by: adjusting the light in the incubation fridge and using mesh bags for achieving a shading gradient according to the CTD PAR measurements.

At the start of the expedition: measure the light in the bottles using the Li-Cor light meter.

Filtrations

Always test for possible leakage of the vacuum pump with MilliQ water.

Make sure to empty the vacuum trap before starting and during the filtrations – the pump should never get water inside! If you forgot to empty it in time, replace the pump protection filter and dry them outside the container. See the detailed protocol for using the SU vacuum pumps in the SO21 vacuum pump SOP.

Day 1

Preparations

1. On Day 1 of the experiment, collect all the material and make the necessary preparations before you get water

2. Prepare the ^{13}C-stock. Make sure that the stock solution is totally dissolved before using it (at least one hour before spiking).

3. Make stock solutions for spiking - MAKE SURE THE LID IS FIRMLY CLOSED BEFORE SHAKING!

   For 33 mL stock: 33 mL MilliQ water+ 0.75g NaH13CO3
   For 44 mL stock: 44 mL MilliQ water + 1g NaH13CO3

Sampling water

4. Get water from the CTD or an ice station

   CTD: Fill 36 polycarbonate incubation bottles (2.3 L) with water from 12 depths, 3 bottles per depth (12× t=0, 12× light, 12× dark). Leave the neck part of the bottle without water.

   ICE: Fill 9 incubation polycarbonate incubation bottles (2.3 L) with water from 3 ice-habitats: melt ponds, brackish brine and ice-seawater interface (3× t=0 + 3× light, 3× dark). Leave the neck part of the bottle without water.

   Always transport the bottles in closed black boxes so that the samples do not experience light

Filtration t=0 samples (Simultaneously – another person)

5. Get the boxes with filled bottles lifted with a crane operated by the *IB Oden* crew to Lab Container 23 (the samples should **never** enter an isotope-enriched container).

6. Filter all t=0 samples for natural levels of ^{13}C, ^{15}N, POC, and PON.

7. Freeze the filters at -20°C (those filtered together) in small plastic zip bags in the PP-labelled white boxes in Container 11.

Spiking

8. In isotope Lab Container 14, spike the bottles with 800 µL ^{13}C – write down time.

9. Mix gently by inverting the bottles.

Incubating the bottles

10. After spiking, transport the bottles to Container 11

11. Place the bottles in the fridges. Make sure that the light bottles are in the designated spaces to assure the right light gradient – write down the exact time of the start of the incubations.

Day 2

Harvesting the incubated bottles

12. After ca. 24 hours incubation time: Harvest the incubation bottles and bring them to Container 14. DO NOT turn off the light in the fridges.
13. Filter all the incubated water volume. Use long filtering times if needed, it doesn't affect the isotope samples. Follow a sequential order with marked tubes: first dark samples and then the light ones. Write down the exact time when you take out each bottle out of the fridge and start the filtration.
14. After filtering, wrap the filters individually in aluminium foil. Write immediately the filter number on the aluminium foil with a thin permanent marker following the protocol sheet. Protect a filter from falling on the floor by always keeping a gloved hand under it. If a filter would fall, discard it and note down what happened.
15. Freeze the filters at -20°C in batches of 12 (those filtered at approximately the same time) in small plastic zip bags in the PP-labelled white boxes in Container 11.

Cleaning up

16. Rinse the tubing between and after every filtration with Milli-Q.
17. Rinse the incubation bottles 3 times with MilliQ water and store them in Container 14.
18. Make sure everything is washed and ready for next time.
19. Double-check that everything is in place for the next incubations after you are ready.

24.14   SO21 SOP: phytoplankton community

SOP developed by Hanna Farnelid (LNU) and Christien Laber (LNU) for the SAS-Oden 2021 expedition

Reviewed by Pauline Snoeijs-Leijonmalm (SU)

Goal
To collect samples for taxonomical identification and community composition of phytoplankton (>5 μm).

Samples

CTD samples: Lugol-fixed phytoplankton samples are taken from each of the CTD bio SAS casts. The sampling depth is the chlorophyll maximum (ChlMax).

ICE habitat samples: Lugol-fixed phytoplankton samples are be taken from three ice-associated water habitats at the SAS Ice Stations: brackish brine (BB), ice-seawater interface (ISI), and melt pond (MP).

All equipment will be brought by WP7 (Farnelid, LNU)

Materials
2 mL Acid Lugol's solution per sample (250 mL needed in total)
500-mL amber glass bottles (120 bottles needed in total)
Funnel
P1000 pipette
P1000 pipette tips

Procedure

• Sample water from the CTD or ice habitat directly into the amber glass bottle.

• Fill the bottle gently from the bottom and up.

• Bring the bottle to the fume hood in the Main Lab (chemistry side) and add 2 mL Acid Lugol's solution.

• Invert the bottle three times.

• Store at 4°C fridge in the Main Lab (biology side).

24.15   SO21 SOP: zooplankton community

SOP developed by Nicole Hildebrandt (AWI) for the SAS-Oden 2021 expedition

Reviewed by Pauline Snoeijs-Leijonmalm (SU)

Goal
To collect depth-stratified multinet samples, as well as bongo net samples, for analysing the abundance, taxonomic composition and biomass of Arctic Ocean meso- and microzooplankton communities. The community samples will be analysed at the AWI after the cruise.

Samples

For mesozooplankton analyses, five standard depth strata are sampled using a multinet (150-µm mesh size). The standard depth intervals are 2000-1000-500-200-50-0 m. Additionally, full-depth casts are planned at some EFICA Master Stations where the water depth is > 2000 m. Here, we will deploy the multinet twice to cover the additional depth strata > 2000 m. Microzooplankton is sampled with bongo net hauls (50-µm mesh size) from 200-0 m.

Materials

5 buckets
200 wide-neck 100-mL Kautex bottles, 50 wide-neck 200-mL Kautex bottles
Sieves (150/50 µm mesh), squeeze bottles
4 L 20% formaldehyde buffered with hexamethylenetetramine
Dispenser
Filtered seawater

Procedure

• Use buckets to safely transport the filled cod ends to the laboratory.

• Pour the sample over a sieve with 150 µm mesh size (mesozooplankton) or 50 µm mesh size (microzooplankton). Rinse the cod end carefully with filtered seawater from a squeeze bottle so that all zooplankton is collected on the sieve. Transfer the zooplankton sample to a (labelled) Kautex bottle.

• If there are large jellyfish (ctenophores or cnidarians), remove them from the sample and note species names and numbers in the station protocol. These organisms will disintegrate in formalin and make post-cruise sample analysis much more difficult. However, make sure to only remove the jellyfish and no other zooplankton organisms such as small copepods, etc.

• Add 20 mL of 20% formalin (buffered with hexamethylenetetramine) to each 100-mL Kautex bottle (or 40 mL to 200-mL Kautex bottles) and fill them up to the neck with filtered seawater (see picture below) to achieve a final concentration of 4% formalin.

Fill the bottles with filtered seawater until this level

• The Kautex bottles are always labelled both on the bottle and on the lid. In addition to the unique sample ID (SAS-Oden 2021, ZOOtax_xxxx), also include date, station number, mesh size and depth interval.

Storage and metadata

• Store the Kautex bottles in a frost-protected place.

• Enter the samples into your metadata excel file.

Appendix A: Research Data Management Policy

SAS 2021

Research Data Management Policy

Background

The Swedish Polar Research Secretariat (SPRS) recognises that high quality research data are valuable products of field work. Data sharing, with fair attribution, is a corner stone for scientific collaboration, not least for the evolvement of interdisciplinary science. Data preservation with open access is fundamental for the legacy of any research activity. Facilitating reuse and re-purposing of research data adds long-term value of the data to scientific research, industry, and society at large.

Building on the data statements of the International Council for Science (ICSU)[1] and the International Arctic Scientific Committee (IASC)[2], SPRS works towards ethical open data publication pertaining supported projects, programmes and research infrastructure.

SPRS seeks to promote high standards and best practice for management of research data. Data originating from research projects on-board Icebreaker Oden during the SAS 2021 expedition are to be published openly, with proper attribution to the data creator, and with minimum delay, unless otherwise agreed upon.

Research data and metadata

Research data refers to any information necessary to validate and reproduce the results of research. This could be e.g. field notes, primary data files, images, or audio-visual materials.

Metadata refers to structured information about the data. This information includes technical information (such as file formats) as well as descriptions of provenance and

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[1] http://www.icsu-wds.org/services/data-sharing-principles

[2] https://iasc.info/images/data/IASC_data_statement.pdf

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context (purpose of study, timeframe, sampling locations, equipment used etc.). The metadata should be descriptive and detailed enough to enable independent interpretation and re-use of the data.

Published research data and metadata should be accurate, complete, identifiable, and openly accessible, and strive to meet the FAIR principles[3]. The dataset together with accompanying metadata should be archived securely and safely in appropriate formats to ensure long-term usability. The data publication should also be given a unique persistent identifier enabling citation, versioning, and proper attribution.

Open access to data and metadata

Data collected during the SAS 2021 expedition can be divided into three categories with corresponding open access policies.

1) *Data originating from installations on Oden funded and/or operated by SPRS:*

   SPRS is owner of data. Metadata and data are to be made available with free, unrestricted, complete access, without charge and with minimum delay in the Swedish National Data Service (SND) data repository[4] after completion of the cruise.

2) *Data collected during the expedition within the scope of a separately funded research project that requires specific installations or activities outside the premises of paragraph 1:*

   Either the organisation of the principal investigator (PI) or the PI is owner of data, depending on applicable regulations or agreements. Metadata and data are to be made available with free, unrestricted, complete access, and without charge within the framework of current legislation. Metadata are to be submitted to the SNDs data repository or other suitable data repository with minimum delay after completion of the cruise. Access to data can be restricted by the PI for a maximum of two years (period of moratorium). If the funder of

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[3] https://www.force11.org/fairprinciples

[4] http://snd.gu.se

2/4

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the research project has stipulated a shorter period of moratorium this has precedence.

3) *Data collected by SPRS in agreement with third party:*

   The agreement should stipulate open access policy and data ownership. SPRS should always strive towards full open access with minimum delay.

The principal investigator is responsible for:

- informing themselves on SPRS research data policy, including which open access policy applies to their data.

- developing appropriate and well documented procedures for data collection, processing and use.

- prior to the expedition, submitting requested information regarding how data will be collected, managed, shared, and openly published.

- ensuring safe and secure storage of data and metadata during and after the cruise. The PI determines which information needs to be retained to support the authenticity of any research results.

- openly publishing data and metadata according to the principles outlined in this document in a format facilitating re-use and re-purposing.

- submitting complete metadata for publication in the SNDs data repository in association with other metadata and data from the SAS 2021 expedition.

- if data is not submitted for publication in SNDs data repository, ensuring that the repository selected for data publication meets requirements of long-term preservation. Links to the published dataset should be submitted to SPRS for publication in SNDs data repository.

- following all agreements with regard to deadlines, publication, data-sharing, moratorium and/or confidentiality.

The Swedish Polar Research Secretariat is responsible for:

- providing PI with advice regarding data management and publication.

- If requested supporting PI in primary publication of metadata and data in the SNDs data repository.

- watching over the long-term preservation of data and metadata published in the SNDs data repository and if needed taking relevant actions regarding data custodianship.

- publishing links to data on polar.se and/or other relevant communication channels controlled by SPRS.

4/4

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Polarforskningssekretariatet är en statlig myndighet som främjar och samordnar svensk polarforskning. Det innebär bland annat att följa och planera forsknings- och utvecklingsarbete samt att organisera och leda forskningsexpeditioner till Arktis och Antarktis.

The Swedish Polar Research Secretariat is a government agency that promotes and coordinates Swedish polar research. This includes to follow and plan research and development, as well as to organise and lead research expeditions to Arctic and Antarctic regions.

polar.se