# CRUISE REPORT

Response of Harmful Dinoflagellates to Climate Change

M/V Le Commandant Charcot, Cruise No. O030622

03.06.2022 – 15.06.2022

Reykjavík (Iceland) – Longyearbyen (Svalbard, Norway)

Cruise participants: Fuat Dursun & Simon Tulatz

*Fuat Dursun, Simon Tulatz*

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# 1 Summary

The cruise started on Friday, 3rd at about 19:00 h UTC from the port of Reykjavík. The scientists arrived at 11:00 h on board at that day and started unpacking their equipment and planning the scientific work schedule together with Daniel Cron and Daphné Buiron, the two crew members responsible for scientific activities on board. The first station was reached on June 4th at about 22:30 h and sampling and sample processing was performed as planned. Within the cruise, a total of 12 stations were sampled (Figure 1), where water samples and net samples were taken.

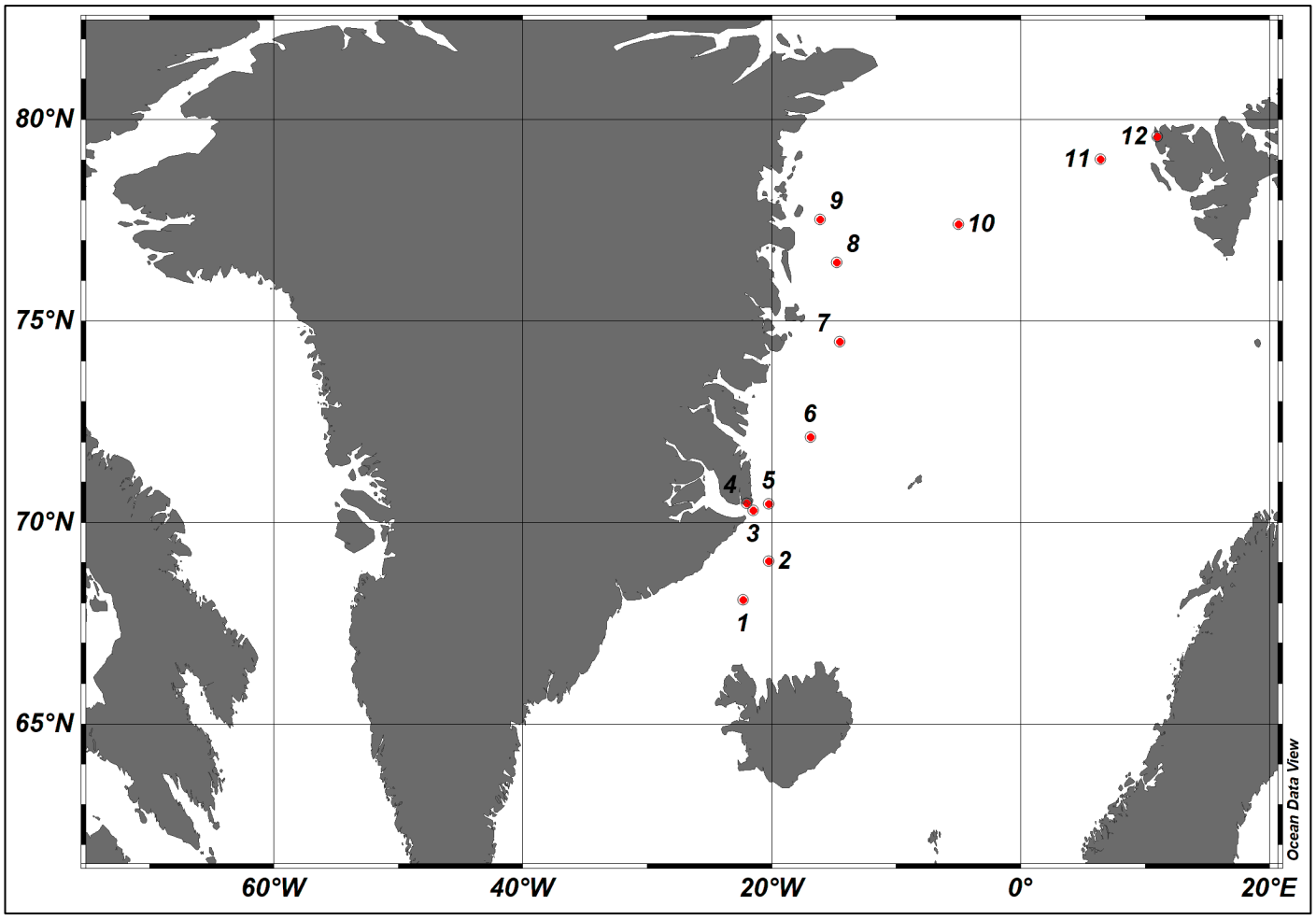


***Figure 1:*** *Map of the 12 stations that were sampled between June 3rd and June 15th on the way from Reykjavík (Iceland) to Longyearbyen (Svalbard, Norway)*

At two of these stations (1 & 5), there was no possibility for water samples and only net tows were performed. All of the stations were sampled from a door in the wet-lab, except station 4 and 9, that were sampled from the sea-ice, in about 500 m distance from the ship.

In addition to stationary work, continuous toxin- and phytoplankton samples were performed within the first four days of the cruise, until the seawater supply of the ship was not blocked by ice (Figure 2). Two SPATT-bags were used for sampling of dissolved toxins. One on a transect during the first day of the cruise and one at station 4, when the ship was stationary for more than 24 h close to Ittoqqortoormiit (Greenland).

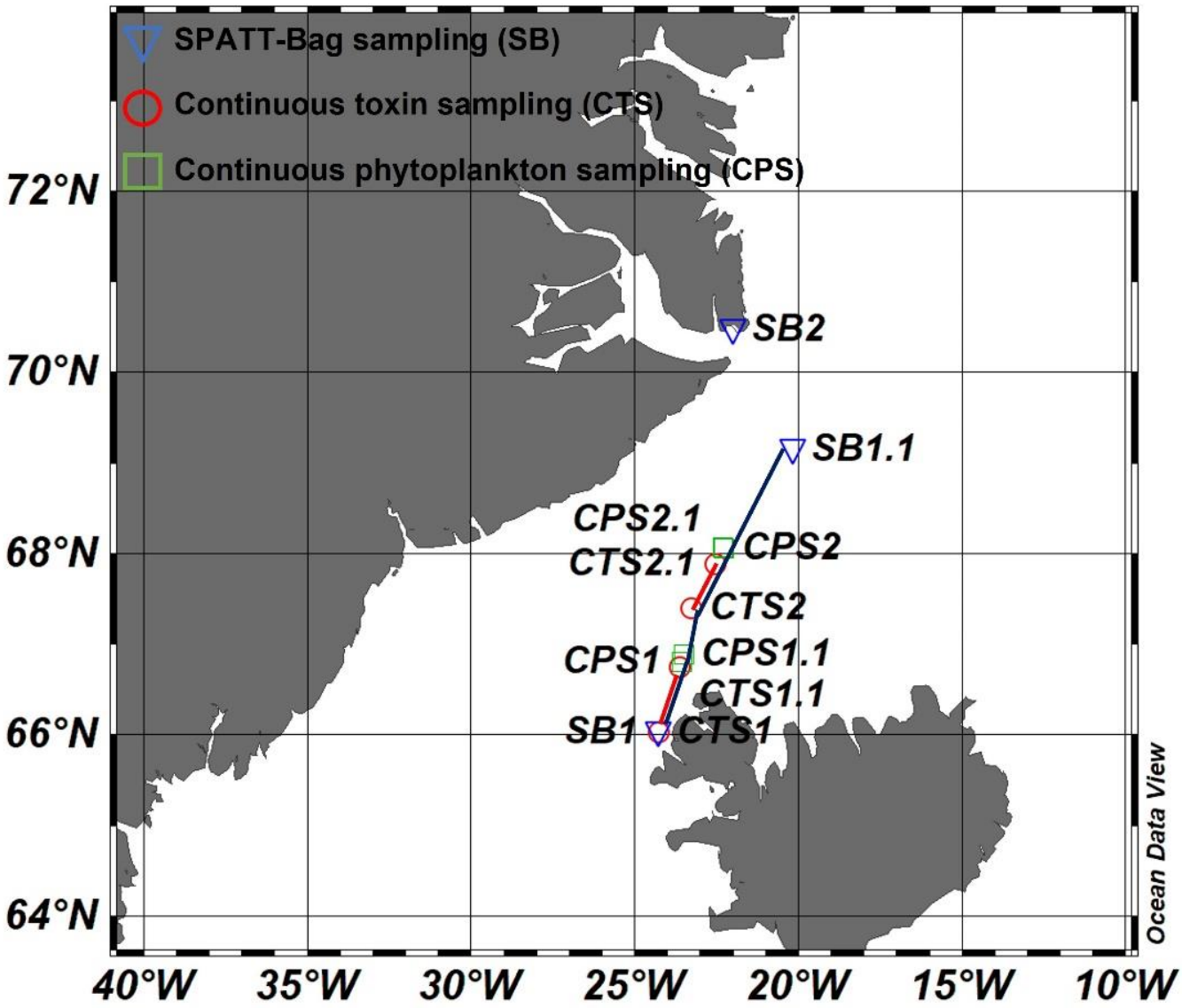


***Figure 2:*** *Map of the areas where continuous toxin-, phytoplankton- and SPATT-Bag sampling was performed. Due to heavy sea-ice, samplings could only be performed at the beginning of the cruise. SB=SPATT-Bag, CTS=Continuous toxin sampling, CPS=Continuous phytoplankton sampling. (The distance between CPS2 and CPS2.1 is to low to be viewable in the map)*

The last station was sampled on June 13th, and the ship entered the port of Longyearbyen late on June 14th. On June 15th, the scientists left the ship together with a part of the crew and flew to Paris via charter flight.

# 2 Research Objectives of the Cruise

The main research objectives of the cruise from Reykjavík to Longyearbyen were:

1) Determination of the presence of toxigenic plankton species in coastal waters of East Greenland and Svalbard by collection of plankton samples with special emphasis on toxigenic dinophyceae.

2) Assessment of phycotoxins presence in the study area.

3) Establishment of monoclonal cultures of potentially harmful algal species for subsequent physiological laboratory experiments under different temperature and pH regimes to assess their potential behaviour in future climatic scenarios.

4) Taxonomic and phylogenetic characterisation of these cultures as well as their phycotoxin profiles.

# 3 Narrative of the Cruise

The scientists from the Alfred Wegener Institute arrived in Reykjavík on June 2nd with the scientific equipment as their personal luggage. They embarked the Commandant Charcot at 11:00 h on June 3rd. After reception and controlling of negative PCR-Tests for COVID-19, the equipment was carried on board and was stored in the wet lab. After lunch, the scientists had a tour through the scientific areas and a safety instruction by the Science Officer Daniel Cron. A safety instruction followed later on the same day together with the passengers. The ship left the port of Reykjavík at 19:00 h.

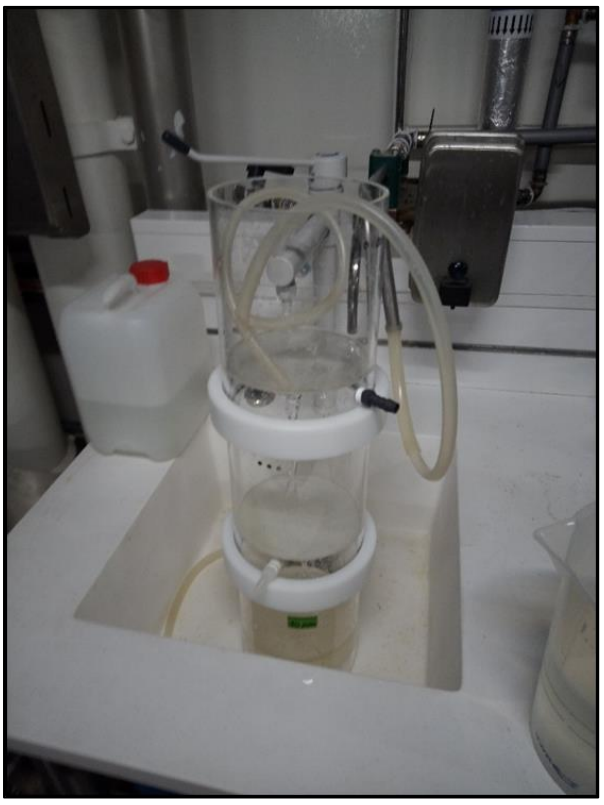

***Figure 3:*** *Continuous toxin sampling from the peristaltic pump. The water was directed to a filter tower with a tube.*

On June 4th at 08:30 h, a solid phase adsorption toxin tracking (SPATT) bag was deployed in a beaker and constantly rinsed with unfiltered seawater from a peristaltic pump at a flow rate of 0.5 L/min for the adsorption of dissolved toxins for 24 h. Furthermore, seawater from the same pump was filtered through a filter-tower with mesh sizes of 200, 20 and 10 µm with a flow rate of about 1 L/min to sample different sizes of phytoplankton for four hours for toxin extraction. After the continuous toxin sampling, the procedure was repeated for 30 minutes for phytoplankton sampling. Both, toxin- and phytoplankton sampling were repeated later that day. Unfortunately, continuous sampling and the deployment of SPATT bags had to be stopped when the ship reached the sea-ice. On the same day, the cruise participants were equipped with waterproof jackets and boots for sampling from the deck, side-door or sea-ice.

The first station was reached at 22:35 h on June 4th and two vertical net hauls with a phytoplankton net (20 µm mesh size) from a depth of 25 m were performed from the side-door of the wet lab. The next station was reached at 05:10 h on the next morning and in addition to the plankton net tows, water samples were taken from the surface with a bucket from the side-door, from a depth of 10 m from the peristaltic pump and from 30 m depth with a Niskin bottle from the aft of the ship, using the mooring winch. Another station was sampled on June 5th at 19:20 h with the same procedure. In addition, a CTD-cast to 80 m was performed

***Figure 4:*** *Plankton net sampling from the side door in the wet lab.*

Early on June 6th, the ship maneuvered into the compact ice-cover close to Ittoqqortoormiit (Greenland) for passenger activities, so sampling from the side-door was not possible at this station. While water samples from 10 and 30 m were taken as mentioned above, samples from the surface and net-tow samples were taken directly from the sea-ice about 500 m behind the ship in a small open water area. A CTD was also lowered to a depth of 80 m from the back of the ship. Furthermore, as the ship was stationary for more than 24 hours with no ice under the ship, another SPATT bag was deployed. After sampling and the processing of the samples, the cruise participants had the chance to walk to the village of Ittoqqortoormiit over the ice.

The ship left the solid ice-cover in the afternoon of June 7th and station work of station 5 was performed at 20:50 h. Due to high ice-density, no Niskin bottes could be deployed from the aft oft the ship and the peristaltic pump could not be used. Therefore, sampling at this station was limited to two net tows from a depth of 25 m from the wet lab. Before the station work started, the cruise participants introduced their research project to the passengers in the theatre. On the 8th of June, at 13:55 h station 6 was sampled in a polynya, big enough for the deployment of Niskin bottles and to use the peristaltic pump. Therefore, net tows and water samples from surface, 10 and 30 m were taken and a CDT cast to 900 m was performed.

***Figure 5:*** *Deployment of a Niskin bottle from the aft of the ship to get water samples from a depth of 30 m.*

In the morning of June 9th, one of the two cruise participants unfortunately had a positive COVID self test, performed by the ship personnel and had to stay in isolation for at least 5 days. Still, at 18:40 h the station work of station 7 was performed. Sampling and sample processing for this and the following stations was possible due to the help of the two crew members responsible for scientific activities. Due to the limited water access from the aft of the ship, sampling only included net tows and water samples from the surface and 10 m depth.

On June 10th, a complete station (station 8) including water samples from all three depths and a CTD-cast to 180 m was performed at 15:50 h from the side door of the wet lab and from the aft of the ship. On June 11th, at 11:00 h the ship was surrounded by a closed sea-ice cover for passenger activities again, leading to differences in sampling procedure. While net tows and surface water samples were taken from the sea ice again, water from 10 and 30 m were sampled from the ship via peristaltic pump and Niskin bottle (station 9). A CTD-cast to 35 m was performed from the ice as well. The sampling for station 10 was performed on the 12th of June at 12:00 h from the ship and contained net tows and water samples from all three depths and CTD-cast to 800 m.

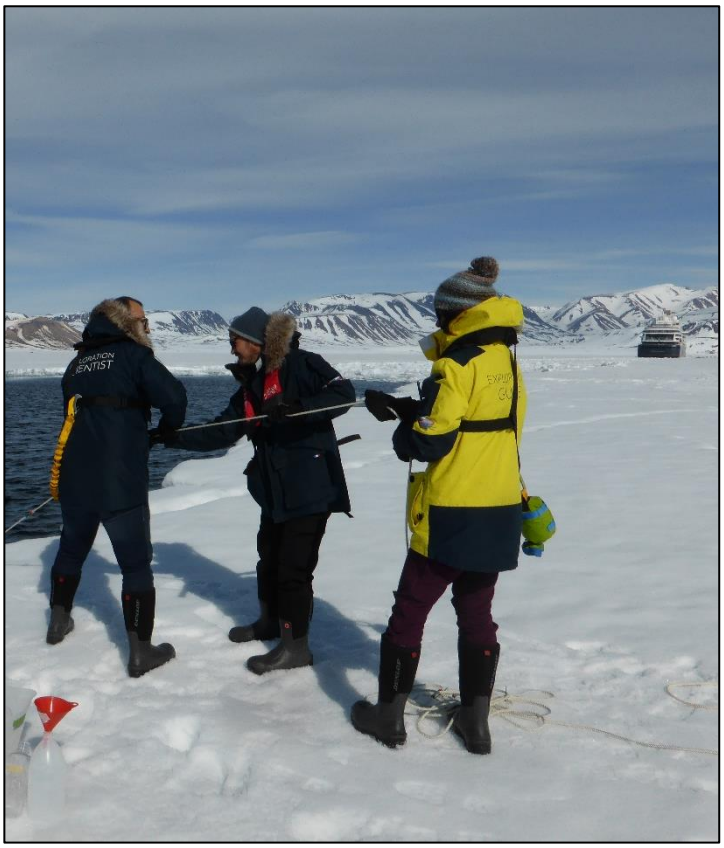

***Figure 6:*** *Plankton net sampling from the sea-ice behind the ship that is seen in the background.*

The last two stations (stations 11 & 12) were sampled on June 13th from the ship. Station work for station 11 was performed at 02:00 h and contained the complete sampling program, including a CTD-cast to 900 m. Station 12 was

done at 12:00 h and was limited to net tows to 25, water samples from the surface and 10 m depths and a CTD-cast to 80 m.

On June 14th, the final cruise day, the cruise participants gave a small presentation about the work on board, development of their projects and preliminary results for the passengers in the theatre. The reminder of the day was used for packing the equipment and cleaning the laboratories. The Commandant Charcot reached the port of Longyearbyen at 20:00 h that evening.

At 10:00 h on June 15th, the cruise participants were transported with their equipment to shore via life boat and further to the airport of Longyearbyen via bus. From there the cruise participants took the charter flight to Paris and a connecting flight to Hamburg the next morning.

After each station, lab work consisted of processing net tow samples and water samples in different ways, to gain subsamples for different toxins, DNA extraction, fixing samples, live samples and cell isolation. Furthermore, the continuous toxin-, phytoplankton samples were processed directly after the sampling was finished.

Continuous toxin sampling

Water from the peristaltic pump was directed through a filter tower of 200, 20 and 10 µm meshes for at least 3 h with a flow rate of about 1 L/min. After that, the 20 and 10 µm filters were rinsed with filtered seawater (FSW) and the samples were transferred to a 50 mL reaction tube, each, with as less water as possible. After that, the reaction tubes were filled with FSW to the defined volume of 45 mL. The samples were divided into 3 subsamples and transferred into 15 mL reaction tubes. One subsample of each size fraction was for the detection of hydrophilic paralytic shellfish toxins (PSP), lipophilic diarrhetic shellfish toxins (DSP) and DNA extraction. The samples were centrifuged at maximum speed for 10 minutes and the supernatants were discarded. Each subsample was transferred to a cryovial with about 1.5 mL of FSW and centrifuged again for 10 minutes at maximum speed. After that, the supernatant was carefully discarded with a pipette and the samples were stored at -20 °C. Extraction and measurement of the toxins will be performed in the laboratories of the Alfred Wegener Institute in Bremerhaven via LC-MS/MS and LC-FLD.

Continuous phytoplankton sampling

Water from the peristaltic pump was directed through a filter tower of 200, 20 and 10 µm meshes for 30 minutes with a flow rate of about 1 L/min. The 20 and 10 µm filters were rinsed with the least volume of FSW possible and were transferred to a glass vial each. The samples were adjusted to a volume of 20 mL with FSW and fixed with 0.5 mL of 37 % formaldehyde for later identification of toxigenic species.

SPATT bag sampling

A SPATT bag was deployed in a beaker and rinsed with unfiltered seawater from the peristaltic pump with a flow rate of 0.5 L/min. After that, the SPATT bag was transferred to a zip bag and stored at 4 °C. Extraction and measurement of the toxins will be performed in the laboratories of the Alfred Wegener Institute in Bremerhaven via LC-MS/MS.

Processing of net tow samples

Two vertical net tows from a depth of 25 m were performed and the samples were collected in sample bottle and the volume was adjusted to a total volume of 2 L. 20 mL of the sample were fixed with 37 % formaldehyde in a 20 mL glass vial. Another 20 mL were filtered through a 200 µm gauze and transferred to a 70 mL culture flask, that was filled up with FSW and stored at 4 °C for post-cruise live microscopy and

cell isolation. 50 mL of the sample were also filtered through a 200 µm net to exclude zooplankton, and transferred to petri dishes. The samples were stored at 4 °C for on board cell isolation.
The remaining sample was filtered over a filter tower of 200, 50 and 20 µm. The 50 µm and 20 µm filters were rinsed with FSW and each fraction was transferred into a 50 mL centrifugation tube. The volume of the samples was adjusted to 45 mL and each sample was divided in three subsamples of 15 mL each in 15 mL centrifugation tubes. The samples were centrifuged at maximum speed for 10 minutes and the supernatants were discarded. Each subsample was transferred to a cryovial with about 1.5 mL of FSW and centrifuged again for 10 minutes at maximum speed again. After that, the supernatant was carefully discarded with a pipette and the samples were stored at -20 °C. Extraction and analysis of PSP, DSP and DNA will be performed post-cruise in the laboratories of the Alfred Wegener Institute in Bremerhaven.

Processing of water samples

3 L of water from the depths of surface, 10 and 30 m (or 4.5 L of surface and 10 m) were pooled in a bucket and filtered over a 20 µm mesh. 1 L of this water was vacuum filtered over a 5 µm polycarbonate filter with a vacuum not higher than 200 mbar. The filter was transferred into a 50 mL centrifugation tube with the lower side to the tube. The filter was rinsed with 500 µL of SL1-Buffer (part of DNA extraction kit) until complete decolouration. The fluid was then transferred to a bead tube, filled with ceramic beads and stored at -20 °C for post-cruise DNA analysis.

The remaining 8 L of water were filtered in the same way and the filter was transferred to a 50 mL centrifugation tube again. The filter was rinsed with 500 µL of 100 % methanol until complete decolouration. The methanol was transferred onto a 0.45 µm spin-filter and centrifuged for 5 minutes at 6500 rpm. The filtered methanol was transferred to HPLC-vial for measurement of azaspiracids (AZAs) in the laboratory in Bremerhaven.

The volume of 1 L of pooled sea-water, that was filtered through a 20 µm mesh was reduced to about 200 mL via gravity filtration. 100 mL were fixed with Lugol's idodine (final concentration 1 %). 50 mL were transferred to glass petri dishes and stored at 4 °C for later cell isolation. The reminder of the water was transferred to a 70 mL culture flask, that was filled completely with FSW, and also stored at 4 °C for live microscopy and cell isolation in the laboratories of the Alfred Wegener Institute.

Cell isolation

Cell isolation was performed to isolate single cells of species of interest from net tow- and sea water samples, to establish monoclonal cultures for later laboratory experiments. Living samples in petri dishes were placed under a binocular and moving cells were isolated wit a gel-loader pipette tip connected to a thin tube. Negative pressure was carefully created with the mouth until the cell of interest was in the pipette tip. After that, the cell was released in a well of a 96-well plate containing filtered FSW enriched with nutrients. The pre-cultures were stored at 4 °C until the transport to Bremerhaven.

***Table 1:*** *Overview of the course of the cruise*

| Area / Station | Date | Time | Activities |
|---|---|---|---|
| Reykjavík | 02.06.2022 | | -Arrival of the cruise participants in Reykjavík |
| Reykjavík | 03.06.2022 | 11:00<br><br><br><br><br>19:00 | -Embarking of the cruise participants on the Commandant Charcot<br>-Tour through scientific areas<br>-Science related safety instruction<br>-Unpacking of equipment and laboratory preparation<br>-General safety instruction with the passengers<br>-Start of the cruise |
| Cruise | 04.06.2022<br>–<br>13.06.2022 | | -Station work<br>-Continuous sampling<br>(see table 2 and 3) |
| | 07.06.2022 | 17:45 | -Introduction of the scientific projects to the passengers |
| | 13.06.2022 | 20:00 | -Scientist go on stage with the crew for gala night |
| | 14.06.2022 | 11:45<br><br>20:00 | -Final presentation of the projects for the passengers<br>-Packing of equipment and cleaning of laboratories<br>-Le Commandant Charcot arrives at Longyearbyen |
| Longyearbyen | 15.06.2022 | 10:00 | -Transport of the cruise participants from the ship to the airport of Longyearbyen via lifeboat and bus<br>-Departure in Longyearbyen via charter flight to Paris |

# 4 Preliminary Results

The results for temperature, salinity, density anomaly, oxygen saturation and oxygen concentration of the CTD-cast at station 6 to 900 m can be found in figures 7 and 8.

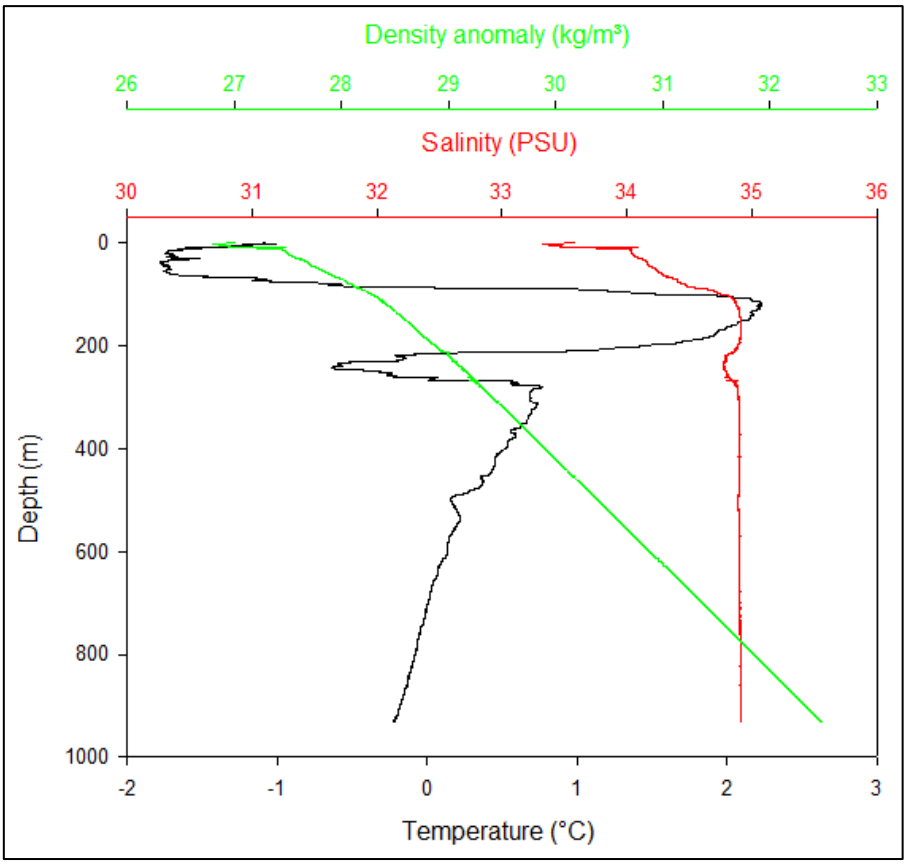


***Figure 7:*** *Results for temperature, salinity and density anomaly of the CTD-cast to 900 m at station 6.*

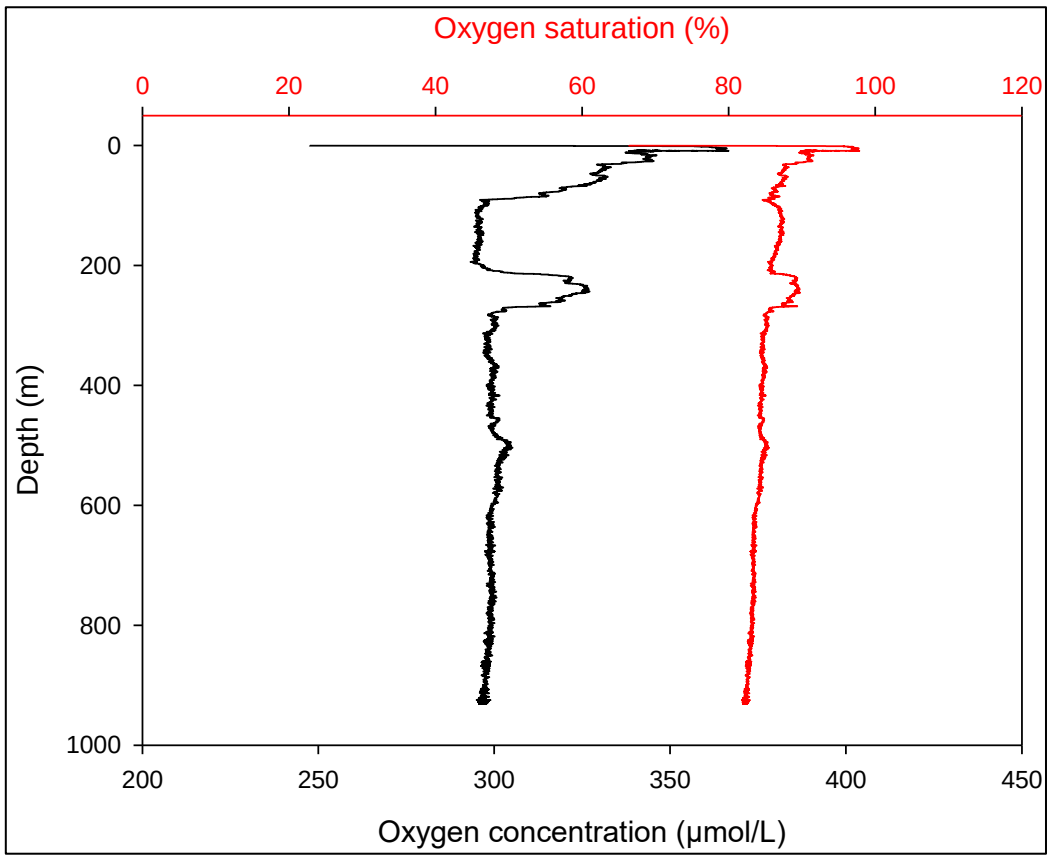


***Figure 8**: Results for oxygen concentration and saturation from the CTD cast to 900 m at station 6.*

The full results of the CTD-casts can be found in the appendix.

# 5 Station Lists

***Table 2:*** *List of stations including time, geographical coordinates and type of samples.*

| Station No. | Date | Time | Latitude | Longitude | Samples | Depths |
|---|---|---|---|---|---|---|
| | 2022 | UTC | | | | |
| 1 | 04.06. | 22:35 | 68°4'3.26"N | 22°18'2.00"W | Plankton Net | 25 m – Surface |
| 2 | 05.06. | 05:10 | 69°2'8.87"N | 20°13'38.74"W | Plankton Net<br>Water samples | 25 m – Surface<br>30 m, 10 m, surface |
| 3 | 05.06. | 19:20 | 70°17.913"N | 21°27.995"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30, 10, 0 m<br>80 m |
| 4 | 06.06. | 11:00 | 70°28.172"N | 22°00.393"W | Plankton Net<br>Water samples<br>SPATT-Bag<br>CTD | 25 m – Surface<br>10 m, surface<br>10 m<br>80 m |
| 5 | 07.06. | 20:50 | 70°26'51.06"N | 20°13'48.60"W | Plankton Net | 25 m – Surface |
| 6 | 08.06. | 13:55 | 72°6'44.65"N | 16°50'56.93"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30 m, 10 m, surface<br>900 m |
| 7 | 09.06. | 18:40 | 74°29'9.57"N | 14°31'42.99"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>10 m, surface<br>180 m |
| 8 | 10.06. | 15:50 | 76°27'12.18"N | 14°44'5.52"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30 m, 10 m, surface<br>180 m |
| 9 | 11.06. | 11:00 | 77°30'25.95"N | 16°5'29.03"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30, 10 m, surface<br>35 m |
| 10 | 12.06. | 11:00 | 77°23'56.29"N | 5°0'29.86"W | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30 m, 10 m, surface<br>800 m |

| | | | | | | |
|---|---|---|---|---|---|---|
| 11 | 13.06. | 00:00 | 79°0'26.53"N | 6°26'38.28"E | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>30 m, 10 m, surface<br>900 m |
| 12 | 13.06. | 10:00 | 79°33'50.76"N | 10°59'40.35"E | Plankton Net<br>Water samples<br>CTD | 25 m – Surface<br>10 m, surface<br>80 m |

***Table 3:*** *List of transects where continuous sampling was performed including times, geographical coordinates and types of samples. CTS=Continuous toxin sampling; CPS=Continuous phytoplankton sampling; SB=SPATT bag*

| Station | Date | Time | Latitude | Longitude | Samples | Remark |
|---|---|---|---|---|---|---|
| | 2022 | UTC | | | | |
| CTS1 | 04.06. | 08:25 | 66°1'52.83"N | 24°16'10.10"W | Continuous toxin sampling | Start |
| CTS1.1 | 04.06. | 12:35 | 66°44'40.71"N | 23°37'10.06"W | | End |
| CTS2 | 04.06. | 17:15 | 67°23'31.85"N | 23°16'52.61"W | Continuous toxin sampling | Start |
| CTS2.1 | 04.06. | 20:25 | 67°52'43.67"N | 22°31'4.40"W | | End |
| CPS1 | 04.06. | 12:55 | 66°47'58.08"N | 23°34'8.42"W | Continuous phytoplankton sampling | Start |
| CPS1.1 | 04.06. | 13:30 | 66°53'2.38"N | 23°30'43.17"W | | End |
| CPS2 | 04.06. | 22:00 | 68°3'14.89"N | 22°16'16.96"W | Continuous phytoplankton sampling | Start |
| CPS2.1 | 04.06. | 22:30 | 68°3'56.45"N | 22°17'47.78"W | | End |
| SB1 | 04.06. | 08:20 | 66°1'22.09"N | 24°16'37.18"W | Continuous dissolved toxin sampling | Start |
| SB1.1 | 05.06. | 08:20 | 69°8'38.52"N | 20°9'7.16"W | | End |
| SB2 | 06.06. | 09:35 | 70°28.172"N | 22°00.393"W | dissolved toxin sampling | Start |
| SB2 | 07.06. | 14:00 | Stationary | Stationary | | End |

# 6 Acknowledgement

The scientists wish to express their gratefulness for the preparation, logistic support and performance of the cruise at the company PONANT and the ARICE office. The scientists especially thank Vladislav Sidorenkov-Duprez for helping with all pre-cruise occurring problems and for organizational and logistic help. Furthermore, the scientists thank Daniel Cron and Daphné Burion for their help during the cruise with all technical, organizational and for helping and improvising a lot to get as much scientific work done as possible. The cruise participants also thank captain Patrick Marchesseau for stopping the ship in order to take scientific samples. Finally, the scientists thank the rest of the crew for making the expedition as comfortable as possible.

# 7 Appendix

Excel file containing the full CTD-Data called CTD data_ReHaDiCC_LeCommandant_Charcot_2022