CRUISE REPORT

RV Simon Stevin Cruise 19-330 (6/05 – 10/05/2019)

Status: FINAL
Datum: 13/5/2019
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CONTENT

1. HISTORY OF DOCUMENT

Version	Name	Date	Info
V1	Jonas Mortelmans	13 May 2019	Draft
V2	Jonas Mortelmans	1 June 2019	Final

2. GENERAL FORM RV SIMON STEVIN

Cruise number: 19-330 (http://www.vliz.be/vmdcdata/midas/report.php?cruise=1943)

Datum/Time: TD 6/05/2019 12:00
TA 10/05/2019 13:00

Chief scientist: Jonas Mortelmans

Participating institutes: Flanders Marine Institute *(VLIZ)*
Centre National de la recherche scientifique, Laboratoire d'océanologie et de Géosciences (CNRS-LOG)
Centre National de la recherche scientifique, Laboratoire d'océanologie et de Géosciences (CNRS-LOG ULCO)
Université Libre de Bruxelles (ULB)

Geographical regions: Thames estuary (http://marineregions.org/mrgid/3241)
Southern bight of the north sea (http://marineregions.org/mrgid/2399)
Eastern English Channel (http://marineregions.org/mrgid/2389)

DIPCLEAR: Dutch waters (approved 16/04/2019)
English waters (approved 15/04/2019)

3. LIST OF PARTICIPANTS

Name	Institute	Email	6/05 ->10/05/2019
Jonas Mortelmans	VLIZ	jonas.mortelmans@vliz.be	Yes
Anouk Ollevier	VLIZ	anouk.ollevier@vliz.be	Yes
Roeland Develter	VLIZ	Roeland.develter@vliz.be	Yes
Reinhoud de Blok	VLIZ	Reinhoud.deblok@vliz.be	Yes
Michèle Pernak	CNRS-LOG	michele.pernak@gmail.com	Yes
Mark van Dijk	CNRS-LOG	markvandijk81@gmail.com	Yes
Eléonore Delecroix	CNRS-LOG ULCO	eleonore.delecroix@etu.univ-littoral.fr	Yes
Fernando Gómez	CNRS-LOG	fernando.gomez@fitoplancton.com	Yes
Jon Lapeyra	ULB	jlapeyra@ulb.ac.be	Yes
Nick Dillen	VLIZ	nick.dillen@vliz.be	Yes
		Total number of participants:	10

Assignment of the cabins by the Chief-Scientist at the start of the campaign.

4. SCIENTIFIC OBJECTIVES

4.1. VLIZ

Zooplankton 4D dynamics: zooplankton imaging by VPR

The Video Plankton Recorder (VPR) will be deployed every night (between 9pm and 6am). Areas of interest are chosen based on zooplankton and turbidity measurements obtained during previous Jerico Next campaigns. Two trajectories are located in the English and French Channel; one in the central part of the Southern Bight, and one north of the Scheldt estuary.

The four trajectories are exactly 45 minutes each, enabling us to tow the VPR back and forth at 12 different depths during the night, resulting in a 4D zooplankton distribution over these trajectories. The first two trajectories in the English and French Channel will mainly try to visualise the gradient between Copepod community structure (mainly Harpacticoida in front of the English coast, and Calanoida in front of the French coast). The third trajectory in the central part of the Southern Bight is dealing with very clear and deep waters. The fourth trajectory just north of the Scheldt estuary will deal with another community structure, existing of mainly *Noctiluca*.

The four trajectories will yield massive amount of in-situ zooplankton distribution data, but also in situ CTD data. The zooplankton data will be compared with the traditional WP2 net sampling and as such, the capacities to use the VPR in the southern bight and Channel water will be fully explored. For optimal comparison, WP2 nets are taken at the start and end of each trajectory. The CTD data from the VPR will be compared to CTD data from the stations (by profiling a carousel).

Ultimately, the data will be used to assess the capabilities of the VPR to obtain 4D community structures. An assessment will be made whether the VPR is capable to record specific taxa (e.g. certain species of gelatinous plankton) that are otherwise lost in traditional net samplings. At this point it is not known whether specific taxa are missing by VPR sampling compared to traditional net sampling, and if obtained density estimations by VPR in relation to density estimations by net sampling. These data will further increase experience and use of the VPR in southern Bight and Channel waters.

Traditional WP2 zooplankton net sampling

WP2-net zooplankton samples are collected at each station, stored and preserved for a- posteriori processing with the ZooScan, as described in Mortelmans *et al.* (2019). These zooplankton measurements will allow us to fully sample and comprehend the base of the food chain on a wide regional scale. Sampling during night, at beginning and end of each VPR track are then used for a best comparison between discrete sampling and in-vivo sampling by VPR.

During previous campaigns, certain stations displayed relatively high abundances of *Noctiluca.* This campaign further explores the temporal occurrences of *Noctiluca* in the southern bight, and these patterns might be explained by linking the wide range of biotic and abiotic parameters that are measured simultaneously to those stations.

Water quality parameters (pigment, nutrient, turbidity)

A set of water quality parameters are collected; including 24 types of pigments, 5 types of nutrients, secchi depth and underway data. These are important abiotic parameters supporting and explaining the drivers of biotic communities.

Marine phytoplankton DNA

Exercises to profile the eukaryotic and prokaryotic phytoplankton communities using universal primers were performed during the 2017 and 2018 cruises. The 2019 samples will be processed using the same

protocol completing this data series and providing opportunity for combined analysis with the FlowCam data.

4.2. ULB

Eukaryotic and prokaryotic biodiversity

Assessing the eukaryotic and prokaryotic biodiversity and link their composition and activity to the chemical and physical environmental parameters is one of the prime targets of the Ecology of Aquatic Systems lab (ULB) onboard the Simon Stevin sampling campaigns.

All samples, including chlorophyll, bacteria, phytoplankton, CH4 and samples for molecular biodiversity (Metabarcoding) and transcriptomics (RNA) will be obtained by collecting the water with the CTD/water-rosette at a depth of 3m. Collected water samples will be size-fractionated into >200µm (discarded) and 200-20µm by gravity-filtration over filter towers and 20-0.22 µm size-fractions in a series of filtration units by vacuum pumping that will be finally collected on polycarbonate filters.

Metabarcoding

PCR gene amplification of the V4 regions of the 18S rRNA (eucaryotes) and 16S V4-V5 (procaryotes) will be performed in the laboratory. Generated libraries will be sequenced by Illumina MiSeq at the Alfred Wegener Institute and the obtained data analysed with already established bioinformatics pipelines. Short sequences (reads) combined with metadata will be published in public databases such as NCBI. Data will be analysed with multifactorial statistics to elucidate the linkage of biodiversity and environmental parameters.

Genomic data obtained of eukaryoplankton/prokaryoplankton will be as well used to study the importance of mixotrophic organisms in the North Sea and at the same time, this will serve to validate already existing biogeochemical models representing the Belgian coastal area.

CH4

Methane is the second most important greenhouse gas contributing to climate warming. Water from Niskin bottles will be used to calculate methane concentrations and study the monthly/yearly variations of this dissolved greenhouse gas, this will be performed following the ULB-Protocol.

Phytoplankton

Water from CTD rosette will be stored in 100ml flasks that will be fixed with Lugol for further analysis by inverted microscope back at the lab.

Bacteria: In order to assess bacterial abundance water from CTD rosette will be store in 50ml falcons and fixed with 0.22µm pre-filtered formaldehyde.

Chlorophyll

In order to construct the relationship between phytoplankton biovolume and chlorophyll and correlate this data to the abundance and diversity of mixotrophic organisms that are being studied in an ongoing project; chlorophyll samples will be taken using GF/F 47mm filters and water coming from the CTD rosette 3m depth.

4.3. CNRS

FRRf profiles

The aim is to perform high quality in-situ phytoplankton productivity measurements integrating all physiological properties of phytoplankton occurring in real time in the water column, in the study area. Proper collection of FRRf profile is not trivial. The FastOcean profiler is not deployed on a rosette but by itself on an independent winch cable on the side of the ship facing the sun. With high current and/or wind speed, the ship can easily turn and the instrument can end up on the shadow side of the ship. Adjustments using the ship's lateral booster can generate high turbulence level in surface-layer and change the vertical gradient of phytoplankton and light. Profiles will be studied in parallel with CTD (if every meter data are available) and Fluoroprobe vertical profiles.

FRRf act2

Furthermore, the aim is to undertake FRRf measurements connected to the underway system. A FRRf Act2 will be installed in the wet lab, connected to a second PC and automated sampling will be performed by the machine every 23 min. In addition some manual water samples are required (20ml from the flow beside the machine) to measure the fluorescence blank of each light-curves (in the evening).

Net and bottle sampling

Discrete samples are collected by a small plankton net (20µm mesh), in order for study the diversity of living phytoplankton by inverted microscopy onboard. Bottle samples will be fixed with Lugol for further analysis by inverted microscope back at the lab.

Fluoroprobe

Profiles will be carried out with a multispectral fluorometer (in which a specific fingerprint of *Phaeocystis* and of diatoms is applied) for characterizing spectral groups in vivo and in situ both at fixed stations as well as before and after the deployment of VPR. The results will be compared with pigmentary groups addressed by HPLC by VLIZ.

4.4. JOINT RESEARCH AND INTERCOMPARISON OBJECTIVES

automated FlowCytoMetry (FCM)

An intercomparison exercise with different Cytobuoy FCM's performing flowcytometric measurements side-by-side was organised by CNRS-LOG, VLIZ and RWSduring the 2018 cruise.
This has led to an intercomparison paper currently under review.
The 2019 cruise wants to address two shortcomings of the 2018 exercise: the appropriate use of calibration beads and the difference in sampling protocol.

During the 2019 cruise, two separate sampling protocols will be run per station by each FCM.

- Protocol 1: protocol used by CNRS-LOG (sampling volume, sensitivity)
- Protocol 2: protocol used by Reinhoud and RWS
- Morning and evening: run calibration beads, preferably the same ones!

FlowCam

FlowCam sampling and analysis protocols (objective, flow rate, flow cell, sample volume, dilution, etc.) need to be comparable in order to perform an intercomparison exercise between in vivo samples with colour FlowCam (CNRS LOG) and Lugol fixed samples with greyscale FlowCam (VLIZ)

FRRf

In-situ and underway FRRf will be used by Michèle Pernak and Fabrice Lizon, CNRS LOG. How comparable are both measured primary production index (JVPII) is a great open question that will be studied for the first time with this 2019 VLIZ cruise; underway-connected FRRf only has info on 3m depth, while the profiler consider all physiological parameters in real light quality in the water column. Such measurements and comparisons in contrasted water bodies are necessary in the context of current research on the integration of primary production into water quality indicators (FW2, OSPAR).

VLIZ is deploying a third FRRf based on the underway system, running simultaneously with the underway FRRf from CNRS. Both systems can be fully compared during the 2019 campaign

5. RESEARCH AREA – SAMPLING STATIONS

5.1. LIST OF SCHEDULED STATIONS

Sampling Station	Y	X
1	51.19	2.75
2	51.12	2.5
3	51.1	2.25
4	51.06	2
5	51.12	1.75
6	50.95	1.66
7	50.83	1.5
8	50.65	1.5
9	50.42	1.5
12	50.37	1.25
13	50.37	1
14	50.63	1
15	50.64	0.75
16	50.86	0.75
17	50.87	1
18	51.06	1.25
19	50.85	1.25
21	51.15	1.5
22	51.39	1.75
23	51.41	1.5
24	51.43	1.25
25	51.55	1
26	51.64	1.25
27	51.83	1.25
28	51.87	1.5
30	51.66	1.75
31	51.88	1.75
33	51.59	2
35	51.36	2.25

36	51.38	2.5
37	51.65	2.5
38	51.61	2.25
42	51.62	2.75
44	51.89	3.25
45	52.12	3.5
46	52.12	3.75
47	52.01	4
48	51.85	3.75
49	51.86	3.5
50	51.65	3.5
51	51.63	3.25
52	51.44	3.5
53	51.39	3.25
54	51.61	3
55	51.39	3
56	51.39	2.75

5.2. MAP OF SCHEDULED STATIONS

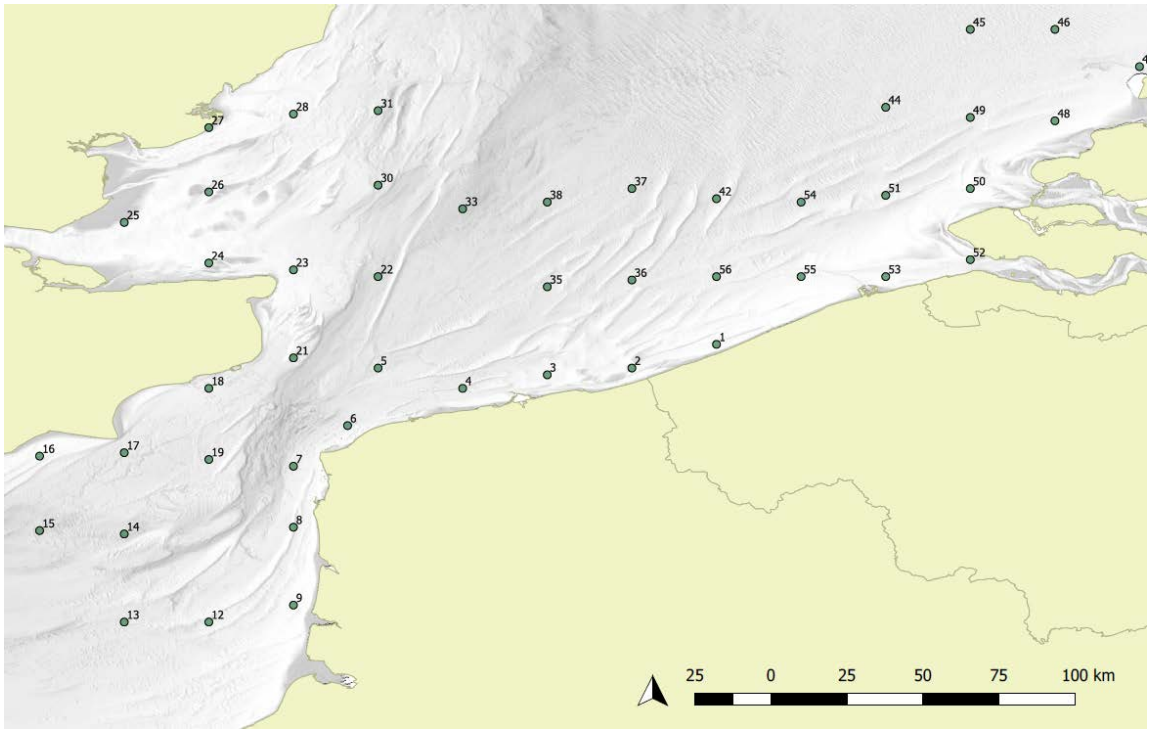

Fig 1: map of the working area (Eastern English Channel, Southern Bight of the North Sea, Thames estuary) with scheduled sampling stations marked from 1 to 56.

6. SAMPLING – ON BOARD ANALYSIS

6.1. LIST OF DISCRETE MEASUREMENTS AND ACTIONS

Group	Sensors and samples	Responsible	Memo
Prokaryotes	DNA, RNA	Reinhoud de Blok	[1]
Eukaryotes	DNA, RNA	Reinhoud de Blok	[1]
Phytoplankton	FCM-CNRS LOG	Mark Van Dijk – CNRS LOG	Protocol 1: CNRS- LOG [2] Protocol 2: VLIZ [2]
	FCM-VLIZ	Reinhoud de Blok	Protocol 1: CNRS- LOG [2] Protocol 2: VLIZ [2]
	Colour FlowCam	Eléonore Delecroix – CNRS LOG	In-vivo discrete samples[3]
	Fluoroprobe	Mark Van Dijk, CNRS LOG	Profiles
	Greyscale FlowCam	Nick Dillen	discrete samples [3]
	In-situ FRRf	Michèle Pernak – CNRS LOG	Vertical profiles in the sunny side of the boat, between sunrise and sunset
	Underway FRRf	Michèle Pernak– CNRS LOG	In parallel with FCM-CNRS LOG, H24
	Binocular microscopy	Fernando Gómez - CNRS LOG	Observation of discrete samples from 20 µm-mesh plankton net tows / Fixation of bottle samples
	Pigments (HPLC)	Jonas Mortelmans, Anouk Ollevier	LifeWatch Protocol [4]
Zooplankton	WP2net (ZooScan)	Jonas Mortelmans	Discrete samples, LifeWatch Protocol [5]
	Video Plankton Recorder (VPR)	Roeland Develter, Anouk Ollevier	LifeWatch Protocol (unpublished)
Abiotic measurements	Nutrients	Jonas Mortelmans, ULB	LifeWatch Protocol [4]
	CTD casts	Jonas Mortelmans, Anouk Ollevier	LifeWatch Protocol (unpublished)
	Underway measurements (incl. turbidity, O2, ..) [6]	Jonas Mortelmans	In-vivo

[1] *filtrated over a 25 mm 0.22 µm polycarbonate filter, extraction and isolation by Muyzer protocol (cf. 2018). 18S rRNA V4 region is amplified by primer TAReuk454FWD1 and TAReukREV3. 16S rRNA v1-v3 region is amplified by primes pA and BKL1. Sampling protocol identical to the 2017/2018 campaign*

[2] *important to run calibration bead in the morning + evening.*

[3] *With comparable sampling and analysis procedure for VLIZ and CNRS-LOG*

[4] *Mortelmans, J.; Deneudt, K.; Cattrijsse, A.; Beauchard, O.; Daveloose, I.; Vyverman, W.; Vanaverbeke, J.; Timmermans, K.; Peene, J.; Roose, P.; Knockaert, M.; Chou, L.; Sanders, R.; Stinchcombe, M.; Kimpe, P.; Lammens, S.; Theetaert, H.; Gkritzalis, T.; Hernandez, F.; Mees, J. (2019a). Nutrient, pigment, suspended matter and turbidity measurements in the Belgian part of the North Sea. Scientific Data 6(1): 22. https://hdl.handle.net/10.1038/s41597-019-0032-7*

[5] *Mortelmans, J.; Goossens, J.; Amadei Martínez, L.; Deneudt, K.; Cattrijsse, A.; Hernandez, F. (2019b). LifeWatch observatory date: zooplankton observations in the Belgian part of the North Sea. Geoscience Data Journal In press: 1-9. https://hdl.handle.net/10.1002/gdj3.68*

[6] *For an overview of underway data, see section 6.3*

For a complete overview of actions taken aboard the RV Simon Stevin, including date and location, see appendix 1

6.2. MAP OF DISCRETE MEASUREMENTS

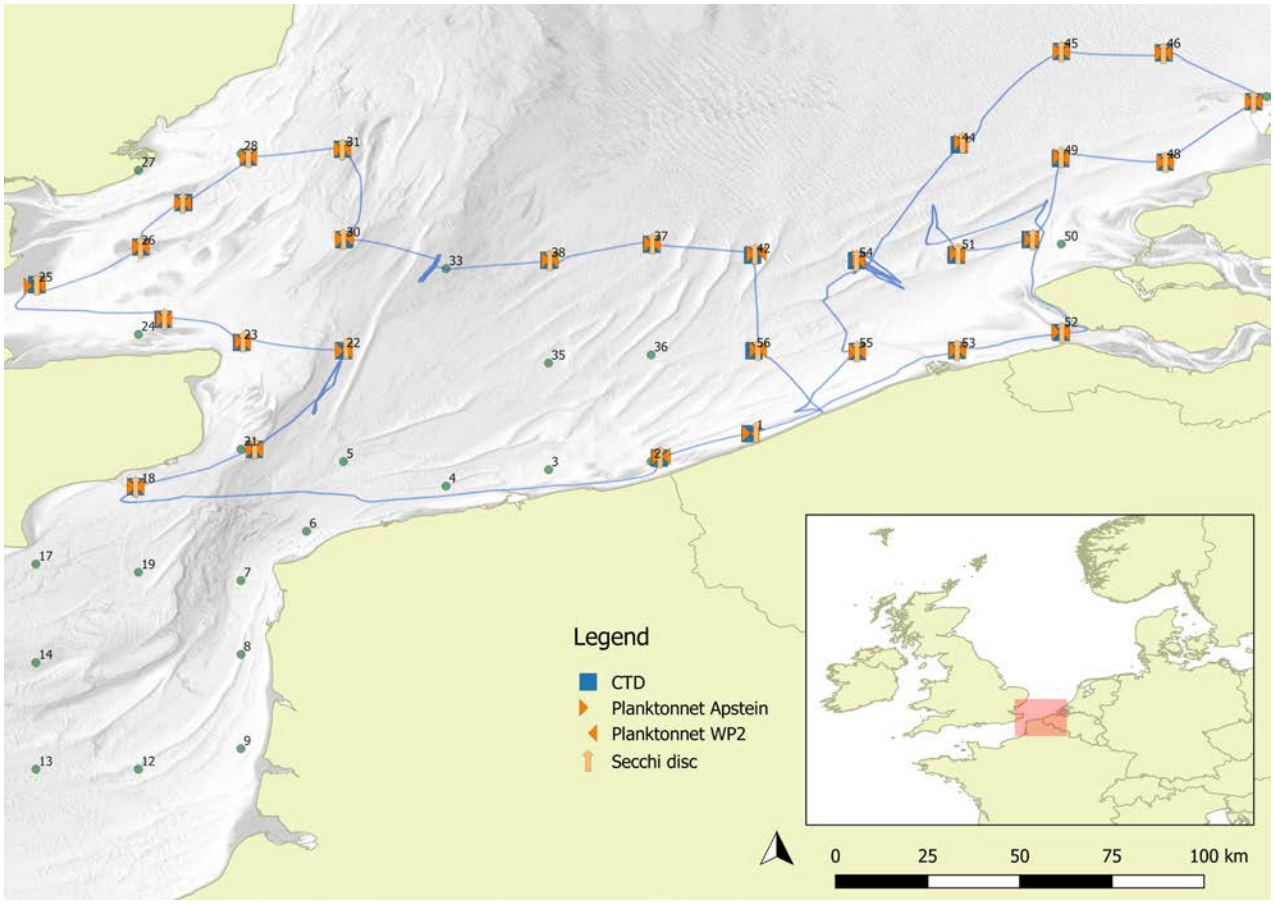

Fig 2: map with discrete measurements plotted (Eastern English Channel, Southern Bight of the North Sea, Thames estuary). Discrete measurements (CTD casts, Secchi, WP2, and Apstein actions).

6.3. LIST OF UNDERWAY MEASUREMENTS

Instrument	Parameter	Data acquisition rate 5 sec	20 min	station
Flow Cytometer (VLIZ)	Phytoplankton densities			x
RTK GPS	Current time, latitude, longitude, depth 200khz, course over ground, speed over ground	x		
?	Octans heading, odom depth 33khz, gpsfix, nav depth 50kzh, speedlog	x		
SBE21	water temperature, salinity, Chlorophyll A, sound velocity	x		
?	Time stamp, FLRTchla,	x		
AWS	Temperature, Relative Humidity, True wind direction, True wind speed, Air pressure, draught	x		
SBE38	Temperature	x		
Flowmeter	Water flow	x		

6.4. MAP OF UNDERWAY MEASUREMENTS

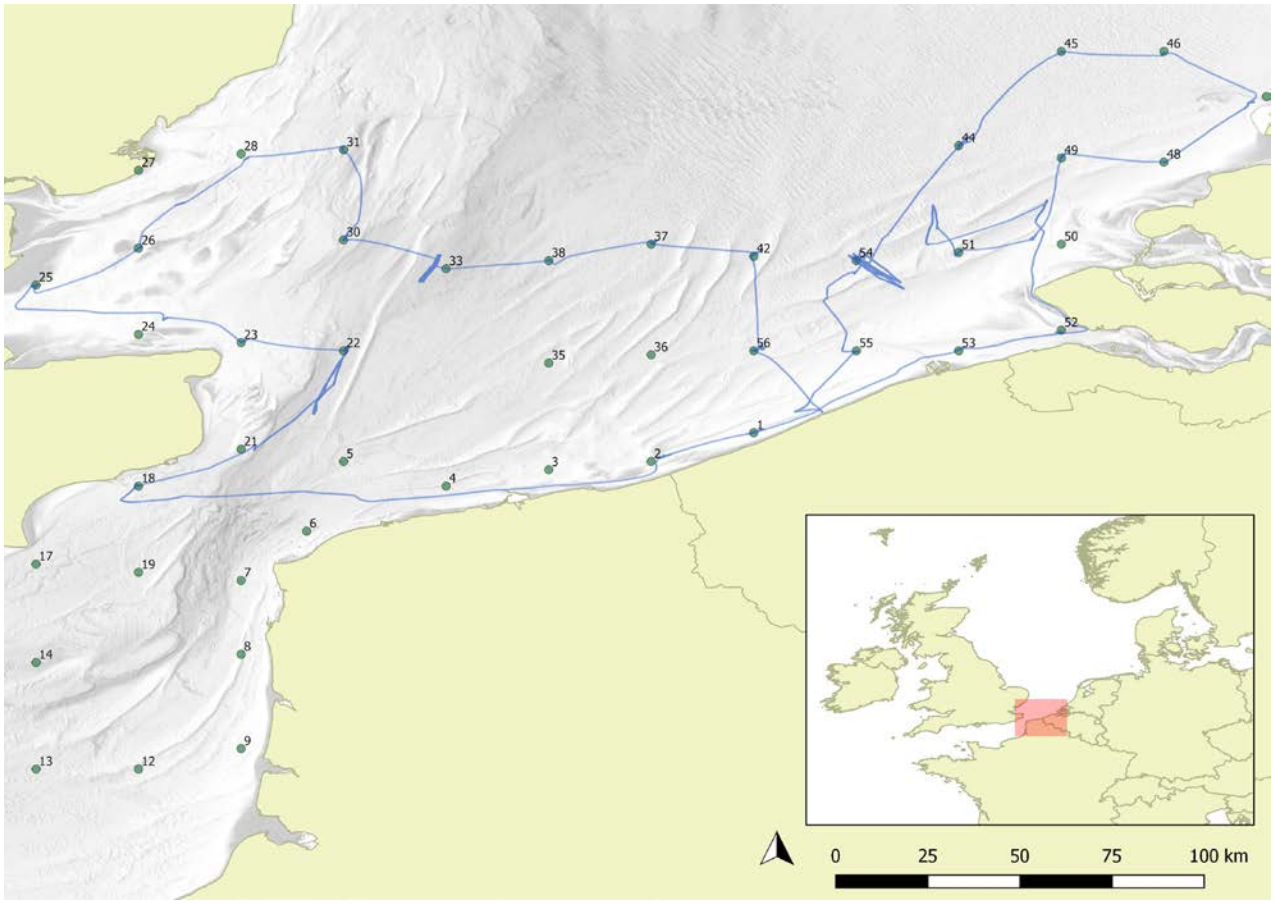

Fig 2: map with the trajectory of the RV Simon Stevin plotted, on this trajectory, underway data was collected (Eastern English Channel, Southern Bight of the North Sea, Thames estuary).

7. CHEMICALS

Several types of chemicals were used, listed in appendix 2: protocol section.

8. ISSUES/TROUBLES/REMARKS

7.1 GENERAL ISSUES

- No technical issues encountered.
- We did not obtain the French permit in time (despite early request!), so all French stations were skipped
- During the complete cruise, we enjoyed relatively rough weather, forcing us to leave the English Channel and head north, to the Netherlands.
- In total 29 (out of 46) stations were successfully sampled during daytime.

7.2 CTD

- No issues.

7.3 WATER QUALITY PARAMETERS

- No issues.

7.4 PLANKTONNET - WP2

- No issues.

7.5 PLANKTONNET - FYTOPLANKTON

- No issues.

7.6 FCM

- The VLIZ Flow cytometer was mainly handled by Reinhoud de Blok and regularly flushed, and beads inserted.
- In general, the scheduler was active. When arriving to a certain station, manual activation of the two protocols.

7.7 VPR

- Three successful tows were performed. The fourth was cancelled for the bad weather.

APPENDIX 1: list of discrete measurements taken during the JericoNext 2019 cruise

ID	Writer	ActionType	Code	StartLat	StartLong	StartDate	EndDate
110446	Dillen Nick	CTD	JN19_56	51.3899262	2.7507462	2019-05-06 T12:15:22	2019-05-06 T12:28:34
110447	Dillen Nick	Niskin Bottle	JN19_56	51.3916829	2.758787	2019-05-06 T12:27:10	2019-05-06 T12:28:28
110448	ollevier anouk	Secchi disc	JN19_56	51.3920043	2.7601167	2019-05-06 T12:28:58	2019-05-06 T12:28:58
110449	Mortelmans Jonas	Planktonnet WP2	JN19_56	51.3920043	2.7601167	2019-05-06 T12:28:58	2019-05-06 T12:28:58
110450	Dillen Nick	Planktonnet Apstein	JN19_56	51.3920043	2.7601167	2019-05-06 T12:28:58	2019-05-06 T12:28:58
110451	ollevier anouk	Planktonnet Apstein	JN19_42	51.6261002	2.753208	2019-05-06 T14:49:28	2019-05-06 T14:49:28
110452	Mortelmans Jonas	Planktonnet WP2	JN19_42	51.6282523	2.7602527	2019-05-06 T15:22:04	2019-05-06 T15:23:34
110453	develter roeland	Secchi disc	JN19_42	51.6260628	2.7531393	2019-05-06 T14:49:22	2019-05-06 T14:49:22
110454	develter roeland	Niskin Bottle	JN19_42	51.6231051	2.7476642	2019-05-06 T14:42:10	2019-05-06 T14:42:10
110455	develter roeland	CTD	JN19_42	51.6229863	2.7474457	2019-05-06 T14:41:52	2019-05-06 T14:43:04
110456	ollevier anouk	CTD	JN19_37	51.6495293	2.5009492	2019-05-06 T16:27:04	2019-05-06 T16:35:58
110457	Mortelmans Jonas	Niskin Bottle	JN19_37	51.6518454	2.504848	2019-05-06 T16:35:52	2019-05-06 T16:35:52
110458	Mortelmans Jonas	Planktonnet WP2	JN19_37	51.6502295	2.5023063	2019-05-06 T16:30:28	2019-05-06 T16:31:58
110459	Dillen Nick	Planktonnet Apstein	JN19_37	51.6511848	2.5027923	2019-05-06 T16:39:04	2019-05-06 T16:39:04
110460	Dillen Nick	Secchi disc	JN19_37	51.6511848	2.5027923	2019-05-06 T16:39:04	2019-05-06 T16:39:04
110461	Lapeyra Jon	CTD	JN19_38	51.6104343	2.2506705	2019-05-06 T18:17:58	2019-05-06 T18:35:52
110462	Mortelmans Jonas	Secchi disc	JN19_38	51.6112086	2.2530933	2019-05-06 T18:36:22	2019-05-06 T18:36:22
110463	Mortelmans Jonas	Planktonnet Apstein	JN19_38	51.6112062	2.2531068	2019-05-06 T18:36:34	2019-05-06 T18:36:34
110464	Mortelmans Jonas	Planktonnet WP2	JN19_38	51.6112051	2.2531133	2019-05-06 T18:36:46	2019-05-06 T18:36:46
110465	Mortelmans Jonas	Niskin Bottle	JN19_38	51.6112012	2.2531378	2019-05-06 T18:36:58	2019-05-06 T18:36:58
110467	Mortelmans Jonas	Niskin Bottle	JN19_30	51.665925	1.755611	2019-05-07 T04:27:46	2019-05-07 T04:27:46
110468	Mortelmans Jonas	CTD	JN19_30	51.6608557	1.7515135	2019-05-07 T04:18:04	2019-05-07 T04:27:46
110469	Mortelmans Jonas	Planktonnet WP2	JN19_30	51.6640413	1.7541232	2019-05-07 T04:24:16	2019-05-07 T04:24:22
110470	Mortelmans Jonas	Planktonnet Apstein	JN19_30	51.6620702	1.7501193	2019-05-07 T04:42:16	2019-05-07 T04:42:22
110471	Mortelmans Jonas	Secchi disc	JN19_30	51.6621217	1.7501387	2019-05-07 T04:42:22	2019-05-07 T04:42:22

110472	Dillen Nick	CTD	JN19_31	51.881061	1.7470768	2019-05-07 T06:37:16	2019-05-07 T06:37:46
110473	Dillen Nick	Niskin Bottle	JN19_31	51.8811078	1.74709	2019-05-07 T06:37:28	2019-05-07 T06:37:28
110474	Mortelmans Jonas	Planktonnet WP2	JN19_31	51.881061	1.7470768	2019-05-07 T06:37:16	2019-05-07 T06:37:16
110475	Dillen Nick	Planktonnet Apstein	JN19_31	51.8810905	1.7470887	2019-05-07 T06:37:22	2019-05-07 T06:37:28
110476	Dillen Nick	Secchi disc	JN19_31	51.8804409	1.7468932	2019-05-07 T06:34:58	2019-05-07 T06:34:58
110477	Mortelmans Jonas	Secchi disc	JN19_28	51.862093	1.5181295	2019-05-07 T07:58:16	2019-05-07 T07:58:16
110478	Mortelmans Jonas	CTD	JN19_28	51.8619464	1.5180315	2019-05-07 T07:59:22	2019-05-07 T08:00:28
110479	Mortelmans Jonas	Niskin Bottle	JN19_28	51.8618469	1.5178752	2019-05-07 T08:00:16	2019-05-07 T08:00:16
110480	Mortelmans Jonas	Planktonnet WP2	JN19_28	51.8619575	1.518045	2019-05-07 T07:59:16	2019-05-07 T07:59:16
110481	Dillen Nick	Planktonnet Apstein	JN19_28	51.8618469	1.5178752	2019-05-07 T08:00:16	2019-05-07 T08:00:28
110482	Dillen Nick	Secchi disc	JN19_27	51.7502776	1.3580648	2019-05-07 T09:09:34	2019-05-07 T09:09:34
110483	Mortelmans Jonas	CTD	JN19_27	51.7511581	1.3606027	2019-05-07 T09:05:58	2019-05-07 T09:19:22
110484	Mortelmans Jonas	Niskin Bottle	JN19_27	51.749429	1.3547207	2019-05-07 T09:14:34	2019-05-07 T09:14:34
110485	Mortelmans Jonas	Planktonnet WP2	JN19_27	51.7503487	1.3583512	2019-05-07 T09:09:10	2019-05-07 T09:09:10
110486	Dillen Nick	Planktonnet Apstein	JN19_27	51.7502945	1.358136	2019-05-07 T09:09:28	2019-05-07 T09:09:28
110487	ollevier anouk	Planktonnet WP2	JN19_26	51.6431682	1.2560022	2019-05-07 T10:14:22	2019-05-07 T10:15:22
110488	develter roeland	Planktonnet Apstein	JN19_26	51.6431682	1.2560022	2019-05-07 T10:14:22	2019-05-07 T10:14:22
110489	develter roeland	Secchi disc	JN19_26	51.6420774	1.2550733	2019-05-07 T10:17:04	2019-05-07 T10:17:04
110490	ollevier anouk	CTD	JN19_26	51.642947	1.2558487	2019-05-07 T10:14:52	2019-05-07 T10:16:40
110491	ollevier anouk	Niskin Bottle	JN19_26	51.6422248	1.255208	2019-05-07 T10:16:40	2019-05-07 T10:16:40
110492	ollevier anouk	CTD	JN19_25	51.5509299	1.0023547	2019-05-07 T11:43:04	2019-05-07 T11:48:46
110493	ollevier anouk	Niskin Bottle	JN19_25	51.5497245	0.9983613	2019-05-07 T11:48:46	2019-05-07 T11:48:46
110494	ollevier anouk	Planktonnet WP2	JN19_25	51.5508444	1.0019837	2019-05-07 T11:43:52	2019-05-07 T11:44:40
110495	develter roeland	Planktonnet Apstein	JN19_25	51.5483219	0.9916557	2019-05-07 T11:56:40	2019-05-07 T11:56:40
110496	develter roeland	Secchi disc	JN19_25	51.550823	1.0019322	2019-05-07 T11:43:58	2019-05-07 T11:43:58
110497	ollevier anouk	Planktonnet WP2	JN19_25	51.5473781	0.990862	2019-05-07 T12:30:10	2019-05-07 T12:30:40
110498	ollevier anouk	Planktonnet WP2	JN19_24	51.4668068	1.3105812	2019-05-07 T14:17:46	2019-05-07 T14:18:52

110499	develter roeland	Planktonnet Apstein	JN19_24	51.4672801	1.313799	2019-05-07 T14:43:04	2019-05-07 T14:43:04
110500	ollevier anouk	CTD	JN19_24	51.4665513	1.3103123	2019-05-07 T14:16:10	2019-05-07 T14:23:04
110501	ollevier anouk	Niskin Bottle	JN19_24	51.4675537	1.311207	2019-05-07 T14:22:52	2019-05-07 T14:22:52
110502	develter roeland	Secchi disc	JN19_24	51.4672801	1.313799	2019-05-07 T14:43:04	2019-05-07 T14:43:10
110503	Mortelmans Jonas	CTD	JN19_23	51.4100687	1.5014022	2019-05-07 T15:48:22	2019-05-07 T15:55:40
110504	ollevier anouk	Niskin Bottle	JN19_23	51.411663	1.5039062	2019-05-07 T15:54:46	2019-05-07 T15:54:46
110505	Mortelmans Jonas	Planktonnet WP2	JN19_23	51.4109961	1.5027967	2019-05-07 T15:52:10	2019-05-07 T15:52:10
110506	develter roeland	Planktonnet Apstein	JN19_23	51.4122205	1.5048778	2019-05-07 T15:56:52	2019-05-07 T15:56:58
110507	ollevier anouk	Secchi disc	JN19_23	51.4122465	1.504926	2019-05-07 T15:56:58	2019-05-07 T15:56:58
110508	ollevier anouk	CTD	JN19_22	51.3903419	1.750294	2019-05-07 T17:15:22	2019-05-07 T17:26:52
110509	ollevier anouk	Niskin Bottle	JN19_22	51.394316	1.7542405	2019-05-07 T17:26:46	2019-05-07 T17:26:46
110510	develter roeland	Secchi disc	JN19_22	51.3959943	1.7562038	2019-05-07 T17:33:46	2019-05-07 T17:33:46
110511	ollevier anouk	Planktonnet WP2	JN19_22	51.3908757	1.7508025	2019-05-07 T17:17:10	2019-05-07 T17:20:34
110512	develter roeland	Planktonnet Apstein	JN19_22	51.3903419	1.750294	2019-05-07 T17:15:22	2019-05-07 T17:26:34
110516	develter roeland	Planktonnet Apstein	JN19_21	51.1494881	1.5322365	2019-05-08 T04:30:16	2019-05-08 T04:37:40
110517	Dillen Nick	CTD	JN19_21	51.1494526	1.5322087	2019-05-08 T04:30:10	2019-05-08 T04:37:40
110518	Dillen Nick	Niskin Bottle	JN19_21	51.1523896	1.5336358	2019-05-08 T04:37:34	2019-05-08 T04:37:40
110519	Dillen Nick	Planktonnet WP2	JN19_21	51.1494881	1.5322365	2019-05-08 T04:30:16	2019-05-08 T04:37:34
110520	develter roeland	Secchi disc	JN19_21	51.15235	1.5336208	2019-05-08 T04:37:28	2019-05-08 T04:37:28
110521	develter roeland	Secchi disc	JN19_18	51.0588861	1.243341	2019-05-08 T06:50:58	2019-05-08 T06:51:04
110522	Dillen Nick	Planktonnet WP2	JN19_18	51.0587555	1.2432382	2019-05-08 T06:51:04	2019-05-08 T06:51:04
110523	develter roeland	Planktonnet Apstein	JN19_18	51.0586253	1.2430967	2019-05-08 T06:51:10	2019-05-08 T06:51:16
110524	Dillen Nick	CTD	JN19_18	51.0587555	1.2432382	2019-05-08 T06:51:04	2019-05-08 T06:51:04
110525	Dillen Nick	Niskin Bottle	JN19_18	51.0587555	1.2432382	2019-05-08 T06:51:04	2019-05-08 T06:51:04
110526	Mortelmans Jonas	CTD	JN19_2				
110527	Mortelmans Jonas	Niskin Bottle	JN19_2				
110528	Mortelmans Jonas	Planktonnet WP2	JN19_2	51.1320952	2.526034	2019-05-08 T12:41:28	2019-05-08 T12:41:40

110529	ollevier anouk	Planktonnet Apstein	JN19_2	51.1306007	2.5219813	2019-05-08 T12:37:52	2019-05-08 T12:37:52
110530	ollevier anouk	Secchi disc	JN19_2	51.1306007	2.5219813	2019-05-08 T12:37:52	2019-05-08 T12:37:52
110531	Mortelmans Jonas	CTD	JN19_2	51.1282987	2.520209	2019-05-08 T12:56:22	2019-05-08 T12:56:22
110532	Mortelmans Jonas	Niskin Bottle	JN19_2	51.1283212	2.5203233	2019-05-08 T12:56:28	2019-05-08 T12:56:28
110533	ollevier anouk	CTD	JN19_1	51.1879817	2.741113	2019-05-08 T14:03:46	2019-05-08 T14:10:04
110534	ollevier anouk	Niskin Bottle	JN19_1	51.1899664	2.7469197	2019-05-08 T14:09:46	2019-05-08 T14:09:46
110535	ollevier anouk	Planktonnet WP2	JN19_1	51.1886502	2.7426758	2019-05-08 T14:05:28	2019-05-08 T14:06:34
110536	Mortelmans Jonas	Planktonnet Apstein	JN19_1	51.1886502	2.7426758	2019-05-08 T14:05:28	2019-05-08 T14:18:40
110537	Mortelmans Jonas	Secchi disc	JN19_1	51.1924711	2.7560662	2019-05-08 T14:18:40	2019-05-08 T14:18:40
110538	ollevier anouk	CTD	JN19_53	51.3909106	3.2451042	2019-05-08 T16:31:52	2019-05-08 T16:39:04
110539	ollevier anouk	Niskin Bottle	JN19_53	51.3919906	3.2492638	2019-05-08 T16:37:58	2019-05-08 T16:37:58
110540	ollevier anouk	Planktonnet WP2	JN19_53	51.3913415	3.2470018	2019-05-08 T16:34:34	2019-05-08 T16:35:34
110541	develter roeland	Planktonnet Apstein	JN19_53	51.3914157	3.2472905	2019-05-08 T16:34:58	2019-05-08 T16:37:40
110542	Dillen Nick	Secchi disc	JN19_53	51.3912048	3.2464125	2019-05-08 T16:33:46	2019-05-08 T16:33:52
110543	ollevier anouk	CTD	JN19_52	51.43464	3.5000915	2019-05-08 T18:10:34	2019-05-08 T18:15:22
110544	ollevier anouk	Niskin Bottle	JN19_52	51.436131	3.4968658	2019-05-08 T18:15:16	2019-05-08 T18:15:22
110545	Mortelmans Jonas	Planktonnet WP2	JN19_52	51.43464	3.5000915	2019-05-08 T18:10:34	2019-05-08 T18:11:46
110546	develter roeland	Planktonnet Apstein	JN19_52	51.4361901	3.4967297	2019-05-08 T18:15:28	2019-05-08 T18:15:28
110547	develter roeland	Secchi disc	JN19_52	51.4341929	3.5013515	2019-05-08 T18:08:34	2019-05-08 T18:08:34
110550	ollevier anouk	Niskin Bottle	JN19_51	51.6270225	3.2481718	2019-05-09 T04:25:22	2019-05-09 T04:25:22
110551	ollevier anouk	CTD	JN19_51	51.6229678	3.2434667	2019-05-09 T04:17:22	2019-05-09 T04:27:40
110552	develter roeland	Planktonnet Apstein	JN19_51	51.6271861	3.2483435	2019-05-09 T04:25:40	2019-05-09 T04:25:46
110553	develter roeland	Planktonnet WP2	JN19_51	51.6236567	3.2443643	2019-05-09 T04:18:52	2019-05-09 T04:30:22
110554	develter roeland	Secchi disc	JN19_51	51.6271324	3.2482877	2019-05-09 T04:25:34	2019-05-09 T04:25:34
110555	develter roeland	Secchi disc	JN19_50	51.6610644	3.4298095	2019-05-09 T05:32:52	2019-05-09 T05:32:52
110556	ollevier anouk	CTD	JN19_50	51.6608218	3.424377	2019-05-09 T05:17:34	2019-05-09 T05:25:52
110557	ollevier anouk	Niskin Bottle	JN19_50	51.6641806	3.4272433	2019-05-09 T05:24:16	2019-05-09 T05:24:16

110558	develter roeland	Planktonnet Apstein	JN19_50	51.6610165	3.4297545	2019-05-09 T05:32:46	2019-05-09 T05:32:46
110559	ollevier anouk	Planktonnet WP2	JN19_50	51.6621067	3.425244	2019-05-09 T05:20:04	2019-05-09 T05:21:16
110560	ollevier anouk	Planktonnet WP2	JN19_49	51.8599683	3.4984615	2019-05-09 T07:05:16	2019-05-09 T07:05:22
110561	develter roeland	Planktonnet Apstein	JN19_49	51.8624674	3.4998423	2019-05-09 T07:11:46	2019-05-09 T07:11:46
110562	ollevier anouk	CTD	JN19_49	51.8585474	3.4978055	2019-05-09 T07:02:10	2019-05-09 T07:11:04
110563	ollevier anouk	Niskin Bottle	JN19_49	51.8620977	3.4996147	2019-05-09 T07:10:52	2019-05-09 T07:10:52
110564	develter roeland	Secchi disc	JN19_49	51.8620977	3.4996147	2019-05-09 T07:10:52	2019-05-09 T07:10:52
110565	develter roeland	Secchi disc	JN19_48	51.8502581	3.7536812	2019-05-09 T08:51:34	2019-05-09 T08:51:34
110566	develter roeland	Planktonnet Apstein	JN19_48	51.8502581	3.7536812	2019-05-09 T08:51:34	2019-05-09 T08:51:34
110567	develter roeland	Planktonnet WP2	JN19_48	51.8496632	3.7534847	2019-05-09 T08:39:46	2019-05-09 T08:40:28
110568	develter roeland	CTD	JN19_48	51.8496059	3.7534027	2019-05-09 T08:38:58	2019-05-09 T08:44:04
110569	develter roeland	Niskin Bottle	JN19_48	51.8499871	3.7534777	2019-05-09 T08:43:58	2019-05-09 T08:43:58
110570	Mortelmans Jonas	Niskin Bottle	JN19_47	51.9953135	3.9621537	2019-05-09 T10:48:10	2019-05-09 T10:48:16
110571	Mortelmans Jonas	Planktonnet WP2	JN19_47	51.9967062	3.9686113	2019-05-09 T10:34:34	2019-05-09 T10:34:40
110572	Dillen Nick	Planktonnet Apstein	JN19_47	51.9967062	3.9686113	2019-05-09 T10:34:34	2019-05-09 T10:34:34
110573	Mortelmans Jonas	CTD	JN19_47	51.9968518	3.9689612	2019-05-09 T10:33:52	2019-05-09 T10:48:16
110574	Dillen Nick	Secchi disc	JN19_47	51.9967237	3.9686617	2019-05-09 T10:34:28	2019-05-09 T10:34:34
110575	Mortelmans Jonas	CTD	JN19_46	52.1161127	3.7501993	2019-05-09 T12:16:40	2019-05-09 T12:24:40
110576	Mortelmans Jonas	Niskin Bottle	JN19_46	52.115136	3.7480992	2019-05-09 T12:24:34	2019-05-09 T12:24:34
110577	Dillen Nick	Secchi disc	JN19_46	52.1156841	3.7487313	2019-05-09 T12:21:04	2019-05-09 T12:21:04
110578	Mortelmans Jonas	Planktonnet WP2	JN19_46	52.1157385	3.748843	2019-05-09 T12:20:34	2019-05-09 T12:20:40
110579	Dillen Nick	Planktonnet Apstein	JN19_46	52.1156952	3.7487533	2019-05-09 T12:20:58	2019-05-09 T12:20:58
110580	Dillen Nick	Planktonnet Apstein	JN19_45	52.1194899	3.4998898	2019-05-09 T13:56:10	2019-05-09 T13:56:10
110581	Mortelmans Jonas	Planktonnet WP2	JN19_45	52.1194949	3.4999055	2019-05-09 T13:55:40	2019-05-09 T13:55:46
110582	Mortelmans Jonas	CTD	JN19_45	52.1194949	3.4999055	2019-05-09 T13:55:40	2019-05-09 T14:03:22
110583	Mortelmans Jonas	Niskin Bottle	JN19_45	52.1191851	3.5000785	2019-05-09 T14:03:16	2019-05-09 T14:03:16
110584	Dillen Nick	Secchi disc	JN19_45	52.1194899	3.4998898	2019-05-09 T13:56:10	2019-05-09 T13:56:10

110585	Dillen Nick	Secchi disc	JN19_44	51.8983213	3.2591707	2019-05-09 T16:43:58	2019-05-09 T16:43:58
110586	Dillen Nick	CTD	JN19_44	51.8922261	3.2512548	2019-05-09 T16:30:40	2019-05-09 T16:34:40
110587	Dillen Nick	Niskin Bottle	JN19_44	51.8940371	3.2535895	2019-05-09 T16:34:28	2019-05-09 T16:34:28
110588	Dillen Nick	Planktonnet Apstein	JN19_44	51.8982798	3.2591138	2019-05-09 T16:43:52	2019-05-09 T16:43:58
110589	Dillen Nick	Planktonnet WP2	JN19_44	51.8922261	3.2512548	2019-05-09 T16:30:40	2019-05-09 T16:30:40
110591	Mortelmans Jonas	CTD	JN19_54	51.6097857	3.0009903	2019-05-10 T05:02:40	2019-05-10 T05:23:34
110592	Mortelmans Jonas	Niskin Bottle	JN19_54	51.6152587	3.0108013	2019-05-10 T05:23:34	2019-05-10 T05:23:34
110593	Mortelmans Jonas	Planktonnet WP2	JN19_54	51.6097857	3.0009903	2019-05-10 T05:02:40	2019-05-10 T05:02:40
110594	Mortelmans Jonas	Planktonnet Apstein	JN19_54	51.6152587	3.0108013	2019-05-10 T05:23:34	2019-05-10 T05:23:40
110595	Mortelmans Jonas	Secchi disc	JN19_54	51.6096925	3.0009395	2019-05-10 T05:02:22	2019-05-10 T05:02:22
110596	Mortelmans Jonas	CTD	JN19_55	51.3881307	3.0026965	2019-05-10 T09:02:28	2019-05-10 T09:07:10
110597	Mortelmans Jonas	Niskin Bottle	JN19_55	51.3878686	3.0014747	2019-05-10 T09:07:10	2019-05-10 T09:07:10
110598	Dillen Nick	Planktonnet Apstein	JN19_55	51.3881329	3.002719	2019-05-10 T09:02:22	2019-05-10 T09:02:22
110599	Mortelmans Jonas	Planktonnet WP2	JN19_55	51.3881307	3.0026965	2019-05-10 T09:02:28	2019-05-10 T09:02:28
110600	Mortelmans Jonas	Secchi disc	JN19_55	51.3879839	3.0019145	2019-05-10 T09:05:28	2019-05-10 T09:05:28

APPENDIX 2: list of used protocols and chemicals on board

Next generation sequencing

Tools

- Vacuum filtration unit
- 50 * 0.2 µm polycarbonate filters
- Niskinbottles
- Tweezers
- Milli-Q-water
- cleaning solutions to remove all DNA (bleach or a DNAse product)
- 50 * 1.5 ml Eppendorf epjes
- Eppendorf stickers and cryogen pen
- tape
- Liquid nitrogen

Protocol

- Clean all used materials before filtration thoroughly with Milli-Q-water
- Rinse syringe and filtercap by pressing a full syringe with Milli-Q-water three times over the filterholder
- To remove all DNA, before sampling, the filtration unit or syringe can be cleaned with bleach or DNAse. Make sure you clean it afterwards with Milli-Q-water to remove all cleaning product
- Place a 0.2 µm polycarbonate filter (25 mm) in de filter holder (vacuum system or syringe) and filtrate as much as possible
- Note the filtrated volume, sampling data and sampling location
- Put the filter with a cleaned (Milli-Q-water, bleach, DNAse or Ethanol) tweezers in a 1.5 ml Eppendorf epje
- Put a sticker with all the relevant information on the Eppendorf epje and wrap scotch tape around the sticker and the lid. Don't forget this, because the sticker will not stick anymore when you put it in liquid nitrogen!!!
- Store the Eppendorf epjes with filters in the liquid nitrogen container until they can be stored -80 °C freezer

HPLC Pigments

Tools

- vacuum pomp and tubing
- 50 * GFF filter
- 50 * 2ml tubes
- roll of paper to dry
- tape
- cryopen
- cryolabels

To do

- done

Protocol

1) Connect vacuum pump to filter unit
2) Place filter and cup on filter unit
3) Fill cup with known amount of collected sample water (eg 500 mL)
4) Open t-valve and turn on vacuum pump to start filtering
5) Refill cup as much as possible, as long the filter is not clogged (for coastal stations, generally around 500mL in total, for deeper water, generally around 2000mL or more)
6) Note total volume of filtered water in excel report
7) Once the filter runs dry, flush sides of cup clean with distilled water and remove cup
8) Remove filter, fold and dry filter on paper tissue (filter has to be very dry!)

9) Store the filter in the specifically designed storage unit.
10) Label storage unit with the cryopen: VLIZ Date Station Chla "Volume filtered" ml (eg VLIZ 20141218_130_500ml_ChlA)
11) Wrap tape around the label to ensure it stays attached in liquid nitrogen.
12) Store in liquid nitrogen.

Clean all used equipment properly by rinsing 3 times with Milli-Q water

Nutrients

Tools

- 50 * 50 mL jars and caps
- 50 * cellulose acetate filters 0.2µm

Protocol

- Connect vacuum pump to Erlenmeyer
- Place cellulose acetate filter and cup on filter unit
- Fill cup (depends on sea state, generally around 300mL)
- Open t-valve and turn on vacuum to start filtering
- When the filter runs dry, pour the filtered water into a nutrient-recipient
- 150ml for official lab nutrient analysis (label with VLIZ, station, date, Nutrients…)
- Clean the Erlenmeyer and all other equipment properly, rinse 3 times with Milli-Q water.
- put in -24

Flowcam

Tools

- 1 * 10 µm net
- 1 * 50µm net
- 1 * 50L barrel
- 50 * 1L recipient (or alternatively: the typical falcons).
- 1 * bucket and rope
- 1 * lugol

To do

- attach strong rope to big bucket.

Protocol

- Filter 50 liters water, from a bucket sample, on 50 µm net
- but contents of the 50µm net into a falcon (or larger recipient).
- Preserve with lugol 2% final concentration
- store in 4°, attach aluminium foil

Dilutions

Volume	Sea Water	Volume	Lugol
1000	mL zeewater	5	mL lugol
500	mL zeewater	2.5	mL lugol
250	mL zeewater	1.25	mL lugol
125	mL zeewater	0.625	mL lugol
62.5	mL zeewater	0.3125	mL lugol

31.25	mL zeewater	0.15625	mL lugol
15.625	mL zeewater	0.078125	mL lugol
7.8125	mL zeewater	0.039063	mL lugol
3.90625	mL zeewater	0.019531	mL lugol
1.953125	mL zeewater	0.009766	mL lugol

Zooplankton

Tools

- WP2 net (200 µm)
- Flowmeter
- gas-water
- formalin

Protocol

- Tell the crew to haul the WP2 net. Ask them to install the flowmeter in order to know the volume of water that passed the net. Note this flow on the excel report file
- Register in MIDAS when the WP2 net is hauled up.
- Once the WP2 net is up, make sure to rinse the outside of the net so all material is certainly in the red flask on the bottom.
- take the red flask on the bottom and try to lose as many water as possible. Pour this material in a 1L flask.
- Rinse the flask very thoroughly with soda water and add it to the recipient
- Dependant on the volume of zooplankton, add 20ml 35% formol, or more! Do this shortly after collecting since zooplankton will predate each other within the recipient.
- In the lab, after fixation, formol will be replaced by 70% ethanol.

Dilutions

Volume Plankton	add formol to obtain 4% concentration (in mL)
100	11.11
200	22.22
300	33.33
400	44.44
500	55.56
600	66.67
700	77.78
800	88.89
900	100.00
1000	111.11