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CRUISE REPORT

Assessment of phycotoxins and their producing species in the Black Sea

R/V TÜBITAK MARMARA, Cruise No. PHYCOB,

11.09.2021 – 17.09.2021, Istanbul (Turkey) – Istanbul (Turkey)

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1 Summary

The cruise started on Saturday, 11th September at 15:00 local time with about two and a half days delay due to bad weather conditions on the Black Sea and the participantion of seven members of the initial PHYCOB consortium. The two TÜBITAK Members Ertuğrul Aslan and Hayati Çalik (on-board nutrient analysis and CTD operation, respectively) complemented the PHYCOB crew. In the next morning the first station was reached and the sampling and sample processing was performed as scheduled. During the entire cruise four stations per day were performed without any hindrance or problems. Deck work consisted of CTD casts including five deep CTD casts to 1000 m depth at stations 1, 4, 12, 15 and 23, two vertical plankton net hauls at all stations and boxcorer sediment samples at the shallower stations 6-11 and 16-19. After completion of all stations in the afternoon of 17th September, R/V TÜBITAK MARMARA returned to Istanbul, where we reached Haydarpaşa Port shortly before midnight of the same day, which was the termination of the cruise.

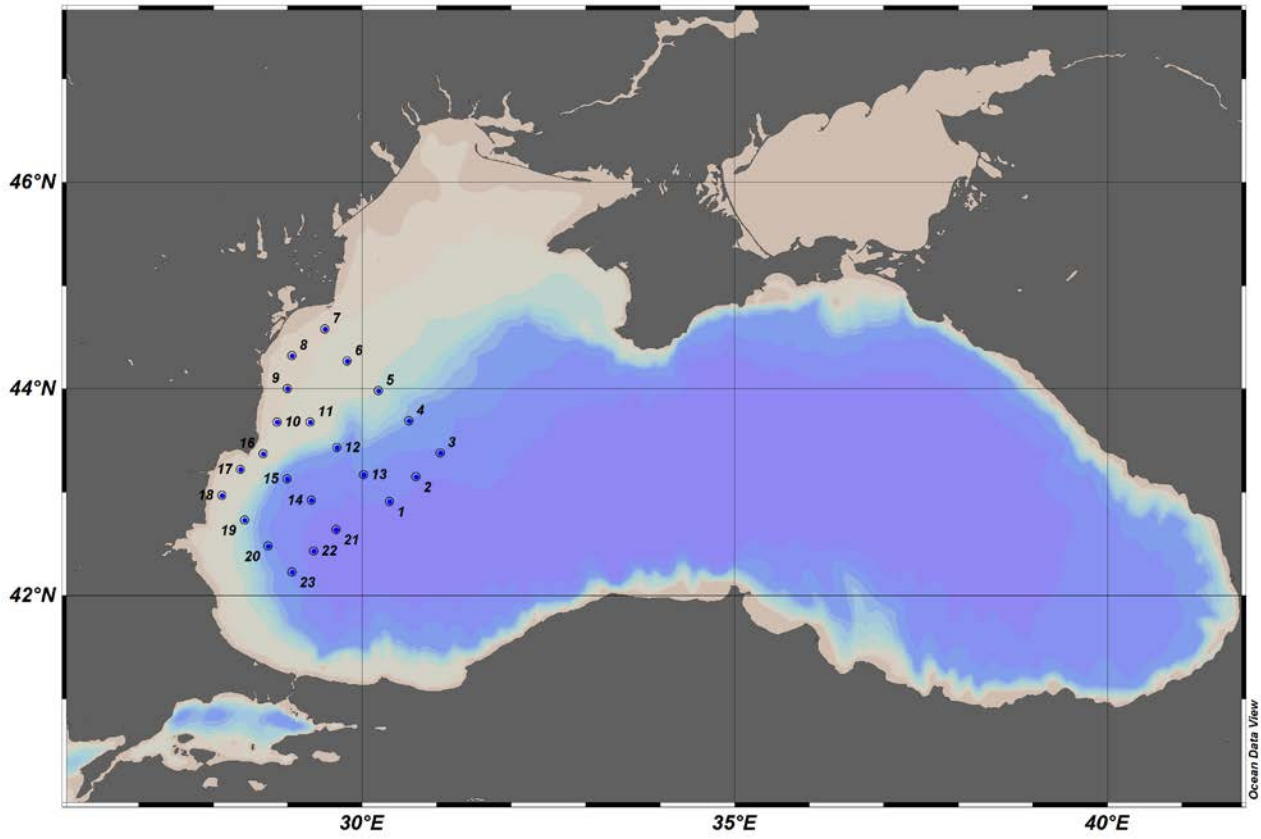

Fig. 1.1 Working area and track chart of R/V TÜBITAK MARMARA Cruise PHYCOB

2 Research Programme/Objectives

The main scientific objectives for the proposed research cruise are: 1) To assess the presence of toxic microalgae in the plankton assemblage of the Western Black Sea. 2) To quantitatively determine the spatial distribution of toxic phytoplankton species and their corresponding toxins in the plankton. 3) To quantitatively describe the spatial distribution of toxic phytoplankton resting stages in surface sediments and determine hotspots of recruitment and bloom initiation. 4) To define correlations among hydrographic and/or meteorological conditions and occurrence of HAB species. 5) To characterize the plankton communities accompanying HAB species together with toxin analysis of size fractionated samples. 6) To identify heterotrophic dinoflagellates, ciliates, or other small zooplankton as possible toxin vectors. 7) To isolate toxic Black Sea microalgal species and

establish monoclonal cultures for characterization of Black Sea strains. 8) To perform an interseasonal comparison of toxigenic plankton species by combining the data sets collected during the R/V Akademik cruise that was performed in May/June 2019 with our data from summer. 9) To compare the data set from the Western Black Sea to the Southern (North-) Western North Sea, Baltic Sea and West Greenland obtained during earlier expeditions for site comparisons.

3 Narrative of the Cruise

In the weeks before the cruise, some participants notified that they were not able to join the cruise for several reasons: 1) Nina Lundholm and Anna Olesen did not get a permit from Copenhagen University to travel to Turkey, due to the covid-19 pandemic. 2) Zlatina Peteva resigned because of personal problems, and 3) Oana Vlas had a surgery and was not able to participate for health reasons. The resulting four free berths could be filled partially with Florian Koch (AWI) covering particulate organic carbon (POC) and nitrogen (PON), chlorophyll-a, and vitamins, Ertuğrul Aslan (TÜBITAK) covering nutrients, dissolved oxygen, and pH, and Hayati Çalik (TÜBITAK) for CTD operation.

Initially the departure of PHYCOB was scheduled for 9th Sep., but due to strongs winds over the Black Sea the captain decided to postpone the departure for two days to 11th Sep. The scientific lead in agreement with TÜBITAK decided not to change the booked flights short term, in order to have time for preparations and last minute adjustments onboard prior to departure. Accordingly, the Bulgarian, German and Romanian team members arrived at Istanbul Airport during 8th September and were transferred to R/V TÜBITAK MARMARA at Haydarpaşa Port by a TÜBITAK shuttle service. After reception and dinner, all participants were tested for covid-19 by PCR and antibody tests. On the same day, the expedition goods sent from AWI arrived at the research vessel.

Fig. 3.1 R/V Tübitak Marmara at Haydarpaşa Port

On 9th Sep. all equipment was unpacked and installed in the ship laboratories. After installation, plankton samples that had previously been taken on 6th Sep. by Fuat Dursun at three stations in the Golden Horn Estuary, Istanbul were inspected by microscopy and extracted for phycotoxin analysis. In the evening the covid-19 test results were received with all participants being tested negative.

The acticvities of the following day started in the morning with a briefing with Captain Tayfun Denizmen for coordination of station order and station work. Thereafter there was free time for all participants for sight-seeing in Istanbul until 17:00 h in the afternoon, when group photos of all participants and representatives were taken

on deck. After dinner onboard there was an informal meeting with the directors of TÜBITAK Marmara Research Center Dr. Selma Ayaz and Dr. Haldun Karan and the cruise participants. During this meeting, some gifts from TÜBITAK and AWI were exchanged followed by an introduction of the PHYCOB project and a presentation of the facilities, projects and ship details by TÜBITAK.

The 11th Sep. started with a safety introduction for all participants given by First Officer Derya Erol. Prior to departure, we were informed by Dr. Özsu that it was not possible to insatal the EARS on-board and departure time was announced for 15:00 h by Captain Denizmen. Directly after departure from dock 3 at Haydarpaşa Port, a solid phase adsorption toxin tracking (SPATT) bag was deployed in a continuous seawater flow of 400 mL min^{-1}. In continuation, R/V TÜBITAK MARMARA sailed through the Bosphorus into the Black Sea and headed for the first station to be reached in the next morning at good weather conditions and calm sea.

Fig. 3.2: continuous sampling of dissolved phycotoxins by SPATT

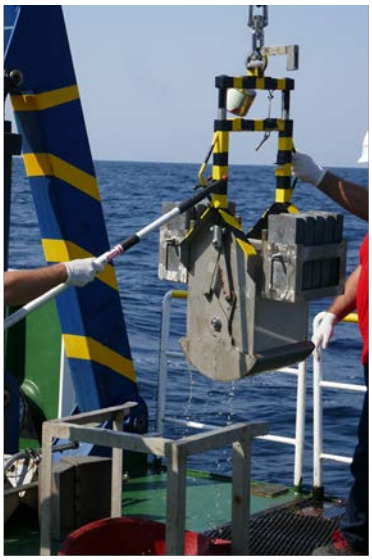

Fig. 3.3 Sediment sampling with box corer

Station work at Station 1 started on 12th Sep. at 7:00 h with a deep CTD cast to 1000 m depth sampling the depths 1000, 500, 250, and 100 m. Subsequently a second cast was performed to 100 m depth and chl-a maximum, thermocline, 10 and 3 m depths were sampled. In addition two vertical net hauls from 30 m depth to surface were performed. The second station started at 11:20 h as a standard station identical to the first one, but without the first CTD cast to depths >100 m. The same scheme applied for the next two stations (3 & 4), which were performed at 14:30 and 17:40 h, respectively. At Station 4 in addition to the standard program, an additional deep CTD cast to maximum depth of 980 m was performed.

On 13th Sep. the shelf was reached with maximum depths shallower than approximately 100 m until station 11. Station work started at 8:00 h at station 5 and followed the same scheme as before with a CTD cast to maximum depth and water sampling at deep chl-a maximum, thermocline and 10 and 3 m depths, followed by two vertical phytoplankton net tows. In addition, at station 6 (11:20 h) and the following stations 7 and 8 (15:00 and 18:30 h, respectively) box corer casts were performed to collect surface sediments for the analysis of immobile resting stages of phytoplankton species. Finally, at 21.00 h the first SPATT bag was exchanged.

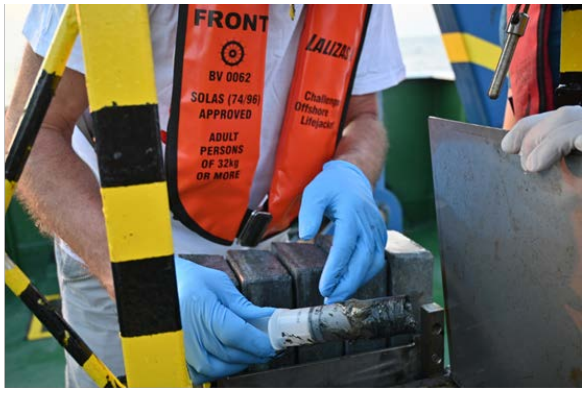

Fig. 3.4 Collection of sediment sample from box corer

On the third day (14th Sep.), the first three stations (9, 10, and 11; 08:00, 10:45, and 13:30 h, respectively) consisted of a CTD cast to bottom, two phytoplankton net tows and sediment sampling with the box corer. At 16:30 h work at station 12 was started and due to large water depth of 1144 m box coring was replaced by a

Fig. 3.5 Collection of plankton samples from net haul

deep CTD cast (1010 m). Deck work was finished at 17:50 h, whereas lab work (filtration, DNA extraction, sample fixations, onboard microscopy, toxin extraction and nutrients analysis continued until 21.00 h.

On 15th Sep., the first two stations (13, 14) were standard stations with CTD casts to 100 m and plankton net. Station 15 at 13:45 h included an additional deep CTD cast to 1000 m depth. The final station of the day 16 was shallow (72 m) and was started at 17:45 h. Standard operations were performed and deck work was concluded with a box corer cast for sediment.

The following day (16th Sep.) stations 17 to 20 were sampled by the standard protocol including box coring were deployed at the first three shallower stations. At station 20 with 1363 m depth only CTD to 100 m and plankton nets were operated.

On the final day (17th Sep.) only three stations (21-23) were left. The first two stations were standard stations consisting of a CTD cast to 100 m depth and two phytoplankton net hauls, whereas at the last station an additional deep CTD cast to 1000 m depth was performed. The deck work of the last station was finished at 14:30 h and thereafter R/V TÜBITAK MARMARA headed back to Istanbul, where we arrived at Haydarpaşa Port the same day shortly before midnight.

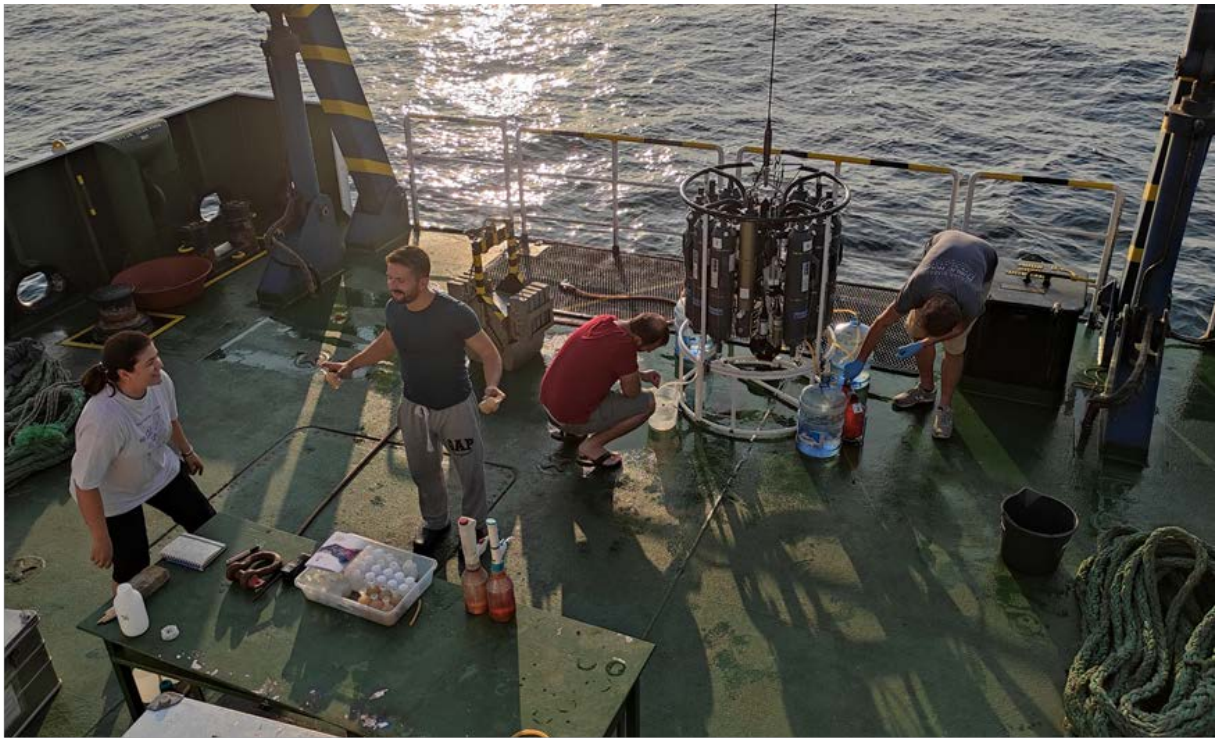

Fig. 3.6 Collection of water samples from rosette sampler

Lab work after each station consisted of processing the bottle water and net tows, whereas sediment samples were only transferred into centrifugation tubes and stored for posterior resting stage analysis and germination at IOW in the fridge at 4 °C. Bottle water was taken for nutrient analysis, dissolved oxygen, pH, dissolved organic matter (DOM), particulate organic carbon (POC) and nitrogen (PON), cobalamins (vitamin B12), plankton species determination and quantification, DNA, live sample inspection by microscopy, cell isolation and phycotoxin determination of smaller dinoflagellates. In contrast, phytoplankton net samples from 30 m depth to surface

were used for species determination and quantification, DNA, live sample inspection by microscopy, cell isolation and phycotoxin determination of bigger dinoflagellates.

Nutrients, dissolved oxygen, pH (Ertuğrul Aslan)

Nitrite and nitrate as nitrogen (NO_3+NO_2), ammonium as nitrogen (NH_4^+), ortho-phosphate as phosphorus (o-PO_4) and silica (SiO_2) analyzes were measured by a QuAAtro39 Continuous Segmented Flow Analyzer consisting of an autosampler, a peristaltic pump, a chemistry manifold, a detector and data acquisition software. This protocol is in accordance with standard methods for the examination of water and wastewater. Dissolved oxygen (DO) was measured by using the Winkler method, in which a divalent manganese solution and strong alkali are added sequentially to the samples. Thus, dissolved oxygen rapidly oxidizes an equivalent amount of divalent manganese to basic hydroxides of higher valency states. With the presence of iodide ions in acidic conditions, the oxidized manganese reverts to its divalent state, and meanwhile, the iodine equivalent to the dissolved oxygen in the sample is released. This released iodine is titrated with a standardized thiosulfate solution and the dissolved oxygen value in the sample is calculated by potentiometric measurement using Metrohm 905 Titrando automatic titrator system. Dissolved oxygen samples are taken and analyzed in duplicate. The relative percent difference (RPD) of duplicates did not exceed 4.3% (Average is 0.62%). All results were also compared with the CTD dissolved oxygen data.

pH measurements of sea waters were measured in lab by Mettler Toledo SevenMulti pH meter. The pH meter was calibrated every two days with the calibration standards at the values of 7.00 and 9.21. During the measurements, the pH values were recorded together with the current sample temperature values.

DOM (Kristof Möller): water samples from the CTD-rosette were taken at various depth (usually 3 m, 10 m, thermocline/Chl-max and 2nd Chl-max. Additionally, at station 1, 4, 10, 15, and 23 deep CTD profiles were taken with additional samples at 100 m, 250 m, 500 m and 1000 m) depth. These samples and filtered through GFF-syringe filters to remove the particulate fraction (<0.7 μm). All used material was thoroughly pre-rinsed 3 times and the filtrate was stored at -20 °C until analysis at AWI in Bremerhaven. The samples will be analyzed for dissolved organic matter, consisting of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON).

Chl-a, POC, PON, B12, flow cytometry (Florian Koch): Samples for size fractionated chlorophyll a and particulate organic carbon and nitrogen (POC/PON) were collected from the surface, pycnocline and the deep chlorophyll a maximum. For POC/PON water was filtered onto pre-combusted glass fiber filters, and stored at -20 °C until subsequent analysis on an elemental analyzer in the home lab. Chl-a samples were collected on GFF and on polycarbonate filters with a 2 μm pore size. At each depth 5 mL of seawater was collected and preserved with buffered formalin (1% final concentration) for flowcytometric analysis at AWI. In addition to the macronutrients, we collected the first vitamin samples in the Black Sea. Samples were filtered through a 0.7/0.2 μm filter cartridge. After particles were removed, the pH was adjusted to 6.2-6.6 with 1.2 N HCl and the water was slowly (<1.5 mL min^{-1}) pulled through C18 containing columns using a microperistaltic pump for preconcentration prior to analysis.. After all of the sample was processed in this way, the columns were rinsed with 10 mL of deinonized water, removing any salts. At AWI vitamins will be eluted from the resin with a small volume of solvent resulting in a 15-20,000 fold concentration factor. Vitamins will be analyzed at AWI by UHPLC-MS/MS.

Species quantification and determination and live microscopy (Laura Biocenco, Urban Tillmann): At each station, lugol fixed subsamples of rosette water samples were taken and stored for later quantitative cell counts. At selected stations, samples were fixed with formaldehyde for later taxonomic studies using electron microscopy. Furthermore, 1 L sea water samples from surface (3m), thermocline and chlorophyll maximum layers were taken

for the investigation of phytoplankton communities (taxonomic structure, numerical abundance, and biomass) from surface (3m), thermocline and chlorophyll maximum layers. The samples were fixed with formaldehyde 37%. Species identification and cell counting of these samples will be done post-cruise under light microscope (Olympus) in the NIMRD laboratory. In addition, on-board analyses by microscopy of live net tow and rosette water samples was performed to characterise the plankton communities, and to document the dinophyte species composition by photo- and video recording. In order to identify and isolate potentially toxin-producing species of dinophytes, rosette water samples were screened by 200 µm gauze, concentrated on 5 µm polycarbonate filters and inspected under the microscope. In addition, various pre-cultures were started by isolating single cells of species of interest. After transportation back to AWI Germany, these samples will be inspected for the growth of algal species of interest, especially for cultures of toxigenic species of *Alexandrium, Lingulodinium, Protoceratium*, or *Azadinium*.

DNA (Nina Dzhembekova): An 1 L aliquot of pooled water from surface water, 10 m depth and the thermocline was filtered through 1 µm pore-size polycarbonate filters. In addition, an aliquot (50 mL) of the net tow concentrate was fixed with formaldehyde for analysis by light microscopy and another aliquot (150 mL) was filtered for DNA. DNA from the filters was extracted with Chelex buffer (5%) and stored for posterior analysis in the home lab.

Phycotoxins (Fuat Dursun, Bernd Krock): An 8 L aliquot of pooled water from surface water, 10 m depth and the thermocline was filtered through 5 µm polycarbonate filters for azaspiracid and karlotoxin determination. The filters were extracted by repeated rinsing with methanol, the methanolic extracts were transferred to centrifugation filters and filtered at a cutt-off of 0.45 µm by centrifugation. Filtrates were transferred into analytical glass vials for posterior LC-MS/MS determination at AWI. The remaining aliquot of the net tow concentrate was filtered through a filter array of 200, 50, and 20 µm meshes. The residue of each mesh was rinsed with filtrated seawater into a centrifugation tube, homogenized by shaking, and divided into two aliquots. Both aliquots of each size fraction were centrifuged at maximum speed for 15 min. After centrifugation, supernatants were decanted and the remaining cell pellets resuspended with a small volume of filtrated seawater and transferred to cryovials containing ceramic beads. The samples were centrifuged again and supernatants carefully removed with a pipette. To one of the two size fraction aliquots 500 µL methanol was added and the same volume of dilute acetic acid to the other. All samples were homogenized with FastPrep instrument by reciprocal shaking at maximum speed and homogenated were centrifuged. After centrifugation, the toxin containing extracts were removed and transferred to centrifugation filters. The extracts were filtered by centrifugation and the filtered extracts were transferred into analytical glass vials for LC-MS/MS and LC-FLD determination, respectively, in the home lab.

Table 3.1: **mobilisation/demobilisation**

Station	**Position**		**Time**	**Operations**
	Latitude (N)	**Longitude (E)**		
Istanbul	40°59.97’	29°0.97’	8.9.2021	Arrival of cruise participants at Istanbul airport and transfer to the ship

Istanbul	40°59.97’	29°0.97’	9.9. - 11.9.2021	Sett up of ship laboratory, Training, Safety instructions
Istanbul	40°59.97’	29°0.97’	11.9.; 15:00 h	Start of the cruise from Haydarpaşa Port
Stations	See Table 7.1	See Table 7.1	12.9.; 7:20 h - 17.9.; 14:30 h	Deep CTD cast, strd CTD cast, net tows, box corer
Istanbul	40°59.97’	29°0.97’	17.9.; 23:45 h	Arrival at Haydarpaşa Port, end of the cruise
Istanbul	40°59.97’	29°0.97’	18.9.	Packing of equipment, organization of shipment of samples to the participants’ labs including frozen samples with cooling chain
Istanbul	40°59.97’	29°0.97’	19.9.; 3:15 h	Transfer of cruise participants from Haydarpaşa Port to Instanbul Airport

4 Preliminary Results

4.1 CTD data (Sabri Mutlu)

CTD data are detailed in Appendix 1 and an example of station 1 is shown in Fig. 4.1

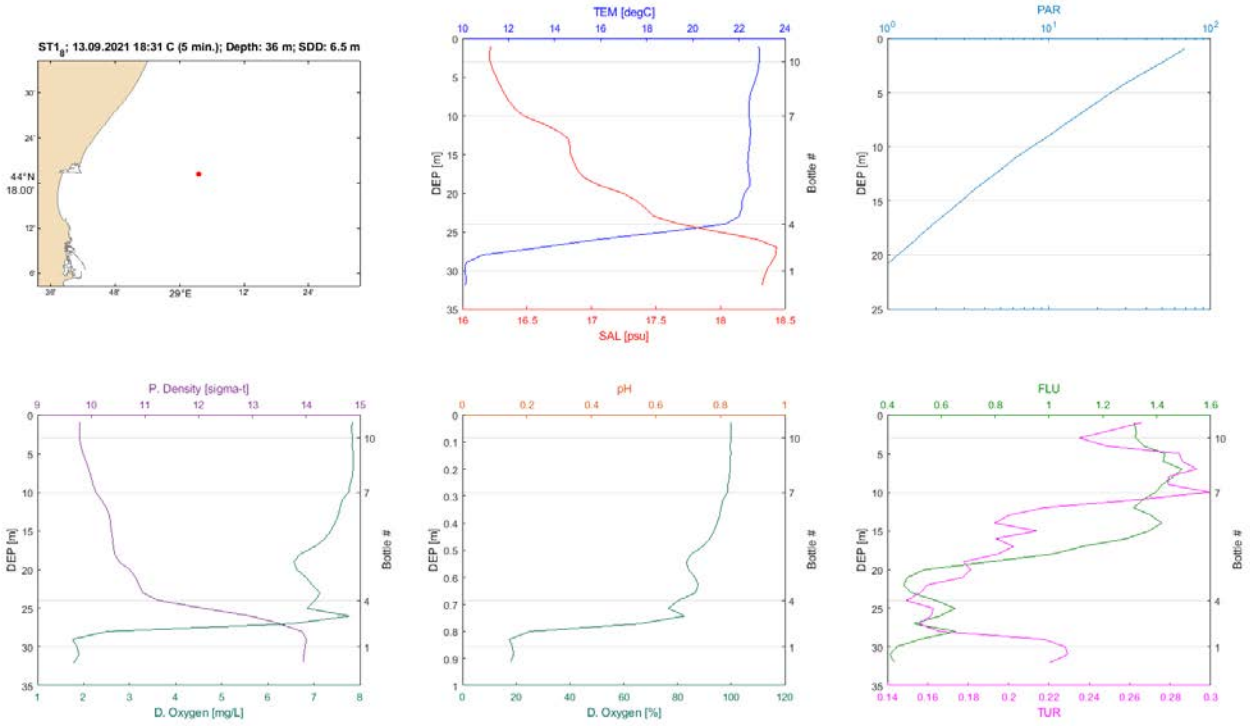

Fig. 4.1 Temperature, salinity, photosynthetic active radiation (PAR), density, oxygen, chl-a fluorescence, and turbidity plot of station 1

4.2 **Nutrient measurements, dissolved oxygen, and pH** (Ertuğrul Aslan)

The following nutrient parameters were measured on-board:

4.2.1 Ortho-phosphate

Tab. 4.2.1 Phosphate concentrations at sampling stations 1-23

	ortho-Phosphate [µM]									
	Depth [m]									
Station #	3	10	TC		CM		100*	250	500	1000
1	<0,02	<0,02	24	0,02	40	0,17	4,74	4,68	5,61	6,92
2	<0,02	<0,02	22	<0,02	38	0,14	nd	nd	nd	nd
3	<0,02	<0,02	25	<0,02	36	0,12	nd	nd	nd	nd
4	<0,02	<0,02	22	<0,02	40	0,08	1,08	4,39	5,58	6,80
5	<0,02	<0,02	32	0,04	40	0,10	nd	nd	nd	nd
6	<0,02	<0,02	25	<0,02	38	0,05	nd	nd	nd	nd
7	<0,02	<0,02	26	0,04	40	0,30	nd	nd	nd	nd
8	<0,02	<0,02	22	0,08	40	0,41	nd	nd	nd	nd
9	<0,02	<0,02	21	0,04	40	0,28	nd	nd	nd	nd
10	<0,02	<0,02	22	<0,02	30	0,08	nd	nd	nd	nd
11	<0,02	<0,02	22	<0,02	40	0,05	nd	nd	nd	nd
12	<0,02	<0,02	22	<0,02	35	0,09	5,48	5,32	6,45	8,21
13	<0,02	<0,02	22	<0,02	40	0,12	nd	nd	nd	nd
14	<0,02	<0,02	25	<0,02	40	0,05	nd	nd	nd	nd
15	<0,02	<0,02	20	<0,02	34	0,05	1,35	4,97	6,46	7,66
16	<0,02	<0,02	20	0,04	36	0,16	nd	nd	nd	nd
17	<0,02	<0,02	18	0,04	27	0,13	nd	nd	nd	nd
18	<0,02	<0,02	20	<0,02	30	0,29	nd	nd	nd	nd
19	<0,02	<0,02	25	<0,02	35	<0,02	nd	nd	nd	nd
20	<0,02	<0,02	30	<0,02	54	0,15	nd	nd	nd	nd
21	<0,02	<0,02	24	<0,02	40	0,07	nd	nd	nd	nd
22	<0,02	<0,02	23	<0,02	42	0,06	nd	nd	nd	nd
23	<0,02	<0,02	27	<0,02	40	0,05	0,92	5,42	6,61	6,96

4.2.2 Silica

Tab. 4.2.2 Silicte concentrations at sampling stations 1-23

	Silica [µM]									
	Depth [m]									
Station #	3	10	TC		CM		100*	250	500	1000
1	<0,06	<0,06	24	1,67	40	4,63	60,76	128,07	195,43	276,07
2	0,25	<0,06	22	1,18	38	4,36	nd	nd	nd	nd
3	<0,06	<0,06	25	1,60	36	4,11	nd	nd	nd	nd
4	<0,06	<0,06	22	1,30	40	3,30	56,06	117,92	182,48	277,34
5	<0,06	0,46	32	2,18	40	3,16	nd	nd	nd	nd
6	<0,06	0,08	25	2,51	38	4,60	nd	nd	nd	nd
7	2,68	1,23	26	12,92	40	19,27	nd	nd	nd	nd
8	1,91	4,60	22	11,48	40	38,80	nd	nd	nd	nd
9	0,45	1,48	21	19,09	40	24,11	nd	nd	nd	nd
10	<0,06	<0,06	22	11,69	30	9,24	nd	nd	nd	nd
11	<0,06	<0,06	22	0,57	40	2,37	nd	nd	nd	nd
12	<0,06	<0,06	22	0,93	35	3,86	63,13	103,91	183,15	275,62
13	<0,06	<0,06	22	1,58	40	4,20	nd	nd	nd	nd
14	<0,06	<0,06	25	0,90	40	2,92	nd	nd	nd	nd
15	<0,06	<0,06	20	0,40	34	2,44	43,65	88,03	195,11	279,31
16	1,15	1,13	20	4,35	36	16,78	nd	nd	nd	nd
17	0,68	0,88	18	2,72	27	14,11	nd	nd	nd	nd
18	0,80	0,80	20	0,25	30	16,61	nd	nd	nd	nd
19	<0,06	<0,06	25	0,77	35	1,80	nd	nd	nd	nd
20	<0,06	<0,06	30	1,98	54	4,89	nd	nd	nd	nd
21	<0,06	<0,06	24	0,81	40	3,59	nd	nd	nd	nd
22	<0,06	<0,06	23	0,74	42	3,68	nd	nd	nd	nd
23	<0,06	<0,06	27	0,86	40	3,01	54,11	131,66	208,21	295,21

4.2.3 Nitrate and nitrite

Tab. 4.2.3 Total nitrate and nitrite concentrations at sampling stations 1-23

	Nitrate + Nitrite [µM]									
	Depth [m]									
Station #	3	10	TC		CM		100*	250	500	1000
1	<0,05	<0,05	24	<0,05	40	0,299	<0,05	<0,05	<0,05	<0,05
2	<0,05	<0,05	22	0,155	38	0,465	nd	nd	nd	nd
3	<0,05	<0,05	25	0,082	36	0,207	nd	nd	nd	nd
4	<0,05	<0,05	22	<0,05	40	0,142	0,062	<0,05	<0,05	<0,05
5	<0,05	<0,05	32	<0,05	40	0,29	nd	nd	nd	nd
6	<0,05	<0,05	25	<0,05	38	0,65	nd	nd	nd	nd
7	0,56	<0,05	26	2,76	40	4,68	nd	nd	nd	nd
8	<0,05	<0,05	22	2,83	40	9,69	nd	nd	nd	nd
9	<0,05	0,12	21	4,26	40	8,19	nd	nd	nd	nd
10	<0,05	<0,05	22	1,82	30	2,30	nd	nd	nd	nd
11	<0,05	<0,05	22	<0,05	40	0,45	nd	nd	nd	nd
12	<0,05	<0,05	22	<0,05	35	0,07	<0,05	<0,05	<0,05	<0,05
13	<0,05	<0,05	22	<0,05	40	0,39	nd	nd	nd	nd
14	<0,05	<0,05	25	<0,05	40	0,17	nd	nd	nd	nd
15	<0,05	<0,05	20	<0,05	34	0,57	4,34	<0,05	<0,05	<0,05
16	<0,05	<0,05	20	0,37	36	5,40	nd	nd	nd	nd
17	<0,05	<0,05	18	0,37	27	0,48	nd	nd	nd	nd
18	<0,05	<0,05	20	0,10	30	1,47	nd	nd	nd	nd
19	<0,05	0,28	25	0,07	35	<0,05	nd	nd	nd	nd
20	0,05	0,21	30	<0,05	54	<0,05	nd	nd	nd	nd
21	<0,05	<0,05	24	<0,05	40	0,35	nd	nd	nd	nd
22	<0,05	<0,05	23	<0,05	42	0,13	nd	nd	nd	nd
23	<0,05	<0,05	27	<0,05	40	0,25	2,06	0,06	0,09	<0,05

4.2.4 Dissolved oxygen

Tab. 4.2.4 Dissolved oxygen concentrations at sampling stations 1-23

	dissolved Oxygen									
	Depth [m]									
Station #	3	10	TC		CM		100*	250	500	1000
1	7,26	7,19	24	9,18	40	8,26	0,16	0,00	0,00	0,00
2	7,24	7,22	22	8,98	38	8,31	nd	nd	nd	nd
3	7,56	7,49	25	9,28	36	8,73	nd	nd	nd	nd
4	7,69	7,70	22	9,42	40	8,90	0,10	0,00	0,00	0,00
5	7,79	8,52	32	8,83	40	8,50	nd	nd	nd	nd
6	7,66	7,62	25	9,42	38	8,41	nd	nd	nd	nd
7	8,23	7,55	26	6,50	40	6,39	nd	nd	nd	nd
8	7,99	7,52	22	6,00	40	1,76	nd	nd	nd	nd
9	7,67	7,26	21	4,87	40	3,93	nd	nd	nd	nd
10	7,67	7,63	22	6,91	30	7,66	nd	nd	nd	nd
11	7,76	7,78	22	8,98	40	8,62	nd	nd	nd	nd
12	7,69	7,81	22	9,24	35	8,70	0,11	0,00	0,00	0,00
13	7,56	7,50	22	9,20	40	8,58	nd	nd	nd	nd
14	7,72	7,76	25	9,32	40	8,94	nd	nd	nd	nd
15	7,72	7,70	20	8,65	34	8,57	0,45	0,00	0,00	0,00
16	7,73	7,56	20	6,44	36	5,38	nd	nd	nd	nd
17	7,45	7,38	18	6,61	27	5,81	nd	nd	nd	nd
18	7,65	7,31	20	7,39	30	5,38	nd	nd	nd	nd
19	7,61	7,46	25	7,78	35	8,53	nd	nd	nd	nd
20	7,69	7,62	30	8,47	54	8,17	nd	nd	nd	nd
21	7,53	7,50	24	9,15	40	8,59	nd	nd	nd	nd
22	7,67	7,63	23	8,57	42	9,00	nd	nd	nd	nd
23	7,53	7,52	27	9,04	40	8,77	0,21	0,00	0,00	0,00

4.2.5 pH

Tab. 4.2.5 pH values at sampling stations 1-23

	pH									
	Depth [m]									
Station #	3	10	TC		CM		100*	250	500	1000
1	8,29	8,26	24	8,12	40	8,04	7,70	7,69	7,66	7,59
2	8,32	8,34	22	8,28	38	8,22	nd	nd	nd	nd
3	8,33	8,33	25	8,24	36	8,23	nd	nd	nd	nd
4	8,33	8,30	22	8,22	40	8,21	7,69	7,72	7,67	7,62
5	8,32	8,23	32	8,17	40	8,16	nd	nd	nd	nd
6	8,34	8,32	25	8,18	38	8,41	nd	nd	nd	nd
7	8,40	8,32	26	8,08	40	8,01	nd	nd	nd	nd
8	8,39	8,35	22	8,08	40	7,72	nd	nd	nd	nd
9	8,39	8,30	21	7,98	40	7,92	nd	nd	nd	nd
10	8,32	8,30	22	8,07	30	8,09	nd	nd	nd	nd
11	8,30	8,27	22	8,20	40	8,16	nd	nd	nd	nd
12	8,27	8,22	22	8,14	35	8,01	7,69	7,67	7,62	7,55
13	8,25	8,23	22	8,06	40	7,98	nd	nd	nd	nd
14	8,30	8,28	25	8,17	40	8,11	nd	nd	nd	nd
15	8,16	8,23	20	8,14	34	8,07	7,67	7,70	7,64	7,57
16	8,32	8,29	20	8,18	36	7,87	nd	nd	nd	nd
17	8,38	8,38	18	8,35	27	8,08	nd	nd	nd	nd
18	8,40	8,38	20	8,35	30	8,01	nd	nd	nd	nd
19	8,35	8,33	25	8,24	35	8,22	nd	nd	nd	nd
20	8,33	8,29	30	8,15	54	8,06	nd	nd	nd	nd
21	8,32	8,30	24	8,20	40	7,97	nd	nd	nd	nd
22	8,30	8,28	23	8,19	42	8,09	nd	nd	nd	nd
23	8,30	8,29	27	8,15	40	8,10	7,68	7,71	7,66	7,62

5 Data and Sample Storage / Availability

A digitalized station protocol already has been shared with all cruise and remote participants. In addition, sediment samples that were taken for remote participant Anke Kremp, IOW were sent to her for dinoflagellate resting stage (cyst) analysis. Furthermore some isolated clonal strains by Urban Tillmann on board of the potenitially toxic pennate diatom genus *Pseudo-nitzschia* were sent to the remote participant Nina Lundholm, UCPH and co-workers, who could not participate in the cruise due to travel restrictions. With these isolates, the Danish colleagues will perform genetic and taxonomic characterization to complement the determination of the toxin profiles, which are currently performed at AWI.

Within the first quarter of 2022, when all cruise samples will have been processed and evaluated, there will be organized a virtual post-cruise meeting with all participants including the remote ones, for mutual data presentation, discussion of results and the development of a joint publication strategy in scientific journals

Data availability and storage will be followed as detailed in the data management plan.

6 Participants

Table 1: Cruise participants; *participants funded by EUROFLEETS+

No.	Name	Early career (Y/N)	Gender	Affiliation	On-board tasks
1	Bernd Krock*	N	M	AWI	Coordination
2	Urban Tillmann*	N	M	AWI	Phytoplankton, Isolation
3	Kristof Möller*	Y	M	AWI	DOM, Vitamins
4	Florian Koch*	Y	M	AWI	POC/PON, Flow cytometry
5	Laura Boicenco*	N	F	NIMRD	Phytoplankon, species determination
6	Nina Dzhembekova*	Y	F	IO-BAS	DNA analysis
7	Fuat Dursun*	Y	M	UIST	Phycotoxins
8	Ertuğrul Aslan	Y	M	TÜB	Nutrients, dissolved oxygen, pH
9	Hayati Çalik	N	M	TÜB	CTD operation

AWI	Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
NIMRD Romania	National Institute for Marine Research and Development “Grigore Antipa”, Constanţa,
IO-BAS	Institute of Oceanology – Bulgarian Academy of Science, Varna, Bulgaria
UIST	Istanbul University, Istanbul, Turkey
TÜB	TÜBITAK Marmara Research Center, Gebze, Turkey

Table 2: Remote participants

No.	Name	Early career (Y/N)	Gender	Affiliation	Post-cruise lab work
1	Nina Lundholm	N	F	UCPH	Establishing diatom cultures
2	Anna Olesen	Y	F	UCPH	Microscopy of diatoms
3	Anke Kremp	N	F	IOW	Sediment sample analysis Anke Kremp
4	Snejana Moncheva	N	F	IO-BAS	Supervision
5	Zlatina Peteva	Y	F	MU-VAR	Data analysis
6	Sabri Mutlu	Y	M	TÜB	CTD data analysis

7	Oana Vlas	Y	F	NIMRD	Phytoplankon, species determination

UCPH Copenhagen University, Copenhagen, Denmark

IOW Institute for Baltic Sea Research, Warnemünde, Germany

IO-BAS Institute of Oceanology – Bularian Academy of Science, Varna, Bulgaria

MU-VAR Medical University, Varna, Bulgaria

TÜB TÜBITAK Marmara Research Center, Gebze, Turkey

NIMRD Romania National Institute for Marine Research and Development "Grigore Antipa", Constanţa,

7 Station List

Tab. 7.1 List of stations, times, geographic coordinates, water depths, and employed gear

Station No.	Date	Time	Latitude	Longitude	Water Depth	Gear	Remarks/Recovery
	2021	[UTC]	[°N]	[°E]	[m]		
1	12.9.	04:20	42°54.80	30°22.31	1946	ROS/CTD Plankton net	1000-500-250-100-40-24-10-3 m 30 m - surface
2	12.9.	08:20	43°09.16	30°43.09	1935	ROS/CTD Plankton net	38-22-10-3 m 30 m - surface
3	12.9.	11:30	43°22.99	31°02.86	1398	ROS/CTD Plankton net	36-25-10-3 m 30 m - surface
4	12.9.	14:35	43°41.39	30°37.89	980	ROS/CTD Plankton net	1000-500-250-100-40-22-10-3 m 30 m - surface
5	13.9.	05:00	43°09.16	30°13.32	102	ROS/CTD Plankton net	40-32-10-3 m 30 m - surface
6	13.9.	08:20	44°16.15	29°48.27	61	ROS/CTD Plankton net Box Corer	38-25-10-3 m 30 m - surface
7	13.9.	12:00	44°34.93	29°48.27	44	ROS/CTD Plankton net Box Corer	40-26-10-3 m 30 m - surface
8	13.9.	15:30	44°19.22	29°03.22	35	ROS/CTD Plankton net	40-22-10-3 m 30 m - surface

						Box Corer	
9	14.9.	5:00	43°59.99	28°59.83	48	ROS/CTD Plankton net Box Corer	40-21-10-3 m 30 m - surface
10	14.9.	7:45	43°40.76	28°51.30	55	ROS/CTD Plankton net Box Corer	30-22-10-3 m 30 m - surface
11	14.9.	10:30	43°41.09	29°18.22	67	ROS/CTD Plankton net Box Corer	40-22-10-3 m 30 m - surface
12	14.9.	13:30	43°25.66	29°39.71	1144	ROS/CTD Plankton net	1000-500-250-130-35-22-10-3 m 30 m - surface
13	15.9.	4:00	43°10.14	30°01.25	1626	ROS/CTD Plankton net	40-22-10-3 m 30 m - surface
14	15.9.	8:00	42°55.33	29°19.09	2030	ROS/CTD Plankton net	40-25-10-3 m 30 m - surface
15	15.9.	10:45	43°07.91	28°59.56	1350	ROS/CTD Plankton net	1000-500-250-100-34-20-10-3 m 30 m - surface
16	15.9.	14:45	43°22.47	28°40.22	72	ROS/CTD Plankton net Box Corer	36-20-10-3 m 30 m - surface
17	16.9.	5:00	43°13.00	28°22.22	41	ROS/CTD Plankton net Box Corer	27-18-10-3 m 30 m - surface
18	16.9.	7:50	42°58.15	28°07.49	33	ROS/CTD Plankton net Box Corer	30-20-10-3 m 30 m - surface
19	16.9.	10:45	42°43,90	28°24.96	235	ROS/CTD Plankton net Box Corer	35-25-10-3 m 30 m - surface
20	16.9.	14:40	42°28.92	28°44.51	1363	ROS/CTD Plankton net	54-30-10-3 m 30 m - surface

21	17.9.	8:00	42°38.16	29°38.92	2154	ROS/CTD Plankton net	40-24-10-3 m 30 m - surface
22	17.9.	10:30	42°26.08	29°21.12	2116	ROS/CTD Plankton net	42-23-10-3 m 30 m - surface
23	17.9.	13:00	42°14.01	29°03.68	1766	ROS/CTD Plankton net	1000-500-250-100-40-27-10-3 m 30 m - surface

8 Acknowledgements

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9 Appendix A

Separate Excel file containing the preliminary CTD data of stations 1-23 named: PHYCOB2021-09_CTD_DATA