DY172 Cruise Report SDC

RRS *Discovery*

*n*POP
New Perspectives on Ocean Photosynthesis

Cape Town, South Africa – Walvis Bay Namibia

21st Dec 2023 – 26th Jan 2024

Principal Scientist:

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2024

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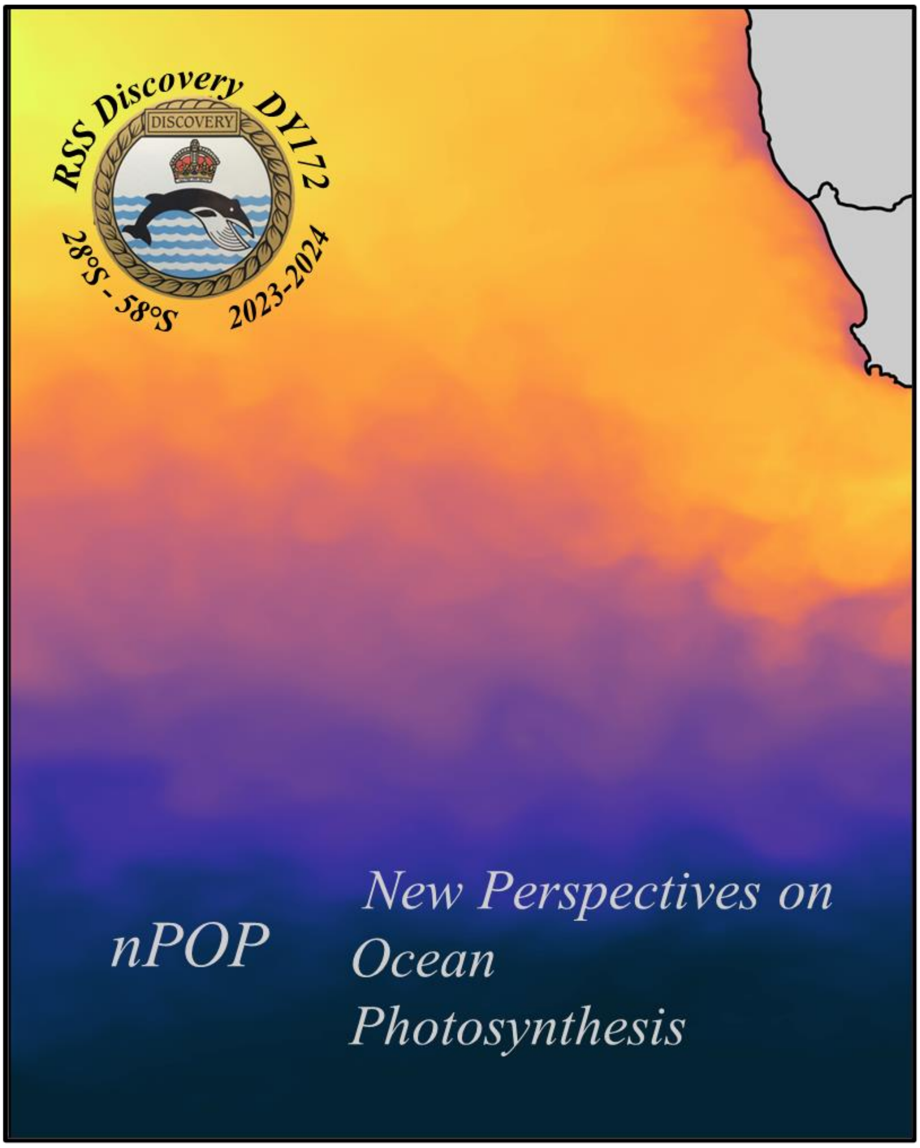
Scientific Background and Objectives



nPOP – Scientific rationale

Light capture by oceanic photosynthesis is a major component of the Earth's system, powering life in the oceans, sustaining fisheries and driving the biogeochemical cycles that regulate climate. Despite the importance of this fundamental metabolic process, we still possess a limited understanding of how photosynthesis actually works in ocean systems. For nearly a century oceanographers have almost exclusively considered phytoplankton photosynthesis as the amount of CO_2 that is 'fixed' into organic compounds using light energy. However, the generation of energy by photosynthesis and the rate of carbon fixation are uncoupled. As such, a focus on carbon as the 'currency' of productivity neglects around 50% of the energy generated by phytoplankton, which is actually used to power their broader cellular metabolism. This unaccounted resource regulates the survival of this crucial microbial group phytoplankton and thus how phytoplankton sustain food production and drive ocean atmospheric interactions. The lack of a fundamental understanding of these non-carbon fixing strategies in natural communities is a major deficiency in our broader understanding of marine primary production, and the focus of nPOP.

Our overarching objective of DY172 is to combine state-of-the-art measurements of photosynthetic physiology and primary production across natural oceanic gradients with experimentally imposed manipulation of the nutrient and temperature environment to collect datasets that will enable us to define how photosynthesis works across oceanic regions that encapsulate most of the world's oceans.

DY172 Cruise objectives

We used a North-South latitudinal survey from the South Atlantic into the Southern Ocean and back. This was selected to encompass gradients in environmental drivers that limit primary production (nitrogen, iron, temperature, light). The carefully selected region sampled across this single research cruise will encompass ecosystems and phytoplankton community and physiological diversity which is representative of the world's surface ocean.

Objective 1: Collect *in situ* measurements of photosynthesis physiology and rates of primary productivity (alongside complementary measurements of the physics, chemistry and microbiology of the system) over natural gradients from:

(i) Oligotrophic waters of the South Atlantic Subtropical Gyre (SASG), a nitrate-limited system (nitrate typically <5 µM) dominated by prokaryotes (Prochlorococcus and Synechococcus) (above 40 °S).
(ii) The iron-limited Southern Ocean (below 50 °S), which is dominated by eukaryotes (diatoms)

Objective 2: Collect water from (i) and (ii) regions to enable on deck manipulation experiments that test the impact of nutrient addition on the photosynthetic physiology and rates of primary production.

Objective 3. To make a number of other observations at sites over the study region that sample the oceanographic gradients encountered and thus giving a broader context.

Objective 4. To deploy 5 gliders for the Met Office in under sampled oceanographic regions

Objective 5. To recover a mooring deployed by NIOZ in Jan 2023

DY172 has been successful in meeting these objectives. Despite losing some time to weather during the transit to 58 °S and on the return, all ships and scientific systems worked well enabling extensive sampling of the prime target regions (low Fe and low N). A total of 31 stations (**Figure 1)** were occupied at which both stainless steel (SS_CTD) and trace-metal-clean (TMC) (titanium) (Ti_CTD) CTDs were deployed for water sample collection; nominal CTD depths were to 1000 m and complemented by targeted Ti_CTDs to full depth to help resolve the sources of nutrients to the surface ocean.

Twelve (48-hr) manipulation experiments and 13 (96-hr) manipulation experiments were conducted across the entire study region (**Figure 1**). These were complemented by underway sampling (every 4 hrs) using the ships system, a TMC (Tow-Fish) deployed from the starboard stern quarter and the use of a suite of continuous (real time) optical measurements of the physiology of photosynthesis communities.

Four stations were occupied for 24 hrs to enable sampling of the same location over diel cycles. Combining these approaches have enabled a study of photosynthesis spatially, temporally and in response to nutrient manipulation.

In addition, the use of SST, Chlorophyll and sea-surface height from satellite data enabled sampling to be targeted to dynamic frontal regions and regions of high chlorophyll concentration, and to study phenomena such as Agulhas Rings.

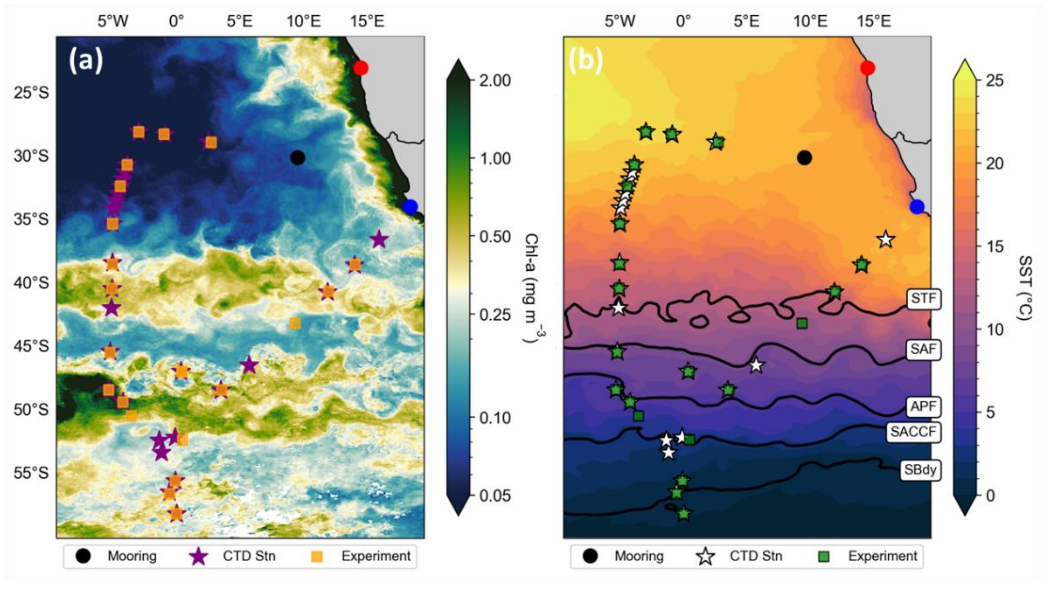


Figure 1: Composite of sea surface chlorophyll (a) and sea surface temperature (b) for the duration of DY172. Locations of CTD stations and locations of experimental set ups are shown on both panels. The location of the frontal systems are also shown in (b). Elevated chlorophyll can be seen to be associated with fronts. (see *Remote Sensing Data*)

DY172 Event Log

Date/ Time (GMT)	Event #	Event	Station	Lat (°)	Lon (°)	Depth (m)	CTD Depth (m)	Wind Speed (m/s)	Air Temp (°C)	PAR Ave	Water Sal (PSU)	Water Temp (°C)
22/12/2023 07:38	1	CTD001_T	1	-36.5	16.0	4633	1000	4.2	18.9	353.5	35.60	20.6
22/12/2023 09:25	2	CTD001_SS	1	-36.5	16.0		1000	7.5	18.8	394.8	35.60	20.4
22/12/2023 11:14	3	Tow Fish Deploy		-36.5	16.0			8.9	18.8	344.4	35.61	20.9
22/12/2023 11:54	4	Tow-Fish Rec		-36.5	15.9			8.9	18.8	361.3	35.61	20.9
22/12/2023 12:50	5	Tow Fish Fully Deploy		-36.5	15.9			7.6	18.6	276.4	35.60	20.9
23/12/2023 04:13	6	CTD002_T	2	-38.5	14.0	4934	1000	8.8	13.7	16.8	35.67	20.2
23/12/2023 05:46	7	CTD002_SS	2	-38.5	14.0		1000	8.2	13.8	77.0	35.67	20.1
23/12/2023 14:00	8	Tow Fish rec		-39.3	13.3			20.1	12.8	363.3	35.62	17.1
23/12/2023 15:00	9	Tow Fish Deploy		-39.4	13.2	4865	1000	4.7	13.1	287.3	35.61	17.1
24/12/2023 04:08	10	CTD003_T	3	-40.6	11.9	4867	1000	5.2	12.0	7.5	34.60	12.7
24/12/2023 05:36	11	CTD003_SS	3	-40.6	11.9			4.8	12.0	81.4	34.61	12.7
25/12/2023 03:51	12	ARGO_1		-43.1	9.3	3934		6.5	7.1	1.9	34.36	10.1
26/12/2023 05:59	13	CTD004_SS	4	-46.4	5.7	4097	1000	7.9	8.1	74.0	33.85	7.1
26/12/2023 07:33	14	CTD004_T	4	-46.4	5.7	4048	4000	7.6	7.9	256.5	33.85	7.1
26/12/2023 11:01	15	ARGO_2		-46.4	5.7	4003		10.5	8.2	387.0	33.85	7.2
27/12/2023 04:25	16	CTD005_T	5	-48.4	3.5	4136	1000	11.5	6.4	6.0	33.78	5.5
27/12/2023 05:47	17	CTD005_SS	5	-48.4	3.5	4160	1000	13.2	5.6	25.5	33.78	5.5
28/12/2023 04:11	18	CTD006_T	6	-46.9	0.3		1000	16.3	8.3	-1.3	33.85	6.9
28/12/2023 05:42	19	CTD006_SS	6	-46.9	0.3		1000	16.0	9.4	13.4	33.85	6.9
30/12/2023 16:10	20	ARGO_3		-50.7	0.1	2996		13.0	5.0	84.3	33.85	4.0
01/01/2024 04:04	21	CTD007_T	7	-52.1	-0.2	0	1000	9.4	3.6	2.0	33.81	3.0
01/01/2024 05:28	22	CTD007_SS	7	-52.1	-0.2	2913	1000	9.6	3.6	56.8	33.81	3.0
02/01/2024 05:03	23	CTD008_SS	8	-55.5	-0.1		1000	6.0	1.6	12.2	33.96	0.9
02/01/2024 07:00	24	CTD008_T	8	-55.5	-0.1		3600	5.8	0.9	45.0	33.96	0.9
03/01/2024 04:58	25	CTD009_SS	9	-58.1	0.0	4372	1000	8.3	0.4	15.2	34.15	0.3
03/01/2024 07:10	26	CTD009_T	9	-58.1	0.0	4384	4368	8.5	0.3	25.6	34.14	0.3
03/01/2024 11:29	27	ARGO_4		-58.1	0.0	4379		14.5	1.4	386.7	34.15	0.4

03/01/2024 11:31	28	ARGO_5		-58.1	0.0			14.2	1.4	373.6	34.14	0.4
04/01/2024 05:03	29	CTD010_SS	10	-56.4	-0.6		1000	10.4	0.7	18.7	34.09	0.7
05/01/2024 05:00	30	CTD011_SS	11	-53.3	-1.2	2753	1000	9.0	0.9	20.8	33.93	1.9
05/01/2024 12:36	31	CTD010_T	12	-52.3	-1.4	2657	F/D	7.0	2.0	465.2	33.82	3.1
07/01/2024 05:15	32	CTD012_SS	13	-49.3	-4.2	3227	1000	12.7	4.2	18.2	33.81	5.8
07/01/2024 07:14	33	CTD011_T	13	-49.3	-4.2	3167	3214	10.6	4.3	85.4	33.81	5.8
09/01/2024 05:00	34	CTD013_SS	14	-48.4	-5.4	4447	1000	4.1	3.3	2.7	33.78	5.7
09/01/2024 06:55	35	CTD012_T	14	-48.4	-5.4	4450	1000	2.7	3.2	58.1	33.78	5.6
10/01/2024 04:10	36	CTD013_T	15	-45.3	-5.2	3632	1000	9.0	9.6	-1.9	33.87	9.1
10/01/2024 05:53	37	CTD014_SS	15	-45.3	-5.2	3633	1000	9.3	9.4	10.4	33.89	9.1
11/01/2024 04:06	38	CTD014_T	16	-41.9	-5.1	4193	1000	4.8	11.0	-2.2	34.66	13.3
11/01/2024 05:38	39	CTD015_SS	16	-41.9	-5.1	4193	1000	5.4	11.2	10.2	34.65	13.4
12/01/2024 04:59	40	CTD016_SS	17	-40.4	-5.1	3728	1000	3.4	13.6	-1.8	34.69	14.3
12/01/2024 07:07	41	CTD015_T_failed	17	-40.4	-5.1	3729		5.5	13.5	51.0	34.68	14.3
12/01/2024 07:52	42	CTD015_T	17	-40.4	-5.1	3728	3696	6.2	13.6	139.1	34.68	14.1
13/01/2024 04:50	43	CTD016_T	18	-38.3	-5.1	3852	1000	5.3	16.8	-1.7	34.81	16.4
13/01/2024 06:13	44	CTD017_SS	18	-38.3	-5.1	3852	1000	5.1	16.6	24.9	34.80	16.3
14/01/2024 04:10	45	CTD017_T	19	-35.3	-5.0	2418	1000	8.9	18.7	-2.0	35.31	19.4
14/01/2024 05:36	46	CTD018_SS	19	-35.3	-5.0	1208	1000	10.5	18.4	6.5	35.31	19.3
14/01/2024 16:08	47	CTD018_T	20	-34.0	-5.0	3946	800	5.9	19.6	345.9	35.66	21.5
14/01/2024 21:51	48	CTD019_T	21	-33.4	-4.8	3520	800	5.3	19.4	-1.0	35.86	22.3
15/01/2024 04:00	49	CTD020_T	22	-32.9	-4.6	4150	800	5.0	19.6	-1.9	36.06	23.0
15/01/2024 09:59	50	CTD021_T	23	-32.3	-4.5	3603	800	3.2	20.4	403.2	35.98	22.3
15/01/2024 16:09	51	CTD022_T	24	-31.7	-4.3		800	2.7	21.0	306.8	35.99	23.4
15/01/2024 22:07	52	CTD023_T	25	-31.2	-4.1	4272	800	4.8	20.9	-1.5	36.05	22.9
16/01/2024 04:08	53	CTD024_T	26	-30.6	-3.9	4042	800	3.8	20.6	-1.5	35.93	22.4
17/01/2024 05:00	54	CTD019_SS	27	-28.0	-3.0		1000	7.8	22.0	-3.0	36.45	24.2
17/01/2024 07:10	55	CTD025_T	27	-28.0	-3.0		4722	8.1	22.0	40.3	36.45	24.3
18/01/2024 04:09	56	CTD026_T	28	-28.2	-0.9		1000	5.2	21.4	-3.2	36.37	23.8
18/01/2024 05:37	57	CTD020_SS	28	-28.2	-0.9		1000	7.3	21.2	4.5	36.37	23.8
19/01/2024 04:43	58	CTD027_T	29	-28.9	2.7	3085	1110	3.7	20.5	-0.7	36.01	22.7
19/01/2024 06:09	59	CTD021_SS	29	-28.8	2.7	3077	1000	30.0	20.5	19.8	36.01	22.7

20/01/2024 04:08	60	CTD028_T	30	-29.5	6.3	5003	600	289.7	20.6	-1.8	35.81	22.1
20/01/2024 05:07	61	CTD022_SS	30	-29.5	6.3		1000	356.0	20.1	3.0	35.81	22.1
21/01/2024 04:20	62	Tow-Fish recovered	31	-30.0	9.5	4906		9.0	20.2	-0.7	35.62	21.7
21/01/2024 05:45	63	Mooring released	31	-30.0	9.5	4914		41.3	20.9	92.4	35.62	21.6
22/01/2024 00:22	64	CTD029_T	31	-30.0	9.5		4940	340.0	20.3	-2.6	35.62	21.7
22/01/2024 05:19	65	CTD023_SS	31	-30.0	9.5	4911	2500	179.8	20.9	16.1	35.64	21.6

DY172 Cruise Narrative

Date	Time (GMT)	Time (ships)	Activity/event	Site/Location
21/12	0500	0700	Shore leave ends	(*-33.9249,18.4241*)
	0900	1100	Sailed Cape Town on route Shake Down Station	
	1100	1300	Science meeting	
	1400	1600	Lifeboat drill	
			Transit continues, Bumpy initial passage	
22/12	0630	0830	Meeting senior staff	
			Tool box talk prior to CTD deployment	
			Shake-down CTDs	
	0738	0938	**Event 001:** Metal-free CTD (CTD001_T) deployed (1000 m) deployment calibration cast and soak for bottles. All bottles fired 1000 m. Standard depths are 1000 m, 750m 500m, 250, 20m Surface (5 m)	**Station 1** (*-36.506, 15.95*)
	0925	1225	**Event 002:** CTD (CTD001_SS) Deployed (1000 m). Standard depths are 1000 m, 750m 500m, 250, 20m Surface (5 m)Calibration of bottles. Two bottles fired at each depth. Measure nutrients from both to test.	
	1114	1314	**Event 003**: TMC Tow-Fish deployment (Test)	
	1154	1354	**Event 004**: TMC Tow-Fish recovered	
	1250	1450	**Event 005:** TMC Tow-Fish deployment (Operational)	
			Underway sampling commenced. Both ships non-toxic and the TMC FISH to be sampled at 02:00, 06:00, 10:00, 14:00, 18:00, 22:00. Great team effort to enable this.	
			Science meeting to plan incubations. Great talk from Tommy outlining the oceanography of Southern Ocean.	
			First day of heavy science activity completed. Some loss of time in places, but not unexpected. Overall all programme completed successfully.	
23/12			**Ship slows to 4kts to sample TMC Tow Fish**	
	0413	0613	**Event 006:** Metal-free CTD (CTD002_T) deployed (1000 m). Bottles fired on the fly. Temperature incubation water collected (60 m)	**Station 2** (*-38.505, 14.016*)
	0546	0746	**Event 007:** CTD (CTD002_SS) Deployed (1000 m). Bottles fired after 1 min stop at target depth.	
			Issues with ships power during deployment. Identified as incubator van switching on. Now resolved	
	1400	1600	**Event 008:** TMC Tow-Fish recovered (not flying well)	
	1500	1700	**Event 009:** TMC Tow-Fish deployment (Operational) issue with loose tubing now resolved.	
			Electrical issue with STAF in lab, cause sea water lacking onto power supply. Now resolved. All science equipment checked to secure power banks above working height of instruments.	
			Celebrate Karel's birthday	
24/12	0235	0435	**Ship slows to 4kts to sample TMC Tow Fish**	
			Station 3, right after crossing the subtropical front	**Station 3** (*-40.628, 11.893*)
	0408	0608	**Event 010:** Metal-free CTD (CTD003_T) deployed (1000 m). 12 bottles fired at 12 depths.	
	0536	0736	**Event 011:** CTD (CTD003_SS) Deployed (1000 m). 24 bottles fired at 12 depths, CTD stopped at 312 m on the way	

			up for ~15min. Issue with autocorrect on winch. Turned off in slight swells. Aerosol sampling starts Water entering main lab – behind bubble (resolved). Windy day – on ships beam. Xmas Quiz.	
25/12	0324 0351	0524 0551	**Ship slows to 4kts to sample TMC Tow Fish** **Event 012:** Argo_1 Deploy Argo Float for Met Office Continue underway sampling at 02:00, 06:00, 10:00, 14:00, 18:00, 22:00, Change Tommy filtration rigs and rubber bungs blocking filters *Merry Christmas*	(*-43.112, 9.319*)
26/12	0559 0733 1101	0759 0933 1301	**Event 013:** CTD (CTD004_SS) Deployed (1000 m). 24 bottles fired at 12 depths **Event 014**: Metal-free CTD (CTD004_T) deployed (4000 m). 24 bottles fired at 24depths. TA/DIC samples were taken **Event 015:** Argo_2 deployed for Met Office close to deep CTD Crossing frontal system. Issue with matching in situ temperature in incubations on deck. Issue with scintillation counter(s). Both not working since started trip. Worse weather forecast may need to wait out before heading further South	**Station 4** (*-46.406, 5.719*)
27/12	0255 0425 0547	0455 0625 0745	**Ship slows to 5kts to sample TMC Tow Fish** **Event 016**: : Metal-free CTD (CTD005_T) deployed (1000 m). 12 bottles fired at 12 depths. **Event 017** CTD (CTD005_SS) Deployed (1000 m). 24 bottles fired at 12 depths Non-toxic underway system leaking in constant temperature lab. Switched off while repaired (approx offline from 1200-1800). Impact on ships underway recording and underway instruments in labs. Issue with Aerosol sampling. May not have been working to date? Alter course to NW for approaching weather.	**Station 5** (*-48.402, 3.479*)
28/12	0222 0411 0542	0422 0611 0742	**Ship slows to 4kts to sample TMC Tow Fish** Station to wait out weather to South **Event 018:** Metal-free CTD (CTD006_T) deployed (1000 m). 12 bottles fired at 12 depths.	**Station 6** (*-46.889, 0.325*)

			Event 019: CTD (CTD006_SS) Deployed (1000 m). 24 bottles fired at 12 depths Proceeding North to Hove to	
29/12			Proceeding to South Hove to – Underway sampling of ships systems as wait out weather Science meeting – some great measurements and data already. Exciting. Scintillation counter working – Loose board fixed. Aerosol sampling working. Not powered correctly. (check date) Pesudo – Diel measurements set up. Generally sunny day (good) but moving through water. 0600, 1000, 1400, 1800)	
30/12			Tow-Fish sampling. Request ship point into swell to prevent backwash from Ship onto FISH. Moving to South through heavy swell. No overside operations possible. Continue to sample underway systems.	
	1610	1810	**Event20**: Argo_3 deployed for Met Office	(*-50.703, 0.06*)
31/12	0321	0521	**Ship slows to 4kts to sample TMC Tow Fish** Reached position approx 52 °S. Still no overside operations possible. Continue to sample ships underway systems. Hove to.	(*-52.307, 0.471*)
	0800	1000	Speed up ship to allow for Tow Fish Sampling	(*-52.406, 0.628*)
1/1	0235	0435	**Ship speeds to 4kts to sample TMC Tow Fish – Samples for Corday**	**Station 7** (*-52.097, -0.15*)
	0404	0604	**Event 21** Deploy metal-free CTD007_T to 1000m, 13 bottles fired at 13 depths, one bottle to soak and other 12 were sampled	
	0528	0728	**Event 22** Deploy Stainless Steel CTD007_SS to 1000m, 24 bottles fired at 12 depths	
2/1	0301	0501	**Ship slows to 4kts to sample TMC Tow Fish**	**Station 8** (*-52.097, -0.15*)
	0528	0728	**Event 23** Deploy Stainless Steel CTD008_SS to 1000m, 24 bottles fired at 12 depths	
	0503	0703	**Event 24** Deploy metal-free CTD008_T to 3600m, Full depth, Ti CTD, 18 bottles at 18 depths. Chart depth only at 2000m, depth from ship ambiguous. Deployed slowly and successfully to 3600m. Issue with wire scrolling. Will try to resolve on next deep Ti_CTD cast. Ship requests swath to be on, Marine mammal watch completed in advance of turning on run by NMF techs. Possible issue with Ships propulsion. To be investigated approx. 7/1. Will require 3hrs stationary with no overside operations. Stay on station until 14:00 for diel sampling. Diel sampling started at 06:00 (every 4hrs) for 36 hrs. Underway sampling continues every 4 hrs.	
3/1	0234	0434	**Ship slows to 4kts to sample TMC Tow Fish** Furthest South, cold sea and grey sky. Some snow in air. Ship slowed during transit due to poor visibility and Ice.	**Station 9** (*-58.136, -0.004*)
	0458	0658	**Event 25** Deploy Stainless Steel CTD009_SS to 1000m, 24 bottles fired at 12 depths, Corday sampled from 1000 m for DOC. Ben to sample from Ti_CTD at corresponding depth for DIC and TA.	
	0710	0910		

	1129 1131	1329 1331	**Event 26** Deploy metal-free CTD009_T to 4368 m, Full depth, Ti CTD, 24 bottles at 24 depths. Issue with wire scrolling resolved. **Event 27 Argo_4** (10059) **Event 28 Argo_5** (10060) **Ships fire drill in Galley for crew at 16:00**	
4/1	0224	0424	**Ship slows to 4kts to sample TMC Tow Fish (02:24 GMT)** Ship not stopped after TMC sampling complete. Hence station location further North than Incubation set up. Station 10 at 04:33 GMT LN maker stopped. Run low on LN. Being looked at. Need to check levels more regularly. LN filling up slowly. Smell in deck lab. Being investigated. Results of ExL_05 suggest strong Fe response.	**Station 10** (*-56.418, -0.576*)
	0503	0703	**Event_29** Deploy Stainless Steel CTD010_SS to 2500m, 24 bottles fired at 12 depths, Corday sampled from deep for DOC.	
	0602	0802	Clean UW end. 3.15V high 4.095V-0.005	
5/1	0336	0536	**Ship slows to 4kts to sample TMC Tow Fish 3:36 (water for Corday)**	**Station 11** (*-53.282, -1.192*)
	0500	0700	**Event_30** Deploy Stainless Steel CTD011_SS to 1000m, 24 bottles fired at 12 depths. Step in mixed layer. Development of subsurface Chl feature. Transit to North – hope to pass front into warmer water. Aim to deploy Ti-CTD here as not sampled on passage south. Ti and SS CTDs deployments out of sync both in location and depth samples. Need to coordinate to align 'party depth' on both systems.	
	1236	1436	**Event_31** Deploy metal-free CTD0010_T to 1000 m, Full depth Transit North to 30S 5W. Alter heading to 50S 4W in order to sample high biomass feature identified by satellite. Associated with front. Lots of Icebergs	**Station 12** (*-52.328, -1.381*)
6/1			Arriving at 50S 4W, watching underway systems to locate high biomass feature. No overside operations. Science meeting for 1500 – Karel to talk. Ship to look into propulsion system when on station. Underway sampling continues. Water clearly greener	
7/1	0232	0432	**Ship speeds up to 4kts to sample TMC Tow Fish** Diel sampling while on station. Reasonable day for this. High cloud.	**Station 13** (*-49.323, -4.246*)
	0515	0715	**Event_32** Deploy Stainless Steel CTD012_SS to 1000m, 24 bottles fired at 12 depths. Double chlorophyll peak. Second is below 1% light. Possibly sinking or subducted water?	
	0714	0914	**Event_33** Deploy TMC CTD011_T to 3214 m. All 24 bottles fired. Features in Chl from Event_32 less clear.	

			Move to NW (47.5S, 6W) . Aim to stay in high biomass water but allow centre of storm to pass South of us. Still some icebergs around.	
8/1			**Ship slows to 4kts to sample TMC Tow Fish** Wind picked up in night. Gusts of 61 mph. Not made waypoint. Moved out of high-biomass water in the night but back in it for incubation set up. Now hove-to in high-biomass water.	
9/1	0302	0502	**Ship speeds up to 4kts to sample TMC Tow Fish. Bottles for Corday. Very dynamic frontal systems. Generally high biomass.**	**Station 14** (*-48.351, -5.365*)
	0500	0700	**Event_34** Deploy Stainless Steel CTD013_SS to 1000m, 24 bottles fired at 12 depths.	
			Repositioned due to currents.	
	0655	0855	**Event_35** Deploy TMC CTD012_T to 1000 m. 12 bottles fired.	
			Iceberg sampling. Calm weather enabled sampling round Iceberg. Underway samples collected. Water freshened in lee of iceberg. Steam to North	
10/1	0300	0500	**Station 15; Ship slows down to 4kts to sample TMC Tow Fish.**	
			Lower biomass, blue water: Raining. Fog	**Station 15** (*-45.346, -5.248*)
	0410	0610	**Event_36** Deploy TMC CTD013_T to 1000 m. 12 bottles fired.	
			Repositioned due to currents.	
	0553	0753	**Event_37** Deploy Stainless Steel CTD014_SS to 1000m, 24 bottles fired at 12 depths.	
			Steam to North Nitrate now not detectable Process to start of Eddy sampling	
11/1			**Station 16**	**Station 16** (*-41.882, -5.149*)
	0406	0606	**Event_38** Deploy TMC CTD014_T to 1000 m. 12 bottles fired. Station 16, 1000m Ti CTD, 12 bottles fired at 12 depths	
			Repositioned due to currents.	
	0538	0738	**Event_39** Deploy SS CTD015_T Station 16, 1000m steel CTD, 24 bottles fired at 12 depths, party depth: 37 m	
	1957	2157	Move to North. Issue with transmissiometer. Also radiometry not working and cloud cover prevents sat images. Hard to identify higher biomass region to sample. Rest of the day's data should be discounted	
12/1	0304	0504	**Station 17; Ship slows down to 4kts to sample TMC Tow Fish.**	**Station 17** (*-40.369, -5.098*)
	0459	0659	**Event_40** Deploy SS CTD016_T Station 16, 1000m steel CTD, 24 bottles fired at 12 depths, party depth: 37 m	
	0707	0907	**Event_41** Deploy TMC CTD015_T to full depth. Recovered due to fault then redeployed.	
	0752	0952	**Event_42** Deploy TMC CTD015_T to full depth. 24 bottles fired at 24depths. 3696 m wire deployed.	

			Diel sampling until 18:00 on station	
13/1	0302	0502	**Station 18; Ship slows down to 4kts to sample TMC Tow Fish**	**Station 18** (*-38.337, -5.066*)
	0450	0650	**Event_43** Deploy TMC CTD016_T to 1000 m. 1000m Ti CTD, 12 bottles fired at 12 depths.	
	0613	0813	**Event_44** Deploy SS CTD017_T 1000m steel CTD, 24 bottles fired at 12 depths, party depth: 20 m	
14/1			**Station 19; Ship slows down to 4kts to sample TMC Tow Fish**	
	0410	0610	**Event_45** Deploy TMC CTD017_T to 1000 m. 14 bottles fired at 12 depths.	**Station 19** (*-35.26, -5.049*)
	0536	0736	**Event_46** Deploy SS CTD018_S 1000m steel CTD, 24 bottles fired at 12 depths, party depth: 67 m. Clear DCM.	
			Nitrate now not detectable Process to start of Eddy sampling	
	1608	1808	**Event_47** Station 20, Eddy 1, 800m CTD018_T, 12 bottles fired at 11 depths	**Station 20** (*-34, -4.998*)
	2151	2351	**Event_48** Station 21, Eddy 2, 800m Ti CTD019_T, 12 bottles fired at 11 depths	**Station 21** (*-33.43, -4.819*)
15/1	0400	0600	**Event_49** Station 22, Eddy 3, 800m Ti CTD020_T, 12 bottles fired at 11 depths	**Station 22** (*-32.869, -4.629*)
	0959	1159	**Event_50** Station 23, Eddy 4, 800m Ti CTD021_T, 13 bottles fired at 11 depths	**Station 23** (*-32.299, -4.45*)
	1609	1809	**Event_51** Station 24, Eddy 5, 800m Ti CTD022_T, 13 bottles fired at 11 depths	**Station 24** (*-31.729, -4.271*)
			Lots of rubbish in water – possibly associated with eddy?	
			Tow fish twisted, caught in pressure under hull, OEG group resolved and made way.	
	2207	0007	**Event_52** Station 25, Eddy 6, 800m Ti CTD023_T, 13 bottles fired at 11 depths	**Station 25** (*-31.169, -4.08*)
16/1	0408	0608	**Event_53** Station 26, Eddy 7, 800m Ti CTD024_T, 13 bottles fired at 11 depths	**Station 26** (*-30.6, -3.9*)
			Sampling eddy success, ADCP suggest passage was through centre. Good use of Sal Chl and SST to target feature.	
			Science meeting 15:00. Nice data emerging. Interesting datasets from Eddy. Nice to see what you can capture with adaptative sampling. Ben gave overview of mooring and plan for upcoming recovery.	
17/1			Furthest north process station.	
	0300	0500	Moved off station for sampling @ 4-5kts for incubation water.	
	0500	0700	**Event_54** Station 27, 800m SS CTD019_SS, to 1000 m	**Station 27** (*-27.999, -3*)
	0710	0910	**Event_55** Station 27, Ti CTD025_T, to full depth 24 bottles fires at 24 depths. max. wire 4722m	
			Crew exercise (fire drill)	
18/1	0233	0433	Slowing down to 4-5kts for incubation water.	
	0409	0609	**Event_56** Station 28, Ti CTD026_T, to 1000 m depth 12 bottles fires at 12 depths	**Station 28** (*-28.214, -0.908*)
	0537	0737	**Event_57** Station 28, SS CTD020_SS, to 1000 m depth 24 bottles fires at 12 depths, party depth: 93m	

			Ships safety meeting 10:30 (ships time)	
			Cleaning non-tox system	
	0613	0813	Ship stopped for 30 min at 11:00 (GMT) to check propulsion.	
			Continue to mooring	
19/1	0250	0450	Slowing down to 4-5kts for incubation water.	
	0443	0643	**Event 58** Station 29, 1000m Ti CTD27, 12 bottles fired at 12 depths	**Station 29** (*-28.85, 2.734*)
	0609	0809	**Event 59** 1000m steel CTD022_SS, 24 bottles fired at 12 depths, party depth: 100m	
			Tool box for mooring	
20/1			**Slowing to 4 knts for water. Final small incubation set up.**	
	0408	0608	**Event 60** CTD028_T, 8 bottles fired at 8 depths. Only 8 bottles as running low on sample bottles.	**Station 30** (-29.473, 6.273)
	0507	0707	**Event 61** Station 30, 1000m steel CTD022_SS, 24 bottles fired at 12 depths	
21/1	0420	0620	**Event 62** Tow Fish Recovery – Last TMC UW sample at 0600 Ships time	**Station 31 - Mooring** (-30.024, 9.516)
	0545	0745	**Event 63** Mooring released	
			No captain meeting.	
			Mooring Recovery – Great job by deck team to recover quickly. Sunny day slight swell Recovery was fast. Samples intact although ones below sediment trap had become loose and bunched. Probably during deployment. Sediment trap unfortunately did not work or recover samples.	
			Steel winch test (not deep enough to resolve issues)	
	0931	1131	Steel Tow Fish Test	
22/1	0022	0222	**Event 64** Station 31, full-depth CTD029_T, 24 bottles fired at 24 depth, bottle 23 misfired, only TA/DIC, pH and nuts samples taken (first bottle fail in over 50 CTD casts, great job from techs and equipment working well)	**Station 31** (-30.019, 9.521)
	0519	0719	**Event 65** Station 31, 2500m steel CTD023_SS, calibration cast mooring instruments, 24 bottles fired at 8 depths (at every depth 2 bottles were fired, remaining ones at surface)	
			End of overside operations	
			Leave station at 10:00 UW sampling non-toxic continues.	
			Heading for chlorophyll filament coming from Benguela upwelling. Outside EEZ	
			Underway Fluorometer seems low and flat. Changed at 0900 (Ships) – Bubble in transmissometer. Watching May stop to find 5000m deep water on route.	
23/1			UW sampling every 4hrs heading to upwelling filament.	
	0600	0800	Swapped UW fluorometer to 246	
			Trying to find deep water to test ships winch. > 5000m.	
24/1			**On filament location** (-22.999, 10.500)	**-22.999 10.500**
			Sampling every 4 hrs	
			Nutrient sampling stops at 10:00 (Ships time)	

			FCM, PABS, Chlorophyll stops at 14:00 (Ships time) Underway continuous measurements run until 20:30 (Ships time) – after sunset BBQ on deck Leave station – sampling stops. Head into EEZ and port.	
25/1			Continue toward port	**-22.964, 11.643**
26/1	0443	0643	**Preparing to dock in Namibia**	-22.847°, 14.429°

DY172 Cruise Overview

The following figures and tables summarise preliminary datasets collected from the ships systems, CTD profiles and samples measured at sea. All data are currently preliminary.

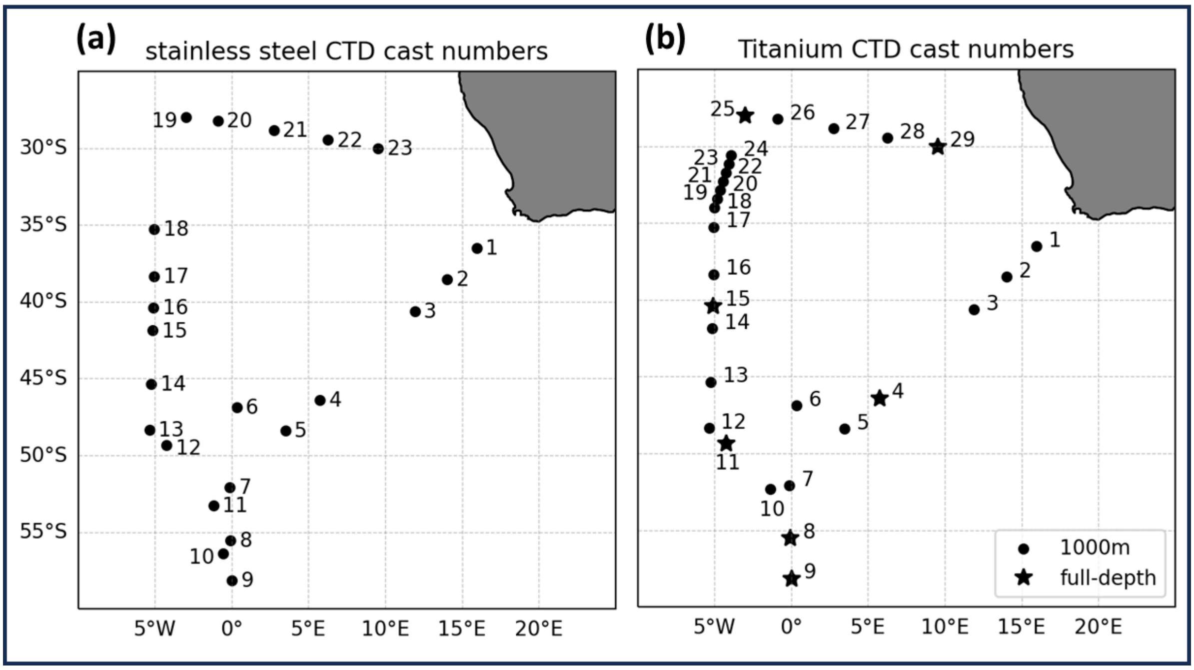
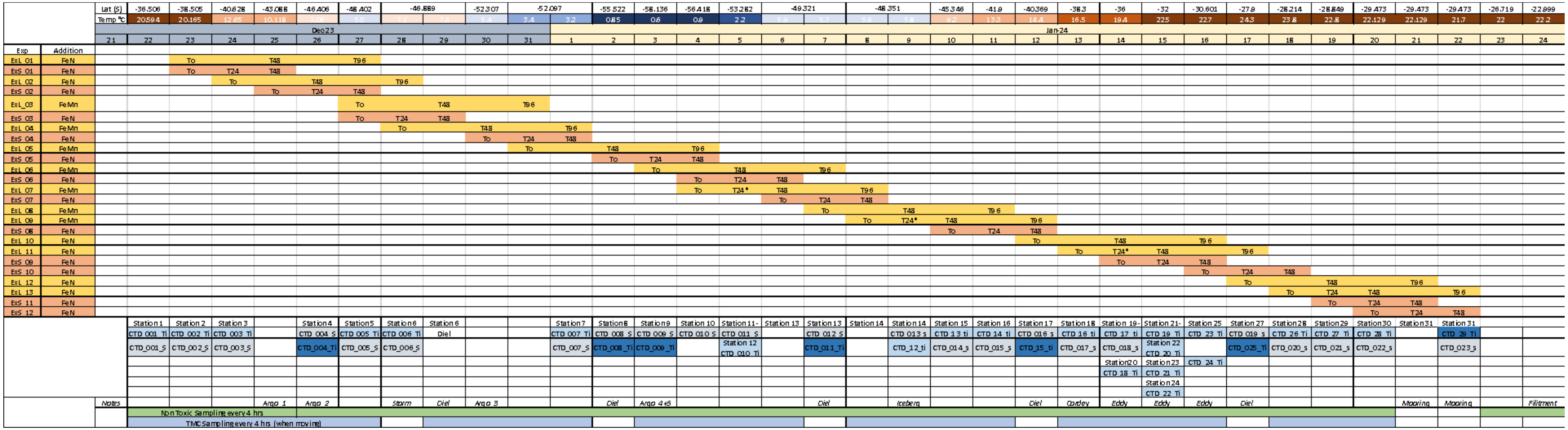


Figure 2 Location and number of Stainless Steel (SS) CTD casts **(a)** and Trace Metal Clean (TMC) Titanium (Ti) CTD casts **(b)** conducted during DY172. The location and number of full depth Ti-CTDs are starred (see event log).



		Lat (S)	-36.506	-38.505	-40.628	-43.088	-46.406	-48.402	-46.889		-52.307	-52.097		-55.522	-58.136	-56.418	-53.282	-49.321		-48.351		-45.346	-41.8	-40.369	-38.3	-36	-32	-30.601	-27.8	-28.214	-28.849	-29.473	-29.473	-29.473	-26.719	-22.899
		Temp °C	20.594	20.165	12.65	10.118	7.06	5.5	7.1	7.4	5.4	3.4	3.2	0.85	0.6	0.9	2.2	5.9	5.7	5.6	5.8	9.2	13.3	14.4	16.5	19.4	22.5	22.7	24.3	23.8	22.8	22.129	22.129	21.7	22	22.2
		Dec-23											Jan-24																							
		21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Exp	Addition																																			
ExL 01	FeN			To		T48		T96																												
ExS 01	FeN			To	T24	T48																														
ExL 02	FeN				To		T48		T96																											
ExS 02	FeN					To	T24	T48																												
ExL_03	FeMn							To		T48		T96																								
ExS 03	FeN							To	T24	T48																										
ExL 04	FeMn								To		T48		T96																							
ExS 04	FeN										To	T24	T48																							
ExL 05	FeMn											To		T48		T96																				
ExS 05	FeN													To	T24	T48																				
ExL 06	FeMn														To		T48		T96																	
ExS 06	FeN															To	T24	T48																		
ExL 07	FeMn															To	T24•	T48		T96																
ExS 07	FeN																	To	T24	T48																
ExL 08	FeMn																		To		T48		T96													
ExL 09	FeMn																			To	T24•	T48		T96												
ExS 08	FeN																					To	T24	T48												
ExL 10	FeN																							To		T48		T96								
ExL 11	FeN																								To	T24•	T48		T96							
ExS 09	FeN																									To	T24	T48								
ExS 10	FeN																											To	T24	T48						
ExL 12	FeN																												To		T48		T96			
ExL 13	FeN																													To	T24	T48		T96		
ExS 11	FeN																														To	T24	T48			
ExS 12	FeN																															To	T24	T48		
			Station 1	Station 2	Station 3		Station4	Station5	Station6	Station 6			Station7	Station8	Station9	Station 10	Station 11-	Station 13	Station 13	Station 14	Station 14	Station 15	Station 16	Station 17	Station 18	Station 19-	Station 21-	Station 25	Station 27	Station 28	Station29	Station30	Station31	Station 31		
			CTD 001 Ti	CTD 002 Ti	CTD 003 Ti		CTD 004 S	CTD 005 Ti	CTD 006 Ti	Diel			CTD 007 Ti	CTD 008 S	CTD 009 S	CTD 010 S	CTD 011 S		CTD 012 S		CTD 013 s	CTD 13 ti	CTD 14 ti	CTD 016 s	CTD 16 ti	CTD 17 ti	CTD 19 Ti	CTD 23 Ti	CTD 019 s	CTD 26 Ti	CTD 27 Ti	CTD 28 Ti		CTD 29 Ti		
			CTD_001_S	CTD_002_S	CTD_003_S		CTD_004_Ti	CTD_005_S	CTD_006_S				CTD_007_S	CTD_008_Ti	CTD_009_Ti		Station 12 CTD 010 Ti		CTD_011_Ti		CTD_12_ti	CTD_014_s	CTD_015_s	CTD_15_ti	CTD_017_s	CTD_018_s	Station 22 CTD 20 Ti		CTD_025_Ti	CTD_020_s	CTD_021_s	CTD_022_s		CTD_023_s		
																										Station20	Station23	CTD 24 Ti								
																										CTD 18 Ti	CTD 21 Ti									
																											Station24									
																											CTD 22 Ti									
		Notes				*Argo 1*	*Argo 2*		*Storm*	*Diel*	*Argo 3*			*Diel*	*Argo 4+5*				*Diel*		*Iceberg*			*Diel*	*Cordoy*	*Eddy*	*Eddy*	*Eddy*	*Diel*				*Mooring*	*Mooring*		*Filitment*
			Non Toxic Sampling every 4 hrs																																	
			TMC Sampling every 4 hrs (when moving)																																	

Table 1

Summary of scientific events during DY172. Dates, types and locations of experimental nutrient addition experiments are shown alongside the water temperature recorded from the underway system in the morning of the day shown. The table also includes station numbers and CTD events. Stainless steel CTDs are in grey, titanium in blue (full depth Ti_CTDs are shown in dark blue)

Composite of vertical profiles collected from CTDs

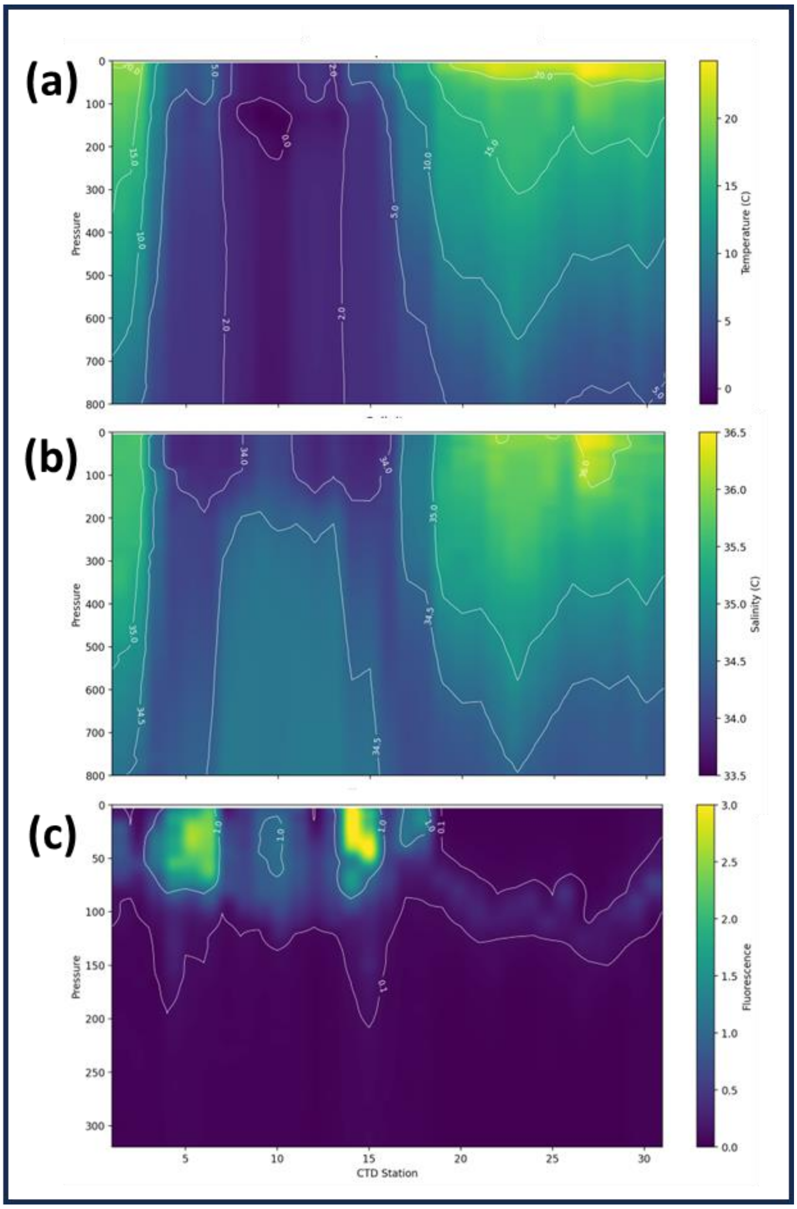


Figure 3 Vertical profiles of Temperature (°C) **(a)**, Salinity **(b)** and Fluorescence **(c)** from sensors mounted on CTD packages. Data is from SS_CTD where both Ti and SS CTDs deployed. Temperature and Salinity data is from surface to 800 m while Fluorescence data is from surface to 300 m. Station number is shown on X-axis. Chlorophyll is concentrated at the surface in high biomass stations to the south, whereas a clear deep-chlorophyll maximum at 80-100 m develops in low biomass regions to the North.

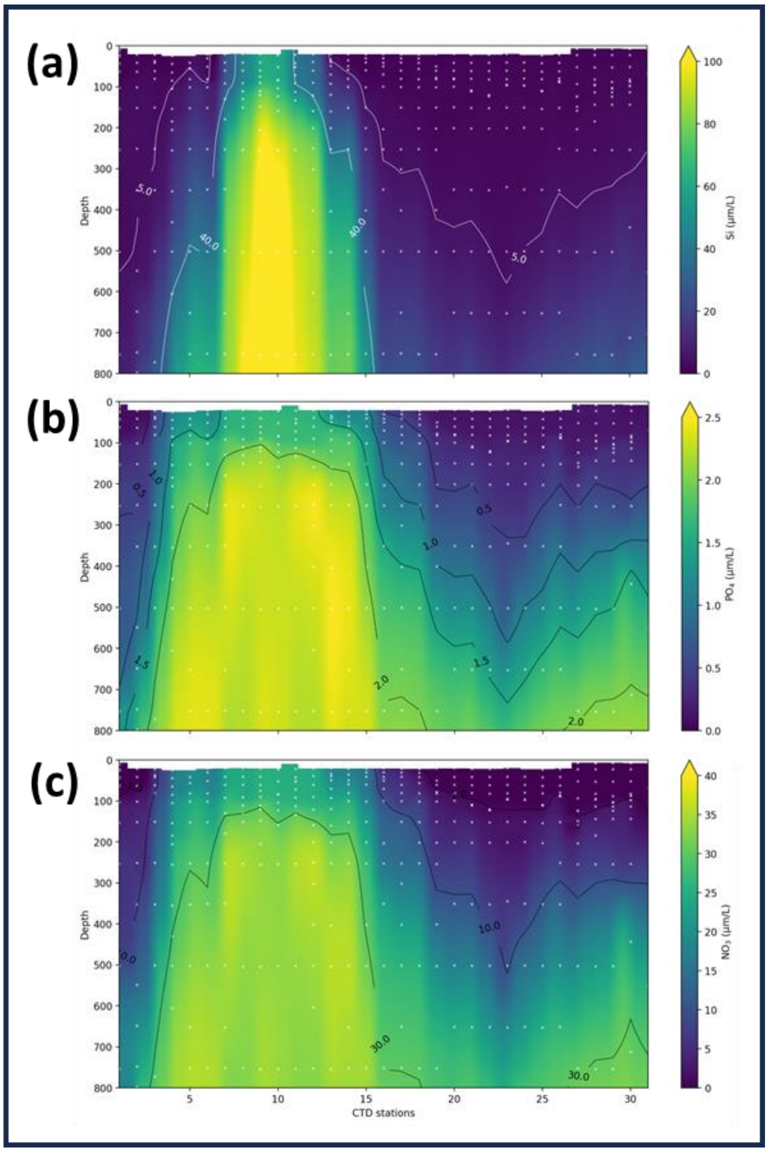


Figure 4 Vertical profiles of macronutrients collected from vertical profiles during DY172: Silica **(a)**, Phosphate **(b)** and Nitrate **(c)**. Data is from surface to 800 m. Clear contrast in macronutrient distribution both vertically and latitudinally where nutrients increase to the South, notably Silica only increases in the very furthest South stations. A clear minimum in macronutrients can be seen at the centre of the Agulhas ring (mode-water-eddy) sampled at station 23-24.

Composite of surface data

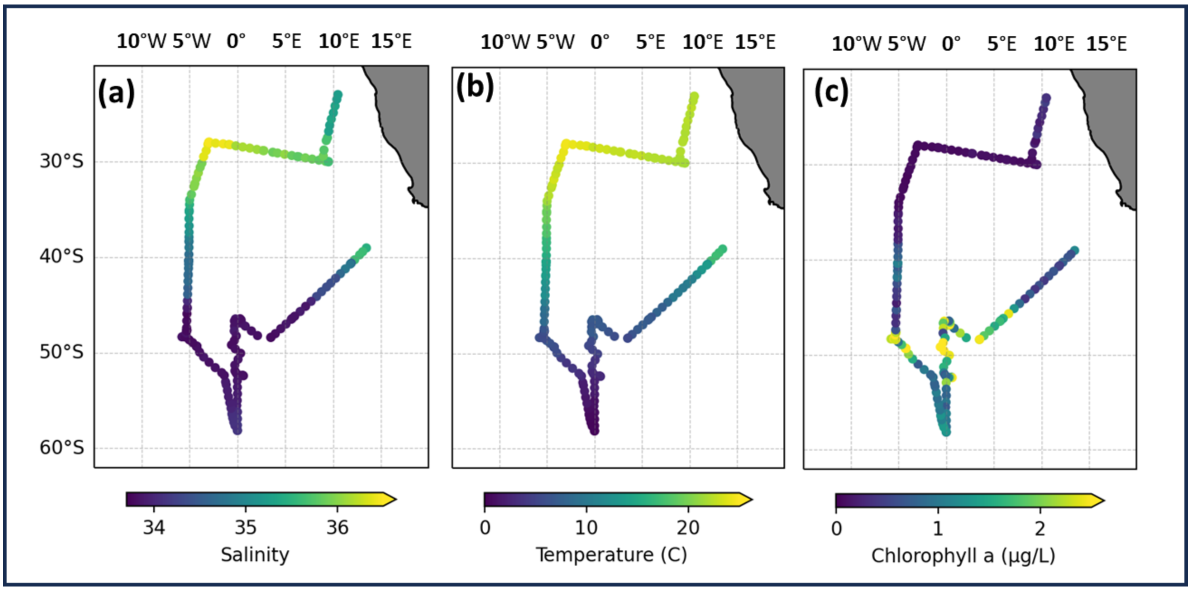


Figure 5 Surface data of Salinity **(a)**, Temperature (°C) **(b)** and Chlorophyll **(c)** (see underway log). Samples collected routinely every 4 hrs during cruise.

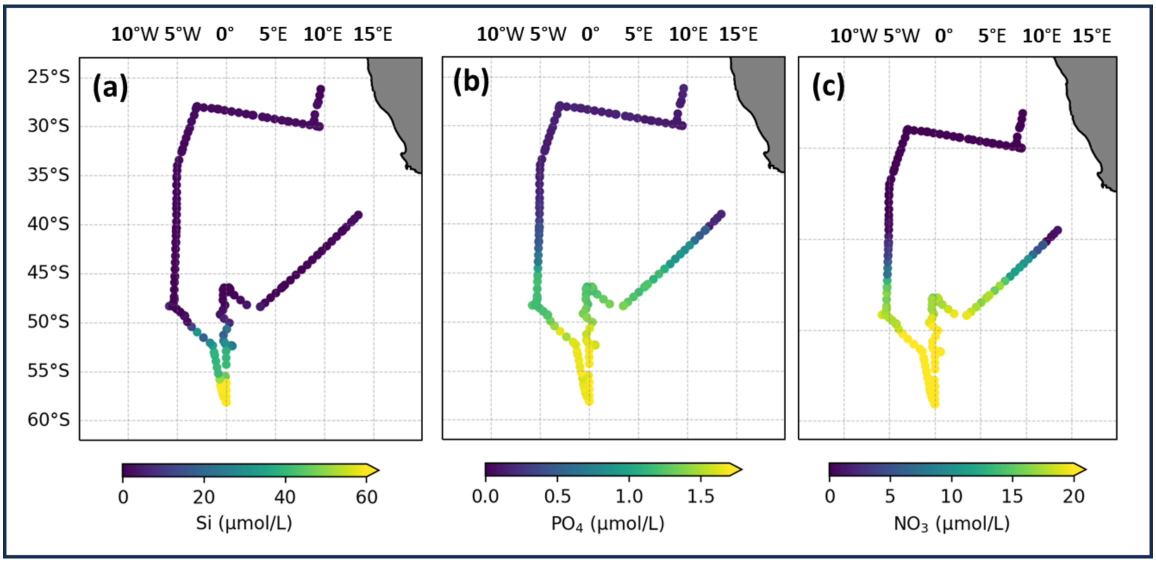


Figure 6 Surface data of Silica **(a)**, Phosphate **(b)** and Nitrate **(c)** (see underway log). Samples collected routinely every 4 hrs during cruise.

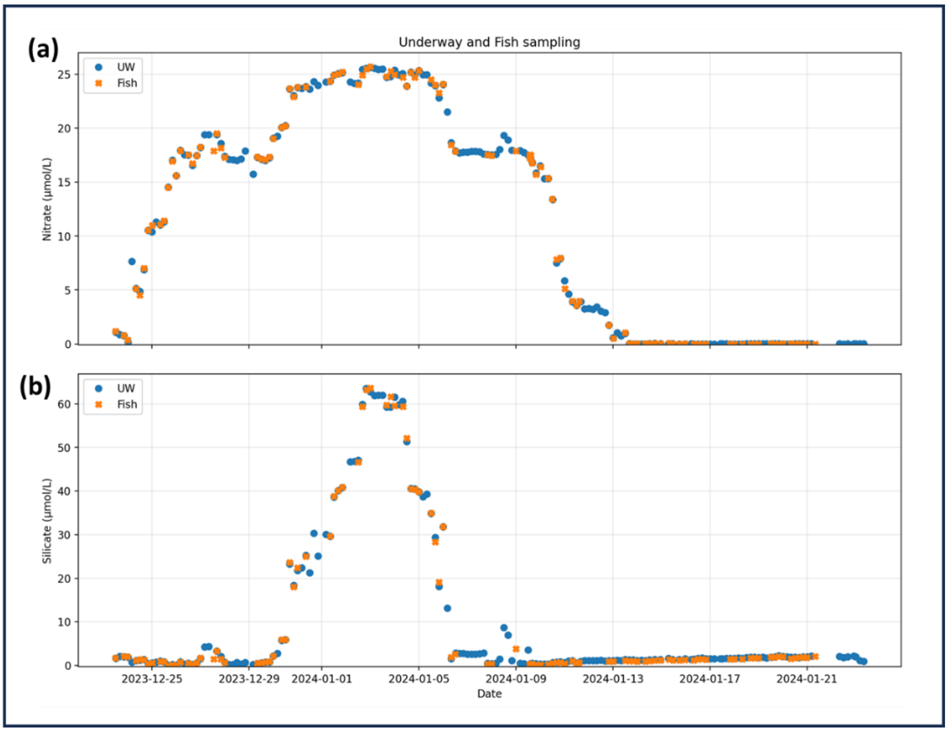


Figure 7 Comparison of Nitrate and Silica data measured from TMC underway sampling (orange) and Ships-Non-Toxic sampling (blue) over cruise.

Agulhas - ring sampling

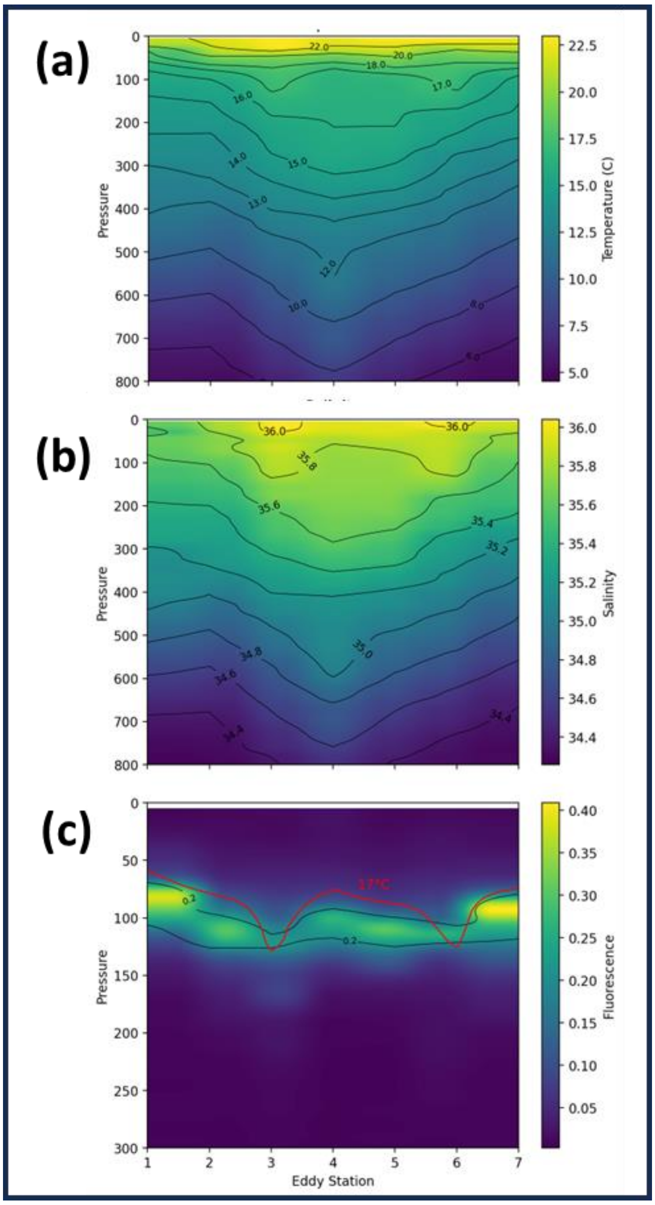


Figure 8 During transect into low-biomass water of South Atlantic Gyre, an Agulhas ring feature was identified in satellite imagery. Given its position, a decision was taken to sample the feature intensively using the Ti_CTD package and CTD profiles were obtained going through the centre of the eddy. Vertical profiles of Temperature (°C) **(a)**, Salinity **(b)** and Fluorescence **(c)** from sensors mounted on CTD packages. Data is from SS_CTD where both Ti and SS CTDs deployed. Temperature and Salinity data are from surface to 800 m, while Florescence data is from surface to 300 m. 'Eddy' station number is shown (see CTD logs). A clear depression of the main thermocline is evident at the centre of the eddy, while a possible uplift of the seasonal thermocline generated a 'mode' water lens. Chlorophyll concentrations appear lower than background through station 3-6 with slight increase in the centre.

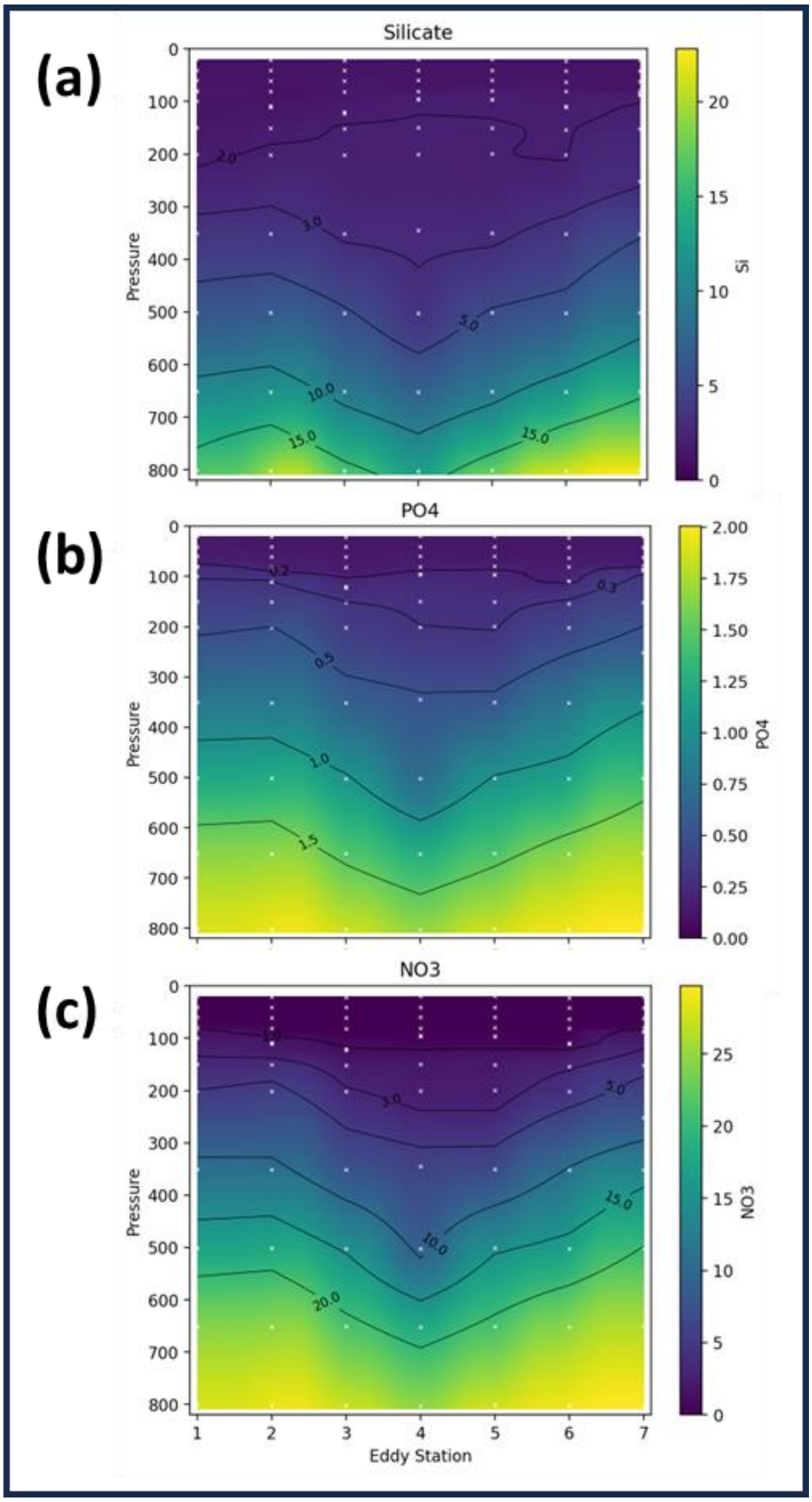


Figure 8 Vertical profiles of macronutrients collected from vertical profiles during sampling of Agulhas ring: Silica **(a)**, Phosphate **(b)** and Nitrate **(c)**. Data are from surface to 800 m. A clear depression of nutrients at eddy centre. Possible evidence of mode-water maintaining nutrients at 100 m.

Iceberg sampling

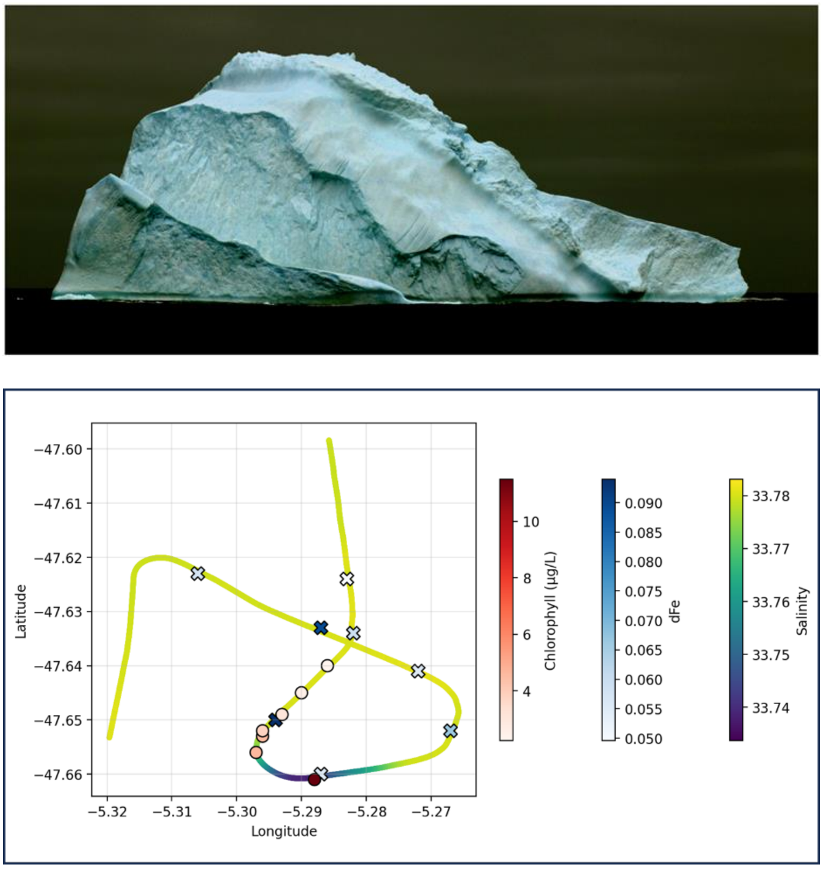


Figure 9 A high concentration of icebergs was encountered during the DY172 during transect to North. Possibly thought to originate from Weddell Sea. Icebergs can be sources of iron, stimulating productivity. Owing to favourable weather, the opportunity was taken to sample the wake of an iceberg to determine iron concentrations associated with fresh water. Figure shows cruise track round iceberg fresh water in the wake.

Underway photosynthetic physiology

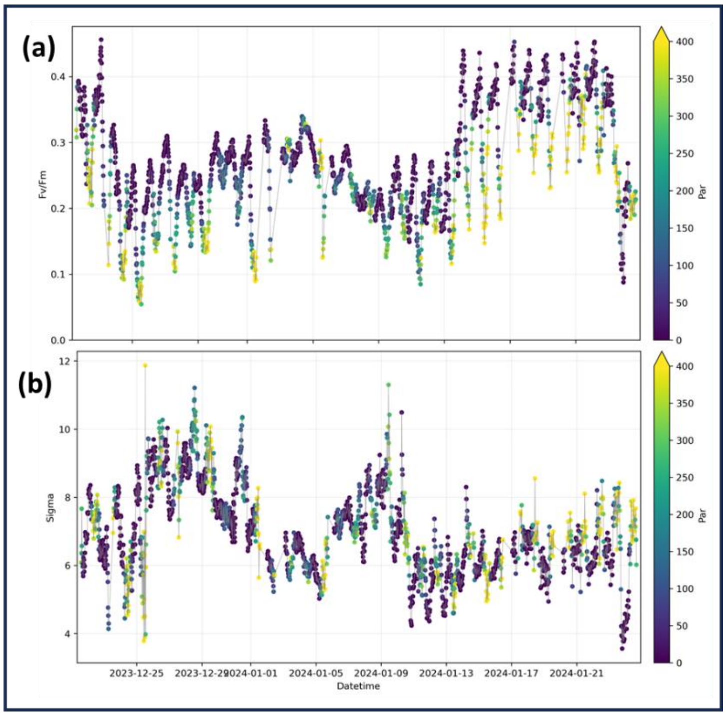


Figure 10 A suite of underway sensors were deployed on DY172 that measure active fluorescence and can be diagnostic of the physiology of the in situ phytoplankton community. An example is shown in this figure from the STAFS instruments run continuously on the cruise measuring the ships-non-toxic water system. Panel (a) shows Fv/Fm, the photosynthetic electron transfer efficiency, and panel (b) shows σPSII, the cross section of light harvesting antenna. Superimposed onto this is the measured light intensity PAR. Data shown clear diel signatures as well as differences with time as samples in different oceanic regions were measured.

Sensors and Moorings

Paul Henderson & Jon Short (National Marine Facilities)

CTD Cast Summary:

Total number of casts: 52

Stainless steel frame with water samplers: 23

Titanium frame casts with water samplers: 29

Deepest casts:

Stainless steel frame – 2500m (Cast 023S)

Titanium frame – 4940m (Cast 029T)

Stainless-Steel CTD:

CTD Wire

CTD Wire 2 was used for all casts. The wire was mechanically and electrically terminated on DY166 and re-load tested to two tonnes at the start of DY172. Resistance and insulation of the cable were checked periodically. The torque setting on the fasteners of the mechanical termination was checked throughout and no slippage was noted. The termination was left on at the end of the DY172 to be used on DY173.

CTD Wire 2 before cast 001S readings:

Resistance 74.8 Ohms Insulation >1000 MOhms @500 VDC

CTD Wire 2 readings after cast 023S:

Resistance 74.3 Ohms Insulation >1000 MOhms @500 VDC

CTD sensor set-up

CTD frame was set-up for DY172 with primary conductivity, temperature and dissolved oxygen sensors on the 9plus and secondary conductivity and temperature sensors on the vane. Other sensors on the frame were altimeter, fluorometer, transmissometer, backscatter and 2 x PAR. Full sensor information can be found on the Sensor Information Sheet – Appendix 1. Backscatter data was spiky/blocky between casts 017S and 020S. This was caused by a fault with the BBRTD cable (+15V wire of the BRTDD cable was reading 129Ohms). This was resolved by swapping out the cable after cast 020S.

Water Samplers

OTE 20L Water Samplers were used on the stainless-steel frame and performed well throughout the cruise with all water samplers firing successfully on every cast. The carousel was removed from the frame at the start of the cruise to wash and exercise the release mechanisms. A number of the water samplers' taps had their O-rings replaced over the cruise.

Trace Metal Free (Titanium) CTD:

CTD Cable and Winch

The 21mm Lebus MFCTD Contingency Winch was set up and maintained by OEG. The cable was terminated and load tested to 1.5 tonnes at the start of DY172. A Yalegrip was used as a backup to the mechanical termination. The termination was left in place at the end of DY172 for use on DY180.

21mm Lebus MFCTD Cable before cast 001T readings:
Resistance 144 Ohms Insulation 380 MOhms @500 VDC
21mm Lebus MFCTD Cable after cast 029T:
Resistance 142 Ohms Insulation 220 MOhms @ 500VDC

CTD sensor set-up

CTD frame was set-up for DY172 with primary conductivity, temperature and dissolved oxygen sensors on the 9plus and secondary conductivity and temperature sensors on the vane. Other sensors on the frame were altimeter, fluorometer, transmissometer, backscatter, pH and 1 x PAR (for casts 019T-024T only). Full sensor information can be found on the Sensor Information Sheet – Appendix 1. Two AMT Deep pH sensors had to be replaced during DY172. 349 was replaced after cast 008T due to poor data shape over a large pH range. 348 was replaced after cast 015T due to a consistent large spike between 400m and 700m.

Water Samplers

10L Trace Metal Free Water Samplers 01 – 24 were used on the titanium frame. Water Samplers were stored, sampled and cleaned in the clean laboratory. For deployment water samplers were affixed to the CTD by the CTD technician and then cocked by the science party. Water sampler 23 did not close on cast 029T. A number of water samplers required minor repairs such as replacing ferules or reseating taps.

SeaBird Data Processing:

Basic post-processing of the CTD cast data was carried out following guidelines established with BODC (ref. Moncoiffe 7th July 2010).

Both the stainless-steel and titanium CTD casts were processed using SBE Data Processing, V7.26.7. The following modules were used to process the data:

- Data Conversion
- Bottle Summery
- Align CTD
- CellTM
- Derive
- Bin Average
- Strip
- ASCII Out

CTD2MET processing was carried out for each cast stainless-steel CTD and sent to the Met Office.
Configuration reports for the SeaSave setup can be found in Appendix 2
All Seabird and associated CTD data and documents were saved to the following folder:
current_cruise\Sensors_and_Moorings\DY172\CTD

Autosal:
A Guildline Autosal 8400B salinometer, S/N: 71185, was used for salinity measurements. The salinometer was located in the Salinometer laboratory. Bath temperature was set at 21°C with the ambient room temperature being approximately 18.5-19.0°C. The salinometer was standardised before the first set of samples. Once standardised the Autosal was not adjusted for the duration of the cruise. A standard was analysed before and after each crate of samples to monitor & record drift. Standards were recorded in the spreadsheets as '0'. Standard deviation was set to 0.0002.
Standards used:
IAPSO Standard Seawater
Batch: P167
Expiry: 21st Feb 2026
K15 = 0.99988
Practical Salinity = 34.995

A program written in Labview called "Autosal" was used to record data for salinity values. Salinity samples were taken and analysed from CTD casts and the results tabulated with CTD salinity data in spreadsheets *DY172_SS_Sanlinity_Data.csv* and *DY172_Ti_Sanlinity_Data.csv*.
Guildline Autosal 8400B salinometer 65764 was set-up as a spare instrument.
All Autosal and associated salinity data were saved to the following folder:
current_cruise\Sensors_and_Moorings\DY172\AUTOSAL

Ship Scientific Systems

Emmy McGarry (Ship Scientific Systems)

Cruise ID	Departure	Arrival	SSS Technician
DY172	21DEC2023	26JAN2024	Emmy McGarry

Cruise Overview:

Ship Scientific Systems (SSS) is responsible for operating and managing the Ship's scientific information technology infrastructure, data acquisition, compilation and delivery, and the suite of ship-fitted instruments and sensors in support of the Marine Facilities Programme (MFP)

The work sites were (1) The South Pacific Gyre and (2) The Iron Limiting HNLC in the Southern Ocean.

The main objectives for SSS in the service of the science party on this cruise were:

1. Acquire underway data and metadata, including sea-surface, meteorological, position and attitude, depth and multibeam swath.
2. Provide services for recording metadata and events and monitoring data streams.
3. Provide basic IT support.

All times in this report are in UTC.

Scientific computer systems:

Underway data acquisition

Data from the suite of ship-fitted scientific instrumentation was aggregated onto a network drive on the ship's file server. This was available throughout the voyage in read-only mode to permit scientists to work with the data as it was acquired. A Public network folder was also available for scientists to share files.

A copy of these two drives are written to the end-of-cruise disks that are provided to the Principal Scientist and the designated data centre.

The designated data centre for this cruise is: British Oceanographic Data Centre

List of logged ship-fitted scientific systems:

/Cruise_Reports/DY172_Ship_fitted_information_sheet.docx

The data acquisition systems used on this cruise are detailed in the table below. The data and data description documents are filed per system in the *Data* and *Documentation* directories respectively within Ship Systems folder on the cruise data disk.

TABLE 1: DATA ACQUISITION SYSTEMS USED ON THIS CRUISE.

Data acquisition system	Usage	Data products	Directory system name
Ifremer TechSAS	Continuous	NetCDF ASCII pseudo-NMEA	/TechSAS/
NMF RVDAS	Continuous	ASCII Raw NMEA	/RVDAS/
Kongsberg SIS (EM122)	Continuous	Kongsberg .all	/Acoustics/EM-122/
Kongsberg EA640	Continuous	None, redirected to Techsas/RVDAS RAM	/Acoustics/EA-640/
UHDAS (ADCPs)	Continuous	ASCII raw, RBIN, GBIN, CODAS files	/Acoustics/ADCP/

Data description documents per system:

/Cruise_Reports/DY172_Ship_fitted_information_sheet.docx

Data directories per system:

/Cruise_Reports/DY172_Ship_fitted_information_sheet.docx

Significant acquisition events and gaps:

On this cruise, the NMF Event Logger was used with CSV records of events saved to the cruise data directory.

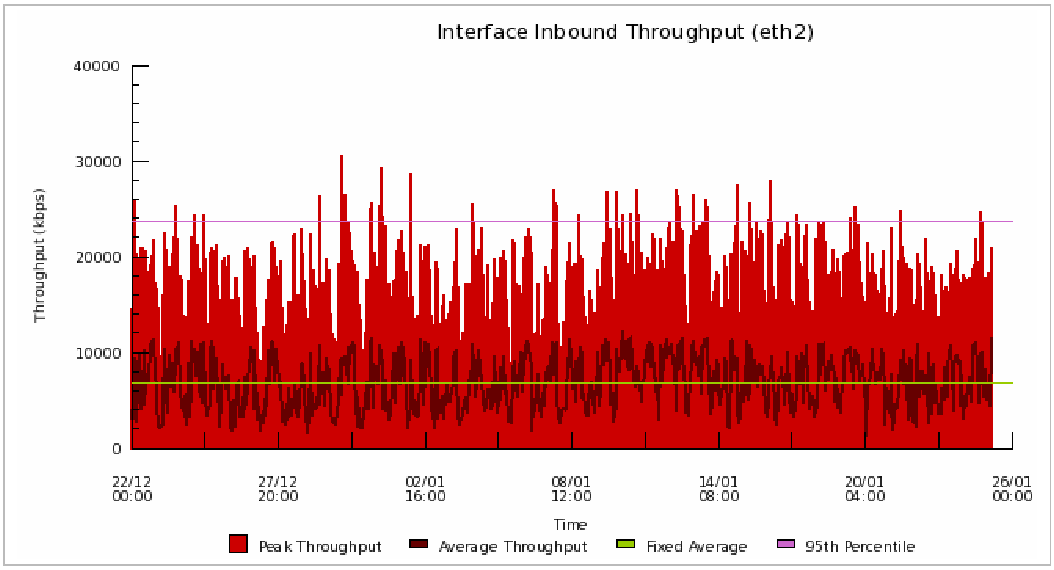
Path and pattern to event log CSV files:

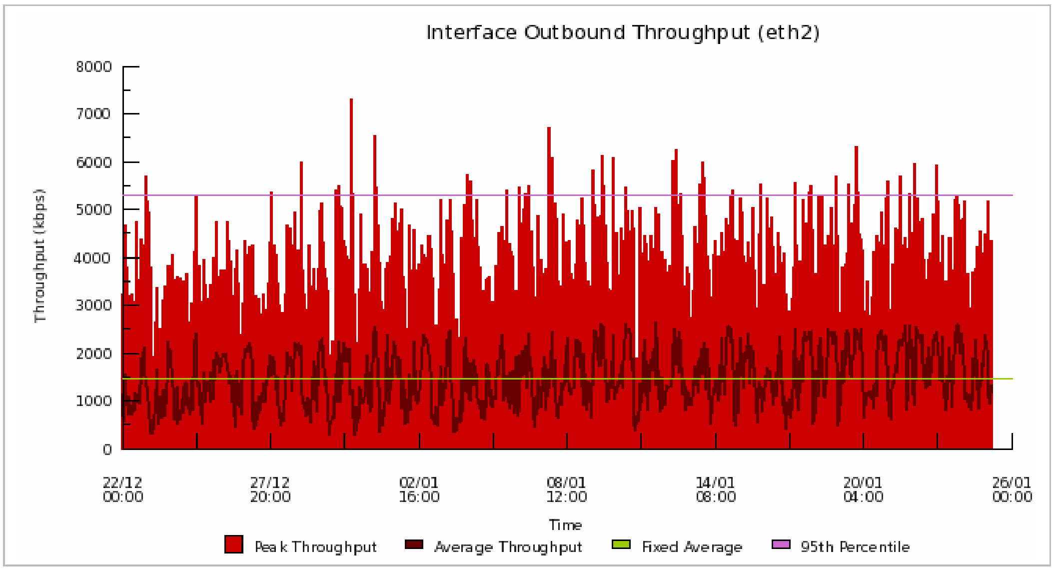
/Cruise_Reports/Event_Logs/backups/csv/[logName]/*.csv

Internet provision:

Satellite communications were provided with both the VSAT and Fleet Broadband systems.

The ship operated with bandwidth controls to prioritise business use. No major loss of signal.





WAN Interface Throughput Summary (eth2)

Data Direction	Total Data (MB)	Throughput Avg (Mbps)	Throughput Max (Mbps)
Inbound	2453884.078	6.78	30.50
Outbound	527205.766	1.46	7.31

Instrumentation:

Coordinate reference

> ***Path to ship survey files:***
> **/Ship_Systems/Documentation/Vessel_Survey**

Origin (RRS Discovery)

> ***All coordinates, unless otherwise specified, use the following convention:*** **Central reference point (0,0,0) at Frame 44, centreline, main deck with sense (X+ fwd, Y+ stbd, Z+ down). This CRP is at (32.4m, 0m, -7.4m) with respect to the ship's absolute stern, centreline, baseline.**

The ship's survey (Parker Maritime, 2013) defines two systems of reference point using two different central reference points (CRPs):

1. (0,0,0) at Frame 0 (aft-most frame, 6m forward from stern), centreline (centre of keel), baseline (ship's bottom-most longitudinal).
2. (0,0,0) at ship's centre of gravity (CG), Frame 44 (26.4m forward from Frame 0 at 0.6m framespacing), centreline (centre of keel), main deck (7.4m up from baseline).
3.

The survey coordinate sense is X is positive forward, Y positive starboard, and Z positive down. The coordinate order in the survey is (Y,X,Z), but unless otherwise noted, all coordinates are given elsewhere as (X,Y,Z).

For all scientific purposes, unless otherwise stated, the coordinate system is referenced using the second system, with the CRP at the CG.

Multibeam:

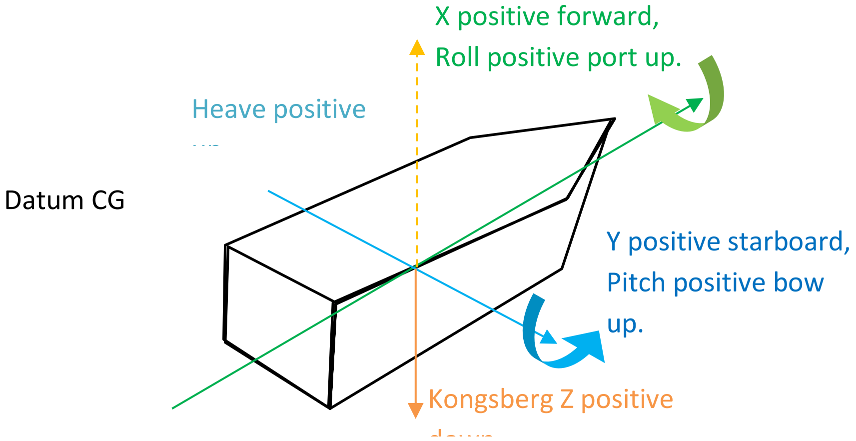


Figure 1: Conventions used for position and attitude. On the Discovery, the Datum is the CRP at the CG.

The Kongsberg axes reference conventions are (see Figure 1) as follows:

1. X positive forward,
2. Y positive starboard,
3. Z positive downward.

The rotational sense for the multibeam systems and Seapath is set to follow the convention of Applanix PosMV (the primary scientific position and attitude system), as per Figure 1.

Primary scientific position and attitude system:

The translations and rotations provided by this system (Applanix PosMV) have the following convention:

1. Roll positive port up,
2. Pitch positive bow up,
3. Heading true positive to starboard,
4. Heave positive up.

Position, attitude and time:

System	Navigation (Position, attitude, time)		
Statement of Capability	/Ship_Systems/Documentation/GPS_and_Attitude		
Data product(s)	NetCDF: /Ship_Systems/Data/TechSAS/NetCDF/ Raw NMEA: /Ship_Systems/Data/RAM/ CSV: /Ship_Systems/Data/RAM/CSV		
Data description	/Ship_Systems/Documentation/TechSAS /Ship_Systems/Documentation/RVDAS		
Other documentation	/Ship_Systems/Documentation/GPS_and_Attitude		
Component	Purpose	Outputs	Headline Specifications
Applanix PosMV	Primary GPS and attitude.	Serial NMEA to acquisition systems and multibeam	Positional accuracy within 1 m.
Kongsberg Seapath 330	Secondary GPS and attitude.	Serial and UDP NMEA to acquisition systems and multibeam	Positional accuracy within 1 m.
Oceaneering CNav 3050	Correction service for primary and secondary GPS and dynamic positioning.	RTCM to primary and secondary GPS	Positional accuracy within 0.15 m.
Fugro Seastar / MarineStar	Correction service for primary and secondary GPS and dynamic positioning.	Corrections to primary and secondary GPS	Positional accuracy within 0.15 m.
Meinberg NTP Clock	Provide network time	NTP protocol over the local network.	

Significant position, attitude or time events or losses:

Date	Time start*	Time end*	Event
27DEC2023	05:42 06:04	05:57 06:33	POSMV thrashing event. Use Position from Seapath
28DEC2023	04:58 06:02	05:59 06:19	POSMV thrashing event. Use Position from Seapath
29DEC2023	04:51 06:02	05:57 06:17	POSMV thrashing event. Use Position from Seapath
02JAN2024	10:39	10:47	POSMV thrashing event. Use Position from Seapath
07JAN2024	09:53 10:00	09:58 10:18	POSMV thrashing event. Use Position from Seapath
08JAN2024	09:11 21:52	10:27 22:30	POSMV thrashing event. Use Position from Seapath
09JAN2024	08:25	10:06	POSMV thrashing event. Use Position from Seapath
14JAN2024	08:51	09:16	POSMV thrashing event. Use Position from Seapath

Ocean and atmosphere monitoring systems:

SURFMET

System	SURFMET (Surface water and atmospheric monitoring)	
Statement of Capability	/Ship_Systems/Documentation/Surfmet	
Data product(s)	NetCDF: /Ship_Systems/Data/TechSAS/NetCDF/ Raw NMEA: /Ship_Systems/Data/RAM/ CSV: /Ship_Systems/Data/RAM/CSV	
Data description	/Ship_Systems/Documentation/TechSAS /Ship_Systems/Documentation/RVDAS	
Other documentation	/Ship_Systems/Documentation/Surfmet	
Calibration info	See Ship Fitted Sensor sheet for calibration info for each sensor.	
Component	Purpose	Outputs
Inlet temperature probe (SBE38)	Measure temperature of water at hull inlet.	Serial to Interface Box.
Drop keel temperature probe (SBE38)	Measure temperature of water in drop keel space.	Serial to Interface Box.
Thermosalinograph (SBE45)	Measure temp. and conductivity at sampling board. Salinity is calculated.	Serial to Interface Box.
Interface Box (SBE90402)	Signals management.	Serial to Moxa.
Debubbler	Reduces bubbles through instruments.	None.
Transmissometer (CST)	Measure of transmittance.	Analogue to NUDAM.
Fluorometer (WS3S)	Measure of fluorescence.	Analogue to NUDAM.
Air temperature and humidity probe (HMP155)	Temperature and humidity at met. platform.	Analogue to NUDAM.
Ambient light sensors (PAR, SKE510; TIR, CMP6)	Ambient light at met. platform.	Analogue to NUDAM.
Barometer (PTB210)	Atmospheric pressure at met. platform.	Analogue to NUDAM.
Anemometer (Windsonic)	Wind speed and direction at met. platform.	Serial to Moxa.
NUDAM	A/D converter.	Serial NMEA to Moxa.
Moxa	Serial to UDP converter.	UDP NMEA to Surfmet VM.
Surfmet Virtual Machine	Data management.	UDP NMEA to TechSAS, RVDAS.

Component	Calibrated product steps
SBE38: Temperature (°C)	No calibration to apply because the residuals are below uncertainty.
SBE45: Temperature (°C)	No calibration to apply because the residuals are below uncertainty.
SBE45: Conductivity (S m^{-1})	No calibration to apply because the residuals are below uncertainty.

CST: Transmission (%)	$\frac{Product=(Data-V_{dark})}{(V_{ref}-V_{dark})}$. Here product has units % and data, V_{dark} and V_{ref} have units V.
WS3S: Fluorescence (µg L^{-1})	$Product = Coefficient \times (Data - Offset)$. Here product has units µg L^{-1}, coefficient has units µg L^{-1} V^{-1}, and data and offset have units V.
HMP155: Temperature (°C)	No calibration to apply because the residuals are below uncertainty.
HMP155: Relative humidity (%)	No calibration to apply because the residuals are below uncertainty.
PTB210: Pressure (hPa)	No calibration to apply because the residuals are below uncertainty.
SKE510: PAR (W m^{-2})	$Product = Data \times \left(\frac{10^6}{Coefficient}\right)$. Here product has units W m^2, data has units 10^{-5} V, the 10^6 scalar has units µV V^{-1}, and coefficient has units µV m^2 W^{-1}.
CMP6: TIR (W m^{-2})	$Product = Data \times \left(\frac{10^6}{Coefficient}\right)$. Here product has units W m^2, data has units 10^{-5} V, the 10^6 scalar has units µV V^{-1}, and coefficient has units µV m^2 W^{-1}.
Windsonic: Wind speed (m s^{-1})	No calibration to apply.
Windsonic: Wind direction (m s^{-1})	No calibration to apply.

Note that while the residuals (difference of reference and measured) are below uncertainty and the output is considered calibrated for the SBE38, SBE45, HMP155, and PTB210 instruments, a regression could still be made between the reference and measured data (see the calibration certificate) if desired. Follow the steps below:

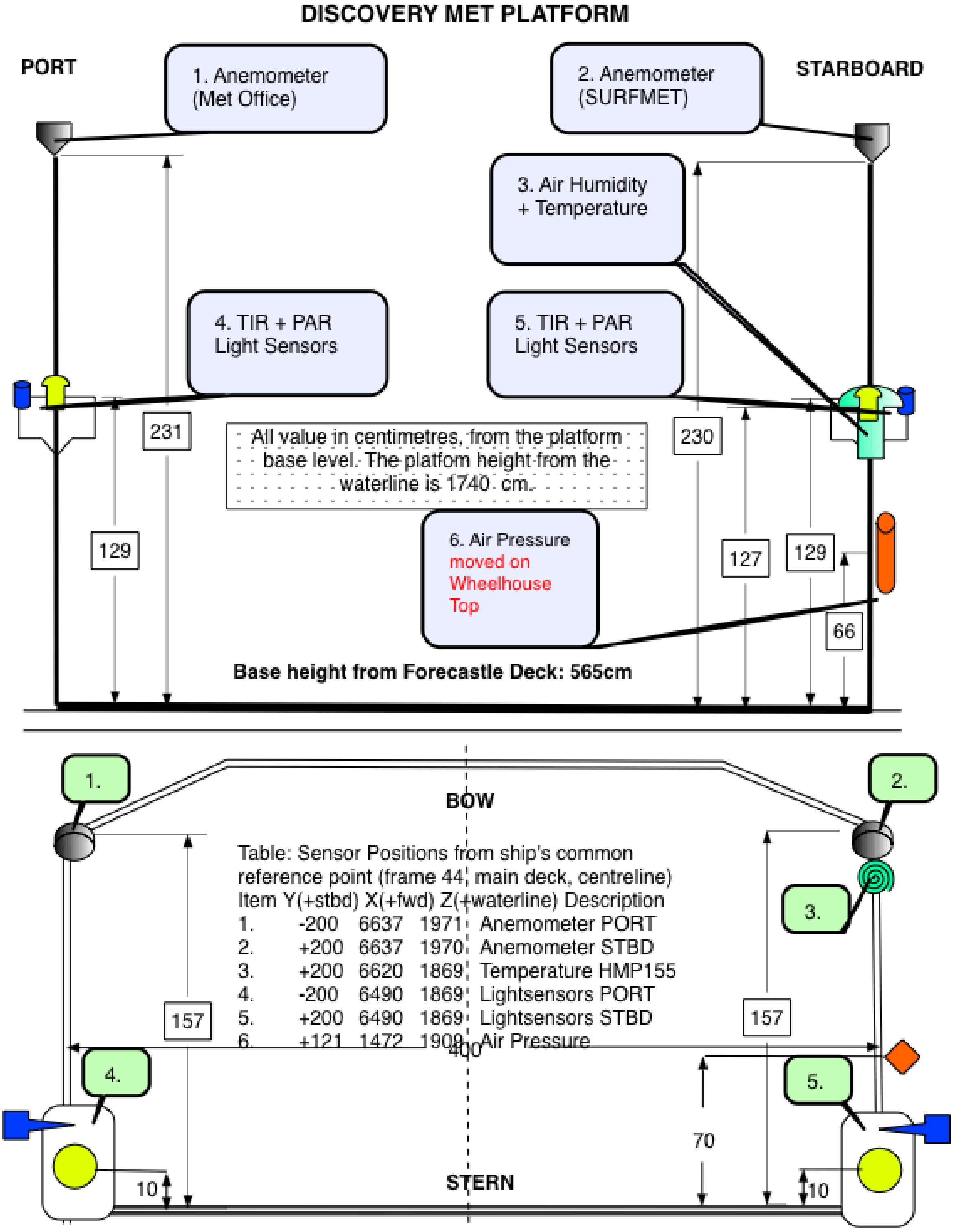
1. Calculate $y = Bx + A$ from calibration data, where x is reference data.
2. $\frac{Product=(Data-A)}{B}$.

The NMF Surfmet system was run throughout the cruise, excepting times for cleaning, entering and leaving port, and whilst alongside. Please see the separate information sheet for details of the sensors used and whether their recorded data have calibrations applied or not.

Surface water sampling board maintenance:

Date	Time start*	Time end*	Event	Trans high (V)	Trans low (V)
21DEC2023	11:23		Underway On		
27DEC2023	10:29	15:55	Broken pipe, underway off		
28DEC2023	05:54	06:08	Underway Clean	4.665	0.05
04JAN2024	05:50	06:02	Underway Clean	4.095	0.05
11JAN2024	05:49	19:57	Underway Clean. Issues with Transmissometer so disruptions through the day	4.88	0.05
18JAN2024	06:13	06:37	Underway Clean	4.75	0.05
22JAN2024	06:00	06:23	Swapped fluorometer		

The system was cleaned prior to the cruise.



All value in centimetres, from the platform base level. The platfom height from the waterline is 1740 cm.

Table: Sensor Positions from ship's common reference point (frame 44, main deck, centreline)

Item	Y(+stbd)	X(+fwd)	Z(+waterline)	Description
1.	-200	6637	1971	Anemometer PORT
2.	+200	6637	1970	Anemometer STBD
3.	+200	6620	1869	Temperature HMP155
4.	-200	6490	1869	Lightsensors PORT
5.	+200	6490	1869	Lightsensors STBD
6.	+121	1472	1908	Air Pressure

Pumped seawater flow rates (ml/min):	1600
Seawater intake depth (m):	5.5

Wave radar:

System	WAMOS Wave Radar	
Statement of Capability	/Ship_Systems/Documentation/Wamos	
Data product(s)	NetCDF: /Ship_Systems/Data/TechSAS/NetCDF/ Raw NMEA: /Ship_Systems/Data/RAM/ CSV: /Ship_Systems/Data/RAM/CSV Raw: /Ship_Systems/Data/Wamos/	
Data description	/Ship_Systems/Documentation/TechSAS /Ship_Systems/Documentation/RVDAS	
Other documentation	/Ship_Systems/Documentation/Wamos	
Statement of Capability	/Ship_Systems/Documentation/Wamos	
Component	Purpose	Outputs
Rutter OceanWaves WAMOS	Measure wave height, direction, period and spectra.	Summary statistics in NMEA to TechSAS and RVDAS. Spectra files.
Furuno Radar	Measures radar reflection on sea surface.	Radar data to WAMOS.

The wave radar magnetron requires bi-annual replacement. Following replacement, WAMOS needs to collect wave data within 5 km of another wave height sensor over the full range of sea-states in order to derive wave height calibration coefficients for the new magnetron. This reference dataset can be derived by examining the ship’s track for wave buoys and downloading their data, or by using the onboard RsAqua Wave Height sensor fitted on the ship’s bow.

Hydroacoustic Systems:

System	Acoustics	
Statement of Capability	/Ship_Systems/Documentation/Acoustics	
Data product(s)	Raw: /Ship_Systems/Data/Acoustics NetCDF (EA640, EM122cb): /Ship_Systems/Data/TechSAS NMEA (EA640, EM122cb): /Ship_Systems/Data/RVDAS CSV: /Ship_Systems/Data/RAM/CSV	
Data description	/Ship_Systems/Documentation/Acoustics	
Other documentation	/Ship_Systems/Documentation/Acoustics	
Component	Purpose	Operation and Outputs
10 kHz Single beam (Kongsberg EA-640)	Primary depth sounder	Continuous, free running NMEA over serial, raw files
12 kHz Multibeam (Kongsberg EM-122)	Full-ocean-depth multibeam swath.	Continuous, free runningBinary swath, centre-beam NMEA, *.all files, optional water column data
Drop keel sound velocity sensor	Provide sound velocity at transducer depth	Continuous, free running/Discrete/Unused Value over serial to Kongsberg SIS.
75 kHz ADCP (Teledyne OS75)	Along-track ocean current profiler	Continuous, free running (via UHDAS)
150 kHz ADCP (Teledyne OS150)	Along-track ocean current profiler	Continuous, free running (via UHDAS)
CARIS	Post-processing	CARIS Project file. CARIS Vessel files

Equipment-specific comments:

EM-122 Configuration and Surveys

Path of Multibeam data on the cruise datastore:

/Ship_Systems/Data/Acoustics/EM-122

Path of EM122 CARIS Vessel Configuration File:

/Ship_Systems/Data/Acoustics/EM-122/CARIS_Processed/VesselConfig

<table>
<tr><th>Attribute</th><th>Value</th></tr>
<tr><td>Number of surveys</td><td>(Run continuously)</td></tr>
<tr><td>Date of patch test</td><td>Not undertaken.</td></tr>
<tr><td>Offsets and rotations</td><td>
<table>
<tr><th>Item</th><th>X (m, + Forward)</th><th>Y (m, + Starboard)</th><th>Z (m, + Down)</th></tr>
<tr><td>Tx transducer</td><td>19.205</td><td>1.830</td><td>6.934</td></tr>
<tr><td>Rx transducer</td><td>14.094</td><td>0.950</td><td>6.932</td></tr>
</table>
<table>
<tr><th>Item</th><th>Roll (deg)</th><th>Pitch (deg)</th><th>Yaw (deg)</th></tr>
<tr><td>Tx transducer</td><td>-0.35</td><td>-0.1</td><td>0.19</td></tr>
<tr><td>Rx transducer</td><td>-0.06</td><td>0.1</td><td>0.15</td></tr>
</table>
</td></tr>
<tr><td>Post-processing undertaken</td><td>None.</td></tr>
</table>

Other systems:

Cable Logging and Monitoring

Winch activity is monitored and logged using the CLAM system.

Remote Sensing Data

Tommy Ryan-Keogh (SOCCO, CSIR)

Remote sensing data was downloaded daily from the Copernicus Marine Data Store, including:

Product Acronym	Name	Units	Spatial Resolution	Temporal Resolution	Weblink
ADT	Absolute Dynamic Topography	m	0.25° × 0.25°	Daily	https://data.marine.copernicus.eu/product/SEALEVEL_GLO_PHY_L4_NRT_008_046/description
SLA	Sea Level Anomaly	m	0.25° × 0.25°	Daily	https://data.marine.copernicus.eu/product/SEALEVEL_GLO_PHY_L4_NRT_008_046/description
SST	Sea Surface Temperature	K	0.05° × 0.05°	Daily	https://data.marine.copernicus.eu/product/SST_GLO_SST_L4_NRT_OBSERVATIONS_010_001/description
CHL	Chlorophyll-a Concentration	mg m^{-3}	4 km	Daily	https://data.marine.copernicus.eu/product/OCEANCOLOUR_GLO_BGC_L3_NRT_009_101/description

Front Calculations

The position of the fronts was calculated from the ADT data using the definitions from Swart et al. (2010), these include:

1. Southern Boundary (SBdy): -1.244 m
2. Southern Antarctic Circumpolar Current front (SACCf): -0.943 m
3. Antarctic Polar Front (APF): -0.480 m
4. Sub-Antarctic Front (SAF): 0.030 m
5. Sub-Tropical Front (STF): 0.350 m

Post-Cruise Analysis

Compiled netCDF files of daily data from 01/12/2023 to 24/01/2024 were produced for a spatial grid of: Lon = 10°W - 20°E, Lat = 20°S to 60°S. These files were stored on the Cruise shared drive for participants to use post-cruise.

References

Swart, S., Speich, S., Ansorge, I. J., & Lutjeharms, J. R. E. (2010). An altimetry-based gravest empirical mode south of Africa: 1. Development and validation. *Journal of Geophysical Research: Oceans*, *115*(C3). https://doi.org/https://doi.org/10.1029/2009JC005299

Bio-optics

Tiera-Brandy Robinson (GEOMAR)

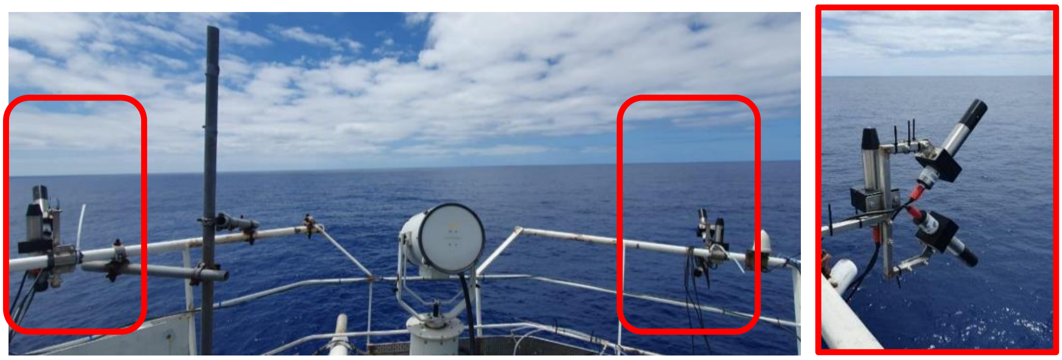


Figure 1. TriOS hyperspectral radiometers and irradiance meters on top of the mast at the bow of RRS Discovery during the cruise DY172.

Objectives:

Satellite radiometric data are used to determine many large scale ocean colour properties. However, measurements are hampered by interference from the atmosphere the signal must pass through. Radiometers installed on ships therefore, bypasses atmospheric interference and can give high resolution in-situ ocean colour data. This can be used both to improve satellite derived ocean colour measurements and to investigate small time and spatial scale changes. Furthermore, radiometers can measure the passive fluorescence of phytoplankton in the ocean as opposed to most onboard sensors which measure active fluorescence, helping to bridge the gap between onboard in situ sampling and satellite data.

Methods:

Ocean Colour Remote Sensing: Automated continuous above-water hyperspectral radiometric quantities were recorded during the whole cruise track of DY172. Two identical radiometer setups were installed. Each with one TriOS RAMSES-ACC hyperspectral cosine irradiance meter to measure incoming solar irradiance Es (λ), and two TriOS RAMSES-ARC hyperspectral radiance meters to measure total sea surface leaving radiance Lsfc (θsfc, Φ, λ) and sky-leaving radiance Lsky (θsky, Φ, λ). The radiometers were installed on the foremast of RRS Discovery at the beginning of the cruise prior to leaving port and required no adjustment during the cruise. The foremast has a height of 5.65 m, putting the radiometers at a relative height of 17.5 m above the sea surface. Lsky and Lsfc, radiance meters were placed at 45° and 135° zenith angles respectively. 50 m cables were connected to each radiometer and ran from the foremast down to a computer in the meteorological lab. Hyperspectral measurements were collected at 1-minute intervals over a spectral range of $\lambda = 320 – 950$ nm. Remote sensing reflectance (Rrs) and water leaving radiance (Lw) can be calculated from the radiometric measurements via the following equation and will be used to investigate changes in fluorescence line height (normalized to Chl a) over the cruise track.

R_rs=L_w/E_s =L_sfc-(ρ_(air-sea)*L_sky)/E_s

The two sets of radiometers (1 ACC and 2 ARC each) were placed at opposing relative azimuth angles (45° and 315°) in order to ensure that at least one set would always have minimal effect from a solar azimuth angles <90°. In addition to the application of sun glint correction, data will be optimized for each timepoint so that the sensor set with the best solar azimuth angles is used. Data processing will be completed as proposed in literature (Garaba and Zielinski, 2013) and after quality control, datasets will be made open-access via PANGAEA.

References:

Garaba, S., and Zielinski, O. (2013). Methods in reducing surface reflected glint for shipborne above-water remote sensing. Journal of the European Optical Society-Rapid publications 8.

Nutrient analysis on R.V. Discovery (Expedition DY172).

Karel Bakker (NIOZ Netherlands)

Introduction:

Nutrient measurements were made on board using a Seal QuAAtro 39 gas-segmented Continuous Flow Analyser (CFA) from the University of Southampton. Measurements were made simultaneously for four channels with Silicate Phosphate, Nitrate with Nitrite together and Nitrite separate. All measurements were calibrated with stock-standards diluted into Artificial Seawater (ASW) in the same salinity range as the samples.

Equipment and Methods:

The colourimetric methods used:

Nitrate plus Nitrite (NO_3+NO_2) is mixed with an NH4CLbuffer at pH 8 and reduced by a copperised cadmium column to Nitrite. The Nitrite is diazotized with sulphanyl-amide and naphtyl-ethylene-diamine to a pink coloured complex and measured at 540nm. Nitrate is calculated by subtracting the Nitrite value of the Nitrite channel from the 'NO3+NO2' value. Described by Grasshof, 1983. (Seawater Methods practical handbook Weinheimverlag).

Nitrite:

Diazotation of nitrite with sulphanilamide and N-(1-naphtyl)-ethylene di-ammonium dichloride to form a pink dye measured at 550nm.

Phosphate:

Ortho-phosphate is measured by formation of a blue reduced Molybdenum-phosphate-complex at pH 0.8. Potassium Antimony tartrate used as the catalyst and ascorbic acid as the reducing agent. The absorbency is measured at 880nm.

Described by J.Murphy and J.Riley, 1962. Analytica Chem.Acta

Silicate (Si) reacts with ammonium molybdate to a yellow complex and after reduction with ascorbic acid, the obtained blue silica-molybdenum complex is measured at 820nm. Oxalic acid is added to prevent formation of the blue phosphate-molybdenum complex (Strickland & Parsons, 1968).

Sample handling:

The samples from the CTD-rosette sampler were collected in 60ml high-density polyethylene syringes connected with a three-way valve and tubing to the Niskin bottles, taken directly from the CTD-rosette bottles without any air contact. After sampling on deck, the samples were left for 2 to 3 hours in the dark at lab temperature to get the same temperature as the calibration or working standards used. The CTD samples were analysed typically within 4 hours, the underway samples collected from the ships-system and from a towed Fish were analysed within 12 hours the same counts for samples from incubation experiments. Analyses were carried out using polypropylene centrifuge tubes with a volume of 15ml as sample cups fitting into the auto-sampler. All tubes were pre-rinsed three times with sample before filled. A total of approximate 1300 samples for four parameters were analysed.

Calibration and Standards:

A sampler-rate of 50 samples per hour was used (sample wash ratio 4). Calibration standards were diluted from stock standards of different nutrients and every day freshly prepared in ASW (ASW consists of 410g NaCl plus an addition of 1.85g NaHCO3 per 10 liter Ultra-Pure Water 18.2MOhm). Artificial Seawater (ASW) was also used as baseline-water for the analysis to wash out in-between the samples. Each run of the system had a correlation coefficient of at least 0.9999 for 10 calibration points, but typical 1.0000 for linear chemistry. The samples were measured from the lowest to the highest concentration in order to keep carry-over in the flow system as small as possible, i.e. from surface to deeper waters. Concentrations were recorded in 'µmol per liter' (µM/L) at an average lab temperature of 22°C. During the cruise each run, a freshly diluted mixed internal nutrient standard (nutrient Antarctic cocktail), containing, phosphate and nitrate was diluted 100 times in ASW and measured. The cocktail sample was used to monitor independently of the standards the performance of the system.

Using the AACE software of the QuAAtro for NO3+NO2 a Quadratic fit was used with the smallest residuals for the target calibration points. For the other channels a linear fit was applied.

Stock standards:

Nutrient primary stock standards were prepared at the lab home by weighing nutrient salts p.a. in de-ionised water. All standards are kept in a so-called 100% humidity box at lab temperature to prevent any concentration change by evaporation, and to be on correct for diluting with pipettes.

Phosphate: by weighing Potassium dihydrogen phosphate in a calibrated volumetric PP flask to make 1mM PO4 stock solution.

Nitrate: by weighing Potassium nitrate in a calibrated volumetric PP flask set to make a 10mM NO_3 stock solution.

Nitrite: by weighing Sodium nitrite in a calibrated volumetric PP flask set to make a 0.5mM NO_2 stock solution.

Si: by weighing in Sodium hexafluoride in a calibrated volumetric PP flask set to make a 20mM Si stock solution.

The calibration, or working standards, were prepared daily by diluting the separate stock standards, using three electronic pipettes, into four 100ml PP volumetric flasks (pre-calibrated at the NIOZ) filled-up to the mark with diluted ASW. The background values of the used ASW were measured on-board before the voyage started and added up to the standard values to compute the final calibration target values and compared with a Certified Reference Material (CRM) batch CE with assigned values very low close to zero for all four channels.

Note; the Silicate background concentration of the ASW was 0.80uM Si, the other parameters 0015uM for NO_2, 0.005uM for PO_4 and 0.025uM for NO_3+NO_2

The cocktail standard, a stock mixture of Silicate Nitrate, and Phosphate preserved with addition of 1ml saturated $HgCl_2$ per litre adjusted with a few pallets of NaOH on pH.

Quality Control:

Statistics

Quality Control

Our standards have already been proven by inter-calibration exercises from ICES and Quasimeme, and since 2006 by the Inter Comparison exercises organised by MRI, Japan.

Our cocktail standard was measured every run for three nutrients during this cruise.

To obtain international comparable results, KANSO CRM's produced by The General Environmental Technos Co., Ltd. Japan were analysed in the runs.

To gain some internal lab accuracy the used Antarctic Cocktail standard is monitored now since 2018, showing in-between runs reproducibility of 0.5 % for NO_3, 0.4% for PO_4 and 0.5% for Si.

Cocktail standard between runs data:

	average µM/L	S.D. µM/L	C.v.(%)	n
(100x dilution):				
PO_4	2.354	0.01	0.40	29 triplicates
NO_3	34.52	0.18	0.51	29 triplicates
Si	129.6	0.59	0.46	29 triplicates

Statistics:

Method Detection Limits (MDL) calculated (EPA norm), as 2.82 x S.D. of a 2% (*from the full range*) spiked samples (**n=10**).

	M.D.L µM/L	*full range µM/L*:	SD dev. µM/L
NO_3+NO_2	0.007	*35.5*	0.0025 µM/L *
NO_2	0.001	*0.5*	0.00042 µM/L
PO_4	0.001	*3.00*	0.00048 µM/L
Si	0.036	140.8	0.0127 µM/L

* Note: For a lot of surface samples NO_3 was below detection limit of 0.007uM, so any value below that in the data should be read as below detection limit.

Some statistics on samples from CTD 001 all bottles closed at one depth: In table below; the typical statistics of analysis of 24 bottles at one depth-level (1000m) taken from the Titanium trace metal frame CTD Station 001 , analyzed in one run, proofs all bottles closed for 100%

CTD001T	NO_2	Si	NO_3+NO_2	PO_4
UNIT	µmol/L	µmol/L	µmol/L	µmol/L
AVERAGE	**0.0063**	**23.00**	**28.26**	**1.926**
STDEV	**0.0013**	**0.08**	**0.043**	**0.0039**
CV %	**21.1**	**0.34**	**0.15**	**0.20**

Precision in a single run: four concentration levels with coefficient of variation (c.v.).

	Level I	SD dev.	*C.v.*	Level II	SD dev.	*C.v.*
	µM/L	µM/L	*%*	µM/L	µM/L	*%*
NO_3+NO_2	7.20	0.004	0.1	14.20	0.21	0.6
NO_2	0.125	0.003	0.2	0.215	0.002	0.1
PO_4	0.60	0.002	0.4	1.200	0.45	0.4
Si	20.8	0.042	0.2	40.8	0.037	0.09

	Level III	*SD dev.*	*C.v.*	*Level IV*	*SD dev.*	*C.v.*
	µM/L	*µM/L*	*%*	*µM/L*	*µM/L*	*%*
NO_3+NO_2	*25.60*	*0.028*	*0.1*	*35.53*	*0.035*	*0.1*
NO_2	*0.365*	*0.001*	*0.2*	*0.515*	*0.003*	*0.5*
PO_4	*2.005*	*0.003*	*0.1*	*3.005*	*0.003*	*0.1*
Si	*100.8*	*0.16*	*0.2*	*140.8*	*0.1*	*0.1*

Accuracy:

To obtain accuracy, certified reference material (CRM) for nutrients were measured at **22.0 °C** containing stable homogeneous values for PO_4, and NO_3 and Si.

The CRMs produced by KANSO lot-CR and CH were used.

CRM CR, n=12 triplicates this voyage:

	µM/L	*full range µM/L:*	StD dev. *µM/L*	C.v.(%)	*uM/kg*	**assigned *uM/kg***
NO_2	1.014	0.5	0.006	0.6	0.990	***0.97***
NO_3	5.45	*35.0*	0.04	0.7	5.319	***5.46***
PO_4	0.402	*3.00*	0.004	1.0	0.392	***0.394***
Si	14.15	*140.8*	0.049	0.3	13.81	***14.00***

CRM CH, n=12 triplicates this voyage:

	µM/L	*full range µM/L:*	StD dev. *µM/L*	C.v.(%)	*uM/kg*	**assigned *uM/kg***
NO_2	0.192	*0.5*	0.003	1.7	0.188	***0.180***
NO_3	17.25	*35.0*	0.08	0.5	16.83	***16.94***
PO_4	1.185	*3.00*	0.006	0.5	1.158	***1.172***
Si	30.28	*140.8*	0.14	0.5	29.55	***29.84***

Evaluation issues encountered during the expedition:

Setting up the QuAAtro39 cost a few days in the harbour before the expedition to get the equipment running, and adjusted for nutrient ranges expected in the Southern Ocean. After the first day of analysis I changed the sample and reagent addition to get an optimal flow with reliable peak-plateaus and had to recalculate all reagents used to be conform to the originals methods from literature.

Same time the polyethylene 1mm internal diameter, connecting the sampler to the QuAAtro, I replaced by a bigger internal diameter of around 1.5mm to avoid the Inter sample air-bubble to break, improving the peak-shape and so resulting in less dispersion over the tubing before arriving to the manifolds of the four channels used. At the most Southern Stations I did a check for evaporation over night with a sampler vial open to the labs atmosphere marked the water line with a marker, next day less than 1% evaporation was observed.

A possible post cruise data normalisation on the Antarctic Cocktail:

In all analytical runs the Antarctic Cocktail was analysed, a normalisation on its average analysed value could be performed after the expedition, what slightly will improve the consistency of the data set.

By implying the average value of the used cocktail from all runs over the expedition, and calculating by a factor with which the used run analyse file data should be multiplied with to obtain this average cocktail-value, normalised data will be obtained.

From all CTD-casts a duplicate from the deep bottle number 1 is reanalysed in the following run; by calculating the difference you get a list of reproducibility in-between the analytical runs or CTD's. The same can be calculated for the duplicate differences within the normalised data on the cocktail with results showed below.

Duplicate in-between CTD's RAW:

	Average µM/L	SD dev. µM/L	C.v.(%)
NO_3+NO_2	32.74	*0.19*	*0.57*
PO_4	2.232	*0.02*	*0.93*
Si	71.59	*0.41*	*0.56*

Normalised on Antarctic Cocktail:

NO_3+NO_2	32.74	*0.17*	*0.53*
PO_4	2.232	*0.02*	*0.90*
Si	71.59	*0.32*	*0.44*

From those reproducibility values from the duplicates in-between the runs it is possible that normalisation on the cocktail for Silicate, might obtain an even more consistent data set over the whole expedition, especially in the deep Souther Ocean near the Weddell Sea border with high silicate values. However for PO_4 and NO_3 no improvement will be seen by normalisation the data.

Note: The cocktail was needed to be diluted each day in ASW (same pipet same volumetric flask), introducing an extra error of 0.15% on precision from this.

Acknowledgement: Thanks to Cynthia Dumousseaud from the University of Southampton for let me use her QuAAtro instrument, she did all the preparations weighing in chemicals and all the packing involved!

Trace metal clean sampling - Dissolved Fe Distribution in the Southern Ocean and the South Atlantic Gyre

Maeve Lohan, Ruth Hawley (University of Southampton)

Objectives:

Iron (Fe) is an essential micronutrient for biological processes during primary production (Morel et al. 1991; Morel and Price 2003). Despite its key roles, Fe is present only at nanomolar concentrations in the ocean as a result of its low solubility under oxic conditions (Boyd & Ellwood 2010). Hence, Fe (co) limitation of primary production has been observed for example in various ocean settings (Boyd et al. 2007). The most notable of these is the Southern Ocean, which is the largest Fe-limited region in the global ocean (Martin, 1990).

To investigate the gradients in the environmental factors that limit primary productivity across the Southern Ocean and into the South Atlantic gyre, and to better constrain the biogeochemical cycling of iron in these regions, dissolved Fe (dFe) (defined as passing through a 0.2µm filter) was measured along the surface gradient and was used to place the nutrient limitation experiments into context.

Methods:

Sampling – dFe samples were collected from Ti-CTD casts at 28 stations. During transit between stations 1 and 28, dFe samples were collected from the underway towed-fish at 4-hour intervals. All sampling took place in a clean laboratory and samples were collected in acid-cleaned (one week soaked in 3 M HCl, one week in 0.5 M HCl, stored in 0.024 M HCl) LDPE bottles by attaching a 0.2 µm Sartobran filter to pressurised OTE bottles. All sampling bottles were rinsed three times with seawater prior to filling. The samples were acidified to pH 1.7 with ultrapure HCl (Romil, UpA) and left to equilibrate for at least twelve hours before analysis.

Analysis – Flow injection analysis with chemiluminescence detection (FIA-CL) was used to determine nanomolar concentrations of dFe in the surface underway samples and incubation experiments (Obata et al. 1993). At least 15 min prior to analysis samples were spiked with 60 µl 0.01 M H_2O_2 to allow any present Fe(II) to be oxidized to Fe(III). The sample was then buffered to pH 3.5 and preconcentrated on a Toyopearl resin. Upon elution by HCl, the Fe entered a reaction stream with luminol, NH_4OH and H_2O_2 to induce the chemiluminescent oxidation of luminol detected by a photomultiplier tube. Each sample was measured in triplicate with a column loading time of 120 s, resulting in a total of approximately 13 min per run.

Results:

A total of 113 surface underway samples and 43 nutrient limitation experiment samples were analysed on board for dFe. Figure 1 displays the surface dFe concentration, with the oceanic fronts overlain. Preliminary results show areas of elevated dFe concentrations (≥ 0.2 nM) coinciding with fronts, indicating that turbulent mixing at fronts across the Southern Ocean acts as an input of dFe to the surface waters, as shown in Figure 1. Away from the fronts, surface dFe concentrations are consistently low throughout the surface waters of the Southern Ocean and the South Atlantic gyre, ranging from 0.02 to 0.15 nM. East of the Meridian Line, dFe concentrations begin to rapidly increase, to a maximum of 0.35 nM. This is likely due to dFe inputs originating from the Benguela upwelling system or from Agulhas rings along the west coast of Africa. The origin of these elevated dFe concentrations will be determined by analysis of dFe profiles from the titanium CTD.

Future work:

The remaining Ti-CTD samples will be analysed for dFe at the University of Southampton using flow injection analysis with chemiluminescence detection, as described above. Additionally, all underway surface samples and Ti-CTD samples will be analysed for a suite of dissolved trace metals (Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn) and total dissolvable trace metals. Fe isotopes and particulate Fe samples from several Ti-CTD casts will also be analysed (see Ti-CTD log for a list of all samples).

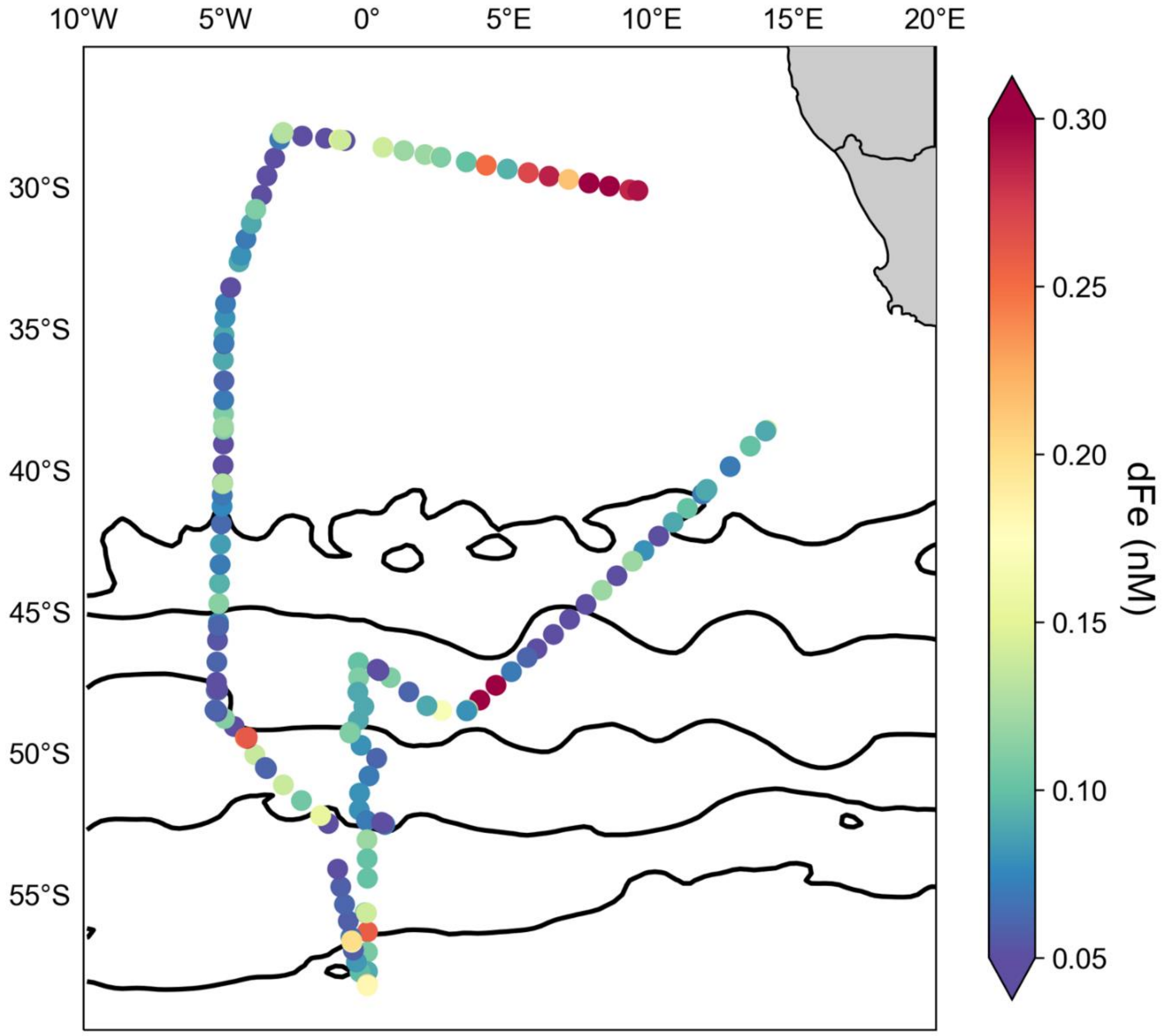


FIGURE 1. MAP OF SURFACE DFE CONCENTRATIONS, RANGING FROM 0-0.35 NM. OCEANIC FRONTS ARE OVERLAIN IN BLACK.

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Aerosol Sampling

Alex Baker (UEA)- Sampling on ship by Ben Cala (NIOZ)

Aerosol samples were collected to determine the amount of trace metal dry deposition to study region. One aerosol collector was set up on monkey island this was connected to an automatic wind sector controller whereby a sonic anemometer was mounted on a pole as high as possible above the deck facing the bow. This controls when the aerosol sampler is switched on.

Filters were loaded into the cassette in a laminar flow hood set up in the constant temperature laboratory on the ship using gloves and plastic tweezers. The filter cassette was then placed inside a holder and plastic bag and brought up to the aerosol sampler on monkey island. The cassette was then placed in the aerosol sampler and the protective cover removed and the lid shut. A new circular chart was placed in the recorder inside the door of the aerosol sampler to monitor when the sampler was switched off when not in the correct wind sector.

After 2 days the aerosol sampler was stopped and the lid opened, the protective cover was placed over the filter cassette and then placed in a plastic bag. The circular chart was also removed and labelled. These were brought down to the constant temperature laboratory and under the laminar flow hood the filter was removed using plastic tweezers and placed in a new labelled plastic bag and stored in the -20ºC freezer until analyses. This processes was repeated throughout the cruise.

Issues: -20ºC Freezer failed between 15th Jan 13;50 (DY172TM010) and the 16th. All samples were moved to another -20ºC freezer on the 16/01/0204.

Blanks were taken on the transit back to Walvis Bay. Two different blanks were collected i) cassette blank and ii) exposure blank. The cassette blank was collected by loading a filtered into the cassette which was placed in a bag for 24 hrs. The exposure blank was collected whereby a new filter was loaded into the cassette and placed in the aerosol sampler for 48 hrs without the motor being turned on.

In total 14 samples were collected from 24/12/2024 to the 23/01/2024 (Table 1).

Table 1. Aerosols samples collected during DY172

Sample id	Latitude (^{o}S) at start	Longitude at start	Date started	Date ended	Counter reading
DY172TM001	41 32.194	10 58.109 oE	24/12/2023	27/12/2023	27403.40
DY172TM002	46 29.0715	0 24.8945 oE	28/12/2023	30/12/2023	27420.49
DY172TM003	50 0.69	0 32 2 oE	30/12/2023	01/01/2024	27464.32
DY172TM004	53 36.5	0 0.15 oW	01/01/2024	03/01/2024	27488.74
DY172TM005	58 0.82	0 0.28oW	03/01/2024	05/01/2024	27522.12
DY172TM006	52 3.28	1 3.81 oW	05/01/2024	07/01/2024	27567.51
DY172TM007	49 3.26	4 2.46 oW	07/01/2024	09/01/2024	27611.83
DY172TM008	47 6.34	5 3.17 oW	09/01/2024	11/01/2024	27639.81
DY172TM009	40 7.8	5 11.4 oW	11/01/2024	13/01/2024	27678.03
DY172TM010	37 6.37	5 0.51 oW	13/01/2024	15/01/2024	27703.26
DY172TM011	31 9.6	4 3.5 oW	15/01/2024	17/01/2024	27739.05
DY172TM012	27 9.98	2 2.99 oW	17/01/2024	19/10/2024	27781.31
DY172TM013	28 5.86	2 7.82oE	19/10/2024	21/01/2024	27835.51
DY172TM014	30 1.016	9 3.13 oE	21/01/2024	23/01/2024	

Chlorophyll analysis

Joe Furby (University of Southampton), Mark Moore (University of Southampton)

In order to provide an index of overall phytoplankton biomass, water samples for the determination of chlorophyll-*a* concentrations were collected from:

i) CTD deployments
ii) Underway samples
iii) Nutrient addition bioasssay experiments (***Error! Bookmark not defined.***)

Further details specific to different sampling types can be found in the corresponding sections of the cruise report, but briefly:

i) CTD samples: The stainless steel CTD was used to collect samples from below the thermocline / deep chlorophyll maximum (deepest depths ~150-200m) to the surface, typically at 8 different depths (See Figure A72). Depending on the biomass, 200-500 mL of seawater were filtered onto Whatman glass fibre GF/F filters for total chlorophyll-*a* concentration.
ii) For the underway samples, 200-500 mL of surface water from the underway system was filtered onto Whatman glass fibre GF/F filters for total chlorophyll-*a* concentration.
iii) For the nutrient addition bioassay experiments between 50 and 100 mL of water from short term and long term nutrient and trace metal addition experiments were filtered onto Whatman glass fibre GF/F filters for total chlorophyll-*a* concentration (see separate report section). Additionally, on a sub-set of the end points from certain experiments, 150 mL of a sub-samples was filtered sequentially through polycarbonate 5 µm and 0.2 µm filters for size-fractionated chlorophyll-*a* (page 85).

In all cases, chlorophyll-*a* was extracted in 6 mL of 90 % acetone over 20 to 24 hours at 4°C in a fridge in the dark. Measurements of chlorophyll-*a* were subsequently made on board using a Turner Designs Trilogy fluorometer set up with a non-acidification kit (after Welschmeyer, 1994). The fluorometer was calibrated against a pure chlorophyll-*a* extract prior to the cruise (calibration date: 17/04/2023). A Turner solid standard (Part No. 8000-952) was used at the start of each set of readings as well as an 90% acetone blank sample to monitor for instrument drift. Blank readings are subsequently used in the calculations to determine chlorophyll-*a* concentrations (see Equation 1).

Chlorophyll-*a* concentrations in mg m^{-3} (µg L^{-1}) were calculated as:

$$Chl\ a = Dilution * (R) * (F - blank) * \left(\frac{v}{V}\right) \qquad \text{Equation (1)}$$

Dilution = 1 (unless required for an over-range sample)
(R) = response factor
F = sample fluorescence
blank = acetone blank reading
v = acetone extracted volume (6 mL)
V = filtered sample volume in mL

A total of 1182 chlorophyll samples were collected and analysed. All data are recorded within the spreadsheet on the drive 'science_public\DY172\Chlorophyll' named 'DY172_Chlorophyll a data FINAL DO NOT AMMEND'.

References:

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Sampling for phytoplankton taxonomy, HPLC pigment analysis and particulate absorption

Sarah Le Besque (University of Oxford)

Aims and Objectives:

- To observe the phytoplankton community structure across an environmental gradient from the Southern Ocean to the subtropical South Atlantic gyre.
- Numerous methods will be used to observe community structure such as pigment analysis, particulate absorption spectra, and flow cytometry, including the use of the CytoSense instrument.

Sampling:

CTD sampling:

From the stainless steel CTD, seawater was collected during every cast from the surface and the “party depth” – another depth of interest in the fluorescence profile such as the deep chlorophyll maximum (DCM). All samples were collected within the photic zone (surface ~200m of the water column).

Seawater samples were filtered for both High Performance Liquid Chromatography (HPLC) analysis of algal pigments and spectrophotometric analysis of particulate absorption (PABS). To observe shifts in phytoplankton community structure, seawater was also preserved using paraformaldehyde (PFA) for analytical flow cytometry (AFC), and using glutaraldehyde for CytoSense analysis. In addition to preserving samples, the CytoSense instrument was used on board to run CTD samples from multiple depths, using its discrete sampling settings.

Additional depths were collected for AFC and CytoSense so as to correspond with the samples taken for DNA/RNA and Protein/77K analysis.

Underway sampling:

The ship's non-toxic underway seawater system was sampled every 4 hours with water collected for PABS and HPLC filtration. Samples were preserved for FCM analysis and the CytoSense instrument ran a pre-set program to automatically measure the underway every 4 hours, creating real-time data files. Underway PABS samples will be used to calibrate the radiometers (Radiometry; GEOMAR) and the MFL instrument as described by Ryan-Keogh et al. (2023) (MFL; SOCCO, CSIR).

Experiments:

For the short nutrient addition experiments (ExS), PABS samples were collected for T0 and from the 48-hour end time point from each nutrient treatment (3 treatments, 1 control). These will be used to calibrate the MFL instrument.

For the long nutrient addition experiments (ExL), samples were collected from T0 and from the 96-hour end time point from the control and nutrient treatment that had the greatest response for AFC and CytoSense analysis. Size fractionated samples were also collected from T0 and the 96-hour time point. 500ml of seawater was filtered through a 10 µm pore filter. The filter was transferred to a 15 mL falcon tube and washed with Milli Q water to resuspend the cells. Subsamples were then preserved with PFA – 1% PFA concentration samples for AFC, and 4% PFA concentration samples for expansion microscopy. The remaining suspension was run through the CytoSense on a slow picture run so as to have an initial idea of what phytoplankton were presents at the start and end of the long experiments.

Sampling Methods:

High Performance Liquid Chromatography (HPLC):

For HPLC, between 0.5 and 4 L of seawater was filtered onto GF/F filters (nominal pore size 0.7 μm) for later extraction and analysis of pigments by HPLC. The seawater samples were filtered, then placed into cryovials, flash frozen in liquid nitrogen and stored in the -80°C freezer for transport back to the UK for further analyses.

Particulate absorption spectra (PABS):

For PABS, seawater samples between 0.125 and 2 L were filtered through 25mm diameter Whatman glass fibre GF/F filters (nominal pore size 0.7 μm), using a glass manifold on the filtration rig. The filters were placed in a plastic petri dish, taped closed, and flash frozen in liquid nitrogen before being stored in the -80°C freezer. The *in vivo* light absorption spectrum of phytoplankton and non-algal particles will be measured using a UV-Vis spectrophotometer back in Oxford, UK. Additional blank filters were collected for analysis from each individual manufacturing lot number.

CytoSense samples:

49.5 mL samples of seawater were collected from the CTD, and the ExL nutrient addition experiments. Samples were fixed with glutaraldehyde to give a final concentration of 0.25%. The samples were left in the fridge for 3 hours to allow the preservative to penetrate the cells before being transferred to the -80°C freezer for analysis in Southampton, UK, at a later date.

Both preserved and underway samples will be processed using the CytoClus software.

AFC:

1.9 mL samples of seawater from the CTD, underway, and ExL nutrient addition experiments, were fixed with PFA to a final concentration of 1% (some samples of 4% PFA were preserved from the experiments). The samples were left in the fridge for 3 hours to allow the preservative to penetrate the cells before being transferred to the -80°C freezer for analysis at a later date.

Table 1: Summary of where samples were collected during CTD sampling. Bold indicates samples for all analysis mentioned were collected (if not bold, then only samples for AFC and CytoSense were collected), italics indicate HPLC/PABS repeats were collected.

Date	CTD cast	Niskin Bottle	Nominal depth (m)	Samples collected
22/12/2023	CTD_001S	**24/23, 20/19**	**5, 20**	HPLC, PABS, AFC, CytoSense (preserved)
23/12/2023	CTD_002S	**24/23**, 17, **15**, 13	**5**, 40, **50**, 60	HPLC, PABS, AFC, CytoSense (preserved)
24/12/2023	CTD_003S	**23**, 19, **17**, 15	**5,** 35, **41**, 55	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
26/12/2023	CTD_004S	***24/23*****, 15**	***5*****, 71**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
27/12/2023	CTD_005S	**23**, **19**	**5, 30**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
28/12/2023	CTD_006S	**23, 19**	**5, 35**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
01/01/2024	CTD_007S	**23, 15**	**8, 75**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
02/01/2024	CTD_008S	**23, 15**	**5, 90**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
03/01/2024	CTD_009S	**23, 19, 15, 11**	**5, 40, 75, 110**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
04/01/2024	CTD_010S	**23, 17**	**8, 80**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
05/01/2024	CTD_011S	**23, 17**	**5, 50**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
07/01/2024	CTD_012S	**23, 17, 15, 13**	**5, 50, 70, 90**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
09/01/2024	CTD_013S	***23*****, 17**	***5*****, 50**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).

10/01/2024	CTD_014S	**23, 17, 13**	**5, 40, 80**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
11/01/2024	CTD_015S	**23, 19**	**5, 37**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
12/01/2024	CTD_016S	**23, 21,** 17	**3, 15,** 40	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
13/01/2024	CTD_017S	**23, 21**	**5, 20**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
14/01/2024	CTD_018S	**23,** 17, **15, 13**	**5**, 55, **67**, 80	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
14/01/2024	CTD_018T	**8**	**80**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
14/01/2024	CTD_019T	**7/8**	**110**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
15/01/2024	CTD_020T	**7**	**121**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
15/01/2024	CTD_021T	**7**	**94**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
15/01/2024	CTD_022T	**8**	**97**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
15/01/2024	CTD_023T	**8**	**107**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
16/01/2024	CTD_024T	**8**	**85**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
17/01/2024	CTD_019S	***24/23,*** 17, **15, 11**	***5,*** 90, **120, 155**	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
18/01/2024	CTD_020S	**23,** 17, **15,** 11	**5,** 80, **93,** 140	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).
19/01/2024	CTD_021S	**23,** 17, **15,** 11	**5,** 85, **100,** 120	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).

20/01/2024	CTD_022S	**23,** 17, **15, 13**	**5,** 85, **90,** 95	HPLC, PABS, AFC, CytoSense (preserved), CytoSense (ran).

Preliminary results – CytoSense CTD Surface:

Samples from the CTD were preserved as stated above, but also run through the CytoSense instrument on the ship. Using the Machine Learning algorithm provided by Fuchs et al. (2022), the surface CTD measurements from the CytoSense instrument were processed on board. The algorithm groups the particles by size and fluorescence. Figure 1 shows the number of cells per µL of each group – Micro-phytoplankton, orange fluorescing nano-phytoplankton, orange fluorescing pico-prokaryotic-phytoplankton, red fluorescing nano-phytoplankton, and red fluorescing pico-phytoplankton. The concentration of cells decreases dramatically as we moved into oligotrophic waters (CTD 18-22), with the highest abundance of cells being in the polar frontal zone (CTD15, CTD16).

Figure 2 shows just the micro-phytoplankton at each CTD at the surface, with the highest abundance of micro-phytoplankton being in the polar frontal zone on the southerly leg of the cruise (CTD4-5). However, this is not seen when we pass through the polar frontal zone as we head north (CTD 15). This could be because of the change in longitude between stations, or an error with the instrument – it will be checked with underway data and the preserved CytoSense samples from the CTD.

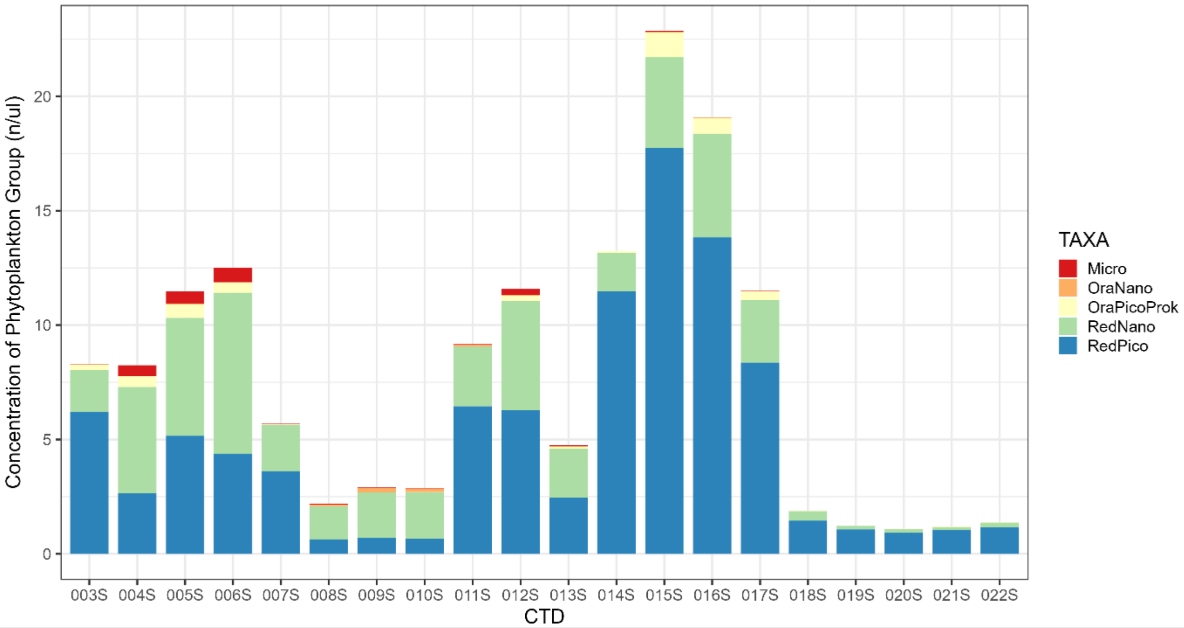

***Figure 1** - Phytoplankton group concentration (n/ µL) produced by applying the Fuchs (2022) ML algorithm to CytoSense data from surface CTD samples.*

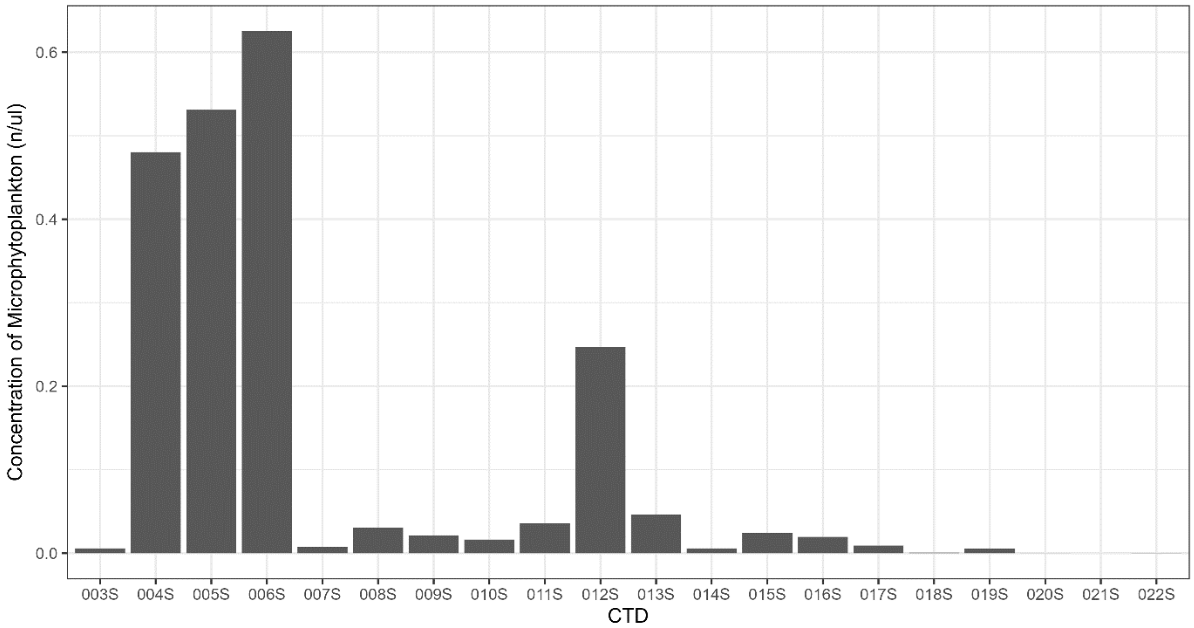

Figure 2 – *Micro-phytoplankton concentration (n/ µL) produced by applying the Fuchs (2022) ML algorithm to CytoSense data from surface CTD samples.*

References:

Ryan-Keogh, T. J., Bone, E. L., Thomalla, S. J., Lain, L., Smith, M. E., Bernard, S., & Vichi, M. (2023). Spatial and temporal drivers of fluorescence quantum yield variability in the Southern Ocean. Limnology and Oceanography. https://doi.org/10.1002/lno.12295

Fuchs, R., Thyssen, M., Creach, V., Dugenne, M., Izard, L., Latimier, M., Louchart, A., Marrec, P., Rijkeboer, M., Grégori, G. and Pommeret, D., 2022. Automatic recognition of flow cytometric phytoplankton functional groups using convolutional neural networks. Limnology and Oceanography: Methods, 20(7), pp.387-399.

Single Turnover Active Fluorometry of Enclosed Samples (STAFES)

Mark Moore (University of Southampton), Tommy Ryan-Keogh (SOCCO, CSIR,) Nina Schuback (Chelsea Technologies)

Chlorophyll active fluorescence analysis, specifically Single Turnover Active Fluorometry (STAF), can provide a useful non-destructive and rapid index of the physiological status of phytoplankton. STAF can be used to measure a suite of parameters pertaining to the photosynthetic physiology of the entire phytoplankton community, most commonly an estimate of the photosystem II photochemical efficiency (Fv/Fm) which can provide a proxy of the overall photosynthetic 'health' of the community. The STAFES technique measures in real time and at high sensitivity.

Instruments summary:

Six LabSTAF instruments were used during DY172.

Serial number	Location on ship	use
19-0105-003	Chem lab	Continuous FLC
19-0105-006	Chem lab	Discrete FLC
19-0105-001	RN van	^{14}C dual incubation
19-0105-001	RN van	^{14}C dual incubation
19-0105-001	RN van	^{14}C dual incubation
21-1834-001	RN van	^{14}C dual incubation

Calibration:

All instruments were calibrated prior to the cruise, calibration files are available on the DY172 shared drive (STAFES\Instruments\calibration).

Software:

Instruments were run on RunSTAF v9.1.10 until very low biomass required updating to RunSTAF v9.1.12 with improved AutoLED and AutoPMT functions. All software versions used are available on the DY172 shared drive (STAFES\Instruments\RunSTAF).

Continuous FLC measurements:

Instrument 19-0105-003 was used to run continuous fluorescence light curves (FLC) using the same protocol throughout the cruise. The instrument was run in AutoFLC mode, with dynamic FLC and AutoHigh activated which results in the light levels of light steps to automatical adjusting during each FLC. The FLC protocol included a low light time of 200s at 20 umol quanta m^{-2} s^{-1} and a dark step of 40s, 8 light levels of 80s each and a final dark step of 60s. A dual waveband measurement (DWM) and photochemical excitation profile (PEP) were automatically run prior to each FLC. The value of Seq/Acq (number of acquisitions to be averaged) was adjusted up to 80 in very low biomass regions. Blanks were run with MQ water and 0.2 um filtrate weekly in high biomass and daily in low biomass regions. Raw data files can be found at STAFES\rawdata\ ml19-0105-003.

Discrete FLC measurements:

Instrument 19-0105-006 was used to run fluorescence light curves (FLC) on discrete samples from CTD casts (surface and 'party depth'), nutrient addition experiment (T0 & Tend of all ExpL) and on size fractionated size samples (T0 of ExpLS and ExpL and Tend of ExpL). A similar FLC setup to the continuous FLC protocol used on instrument 19-0105-003 was used (8 light steps and 1 dark step, DWM and PEP). Raw files can be found at STAFES\rawdata\ ml19-0105-006.

Quench experiment:

Starting on 01.01.2024, instrument 19-0105-006 was run with automated sample exchange to assess the rate and magnitude of relaxation from fluorescence quenching after transfer from an in situ sample into the dark sampling chamber. The instrument was run with no PEP or DWM and an FLC of one light step at 0 irradiance for 1 h. Raw files can be found at STAFES\rawdata\ ml19-0105-006.

^{14}C-dual incubation experiments:

The 4 instruments used for parallel measurements of STAF and ^{14}C-fixation were run in manual mode with protocols described in detail in the cruise report section on ^{14}C. Raw data of STAF measurements can be accessed at STAFES\rawdata\rad19-0105-001, STAFES\rawdata\rad19-0105-002, STAFES\rawdata\rad19-0105-004, STAFES\rawdata\rad21-1345-003.

Resources (LabSTAF instrument):

More information about the instrument and approach can be found in the instrument handbook, available on the Ocean Best Practice Repository: https://repository.oceanbestpractices.org/handle/11329/1531.4

The latest version of the instrument software, required to open raw files, can be downloaded at https://1drv.ms/u/s!AkUtV8PHZSmVvJ9wFOm-fSR1FbwYGQ?e=

A zenodo repository of continuous FLC data from previous cruises can be accessed on: https://zenodo.org/communities/staf_underway?q=&l=list&p=1&s=10&sort=newest

FastOcean FRRf measurements:

In addition to the STAFES measurements, single turnover active chlorophyll fluorescence measurements were also made using a Chelsea Technologies Group (CTG) FastOceanTM fluorometer on sub-samples from the nutrient addition experiments (*Nutrient Amendment Incubation Experiments*).

The instrument (SN 15-0093-002) was set up with a saturating sequence of 100 flashlets (at 2µs pitch) and a relaxation sequence of 20 flashlets (at 60 µs pitch), with 110 sequence repeats at a sequence interval of 100ms. Data was downloaded daily and analysed with ‘FastPro8’ software to derive fluorescence parameters (see above). Raw data and a scan of the completed log book are available on the ships drive at the location ‘\\dynetapp.discovery.ad.noc.ac.uk\science_public\DY172\Nutrient addition experiments\FRRf mkIII’. Processed data are available on the ships drive within the data files for the nutrient addition experiments at location: ‘\\dynetapp.discovery.ad.noc.ac.uk\science_public\DY172\Nutrient addition experiments’.

Multi-Excitation Absolute Fluorescence Quantum Yield

Tommy Ryan-Keogh (SOCCO, CSIR) and Sarah Le Besque (University of Oxford)

Aims and Objectives:

- Measure and quantify in situ variability of absolute fluorescence quantum yield – complementing extensive measurements previously performed in the region.
- Measure the effects of different nutrient stress relief on changes of absolute fluorescence quantum yield.

Sampling protocols:

A JFE Advantech Multi-excitation fluorometer (MFL; Serial Number 0013) was deployed to discriminate between phytoplankton species based on their accessory pigment composition. It is equipped with a high sensitivity chlorophyll fluorescence detector and 9 excitation LEDs (375nm, 395nm, 420nm, 435nm, 470nm, 490nm, 535nm, 570nm and 590nm), measuring phytoplankton biomass and estimating species composition. The MFL was run in continuous mode for the duration of the cruise, sampling seawater from the ship's scientific underway supply, by having it pumped under positive pressure to fill the MFL sampling chamber. The software was then initiated to record a measurement every second.

The instrument was disconnected from the ship's scientific underway supply when measurements were performed from the short-term ExS experiments (ExS01 to ExS11) or from the "party" depth of the CTD profile (from CTD08S onwards and the Eddy CTD stations). The MFL chamber was drained and using a tubing connected to a funnel it was filled with either the CTD or experimental sample until between 0.5-1 times the volume of chamber (<1.0 L) had been rinsed through. The discrete measurements were recorded for between 3-5 minutes to allow the sample to stabilise in the chamber.

The MFL was cleaned regularly during the cruise with the following measurements to check for instrument drift and biofouling:

1. Dirty measurement – fill the chamber with MilliQ water and perform measurement.
2. Empty measurement – clean and dry the chamber and perform measurement.
3. MilliQ measurement – fill chamber with MilliQ water and perform measurement.
4. Filtered seawater measurement – fill chamber with filtered seawater and perform measurement.

Post-Cruise Processing:

The MFL will undergo a post cruise calibration using the ATTO490LS dye following the protocol of Griffith et al. (2018). The MFL measurements following calibration will be combined with the phytoplankton specific absorption measurements (PABS; University of Oxford) to derive measurements of absolute quantum yield as described in Ryan-Keogh et al. (2023). Measurements for phytoplankton specific absorption measurements were collected every 4 hours from the ship's scientific underway supply, from 2 depths on all stainless and Eddy CTD casts and from the initial and treatment conditions of the ExS experiments. Please refer to PABS section for details on collection and volumes filtered.

Measurement Inventory:

UW Measurements

Measurements missing for the following UW numbers:

UW101-UW106 – Samples only collected for chlorophyll-a

UW117-UW121 – Laptop had crashed and data is unrecoverable

Measurements were stopped after UW172.

CTD Measurements

CTD #	Surface	DCM	CTD #	Surface	DCM
01S	✓	×	16S	✓	✓
02S	✓	×	17S	✓	✓
03S	✓	×	18S	✓	✓
04S	✓	×	18T	✓	✓
05S	✓	×	19T	✓	✓
06S	✓	×	20T	✓	✓
07S	✓	×	21T	✓	✓
08S	✓	✓	22T	✓	✓
09S	✓	✓	23T	✓	✓
10S	✓	✓	24T	✓	✓
11S	✓	✓	19S	✓	✓
12S	✓	✓	20S	✓	✓
13S	✓	✓	21S	✓	✓
14S	✓	✓	22S	✓	✓
15S	✓	✓			

Experiment Measurements

Experiment #	MFL Measurement	PABS Sample	Treatment
ExS01	✓	✓	Control, Fe, N FeN
ExS02	✓	✓	Control, Fe, N FeN
ExS03	✓	✓	Control, Fe, N FeN
ExS04	✓	✓	Control, Fe, N FeN
ExS05	✓	✓	Control, Fe, N FeN
ExS06	✓	✓	Control, Fe, N FeN
ExS07	✓	✓	Control, Fe, N FeN
ExS08	✓	✓	Control, Fe, N FeN
ExS09	✓	✓	Control, Fe, N FeN
ExS10	✓	✓	Control, Fe, N FeN
ExS11	✓	✓	Control, Fe, N FeN

References

Griffith, D. J., Bone, E. L., Thomalla, S. J., & Bernard, S. (2018). Calibration of an in-water multi-excitation fluorometer for the measurement of phytoplankton chlorophyll-a fluorescence quantum yield. *Optics Express*, *26*(15), 18863. https://doi.org/10.1364/OE.26.018863

Ryan-Keogh, T. J., Bone, E. L., Thomalla, S. J., Lain, L., Smith, M. E., Bernard, S., & Vichi, M. (2023). Spatial and temporal drivers of fluorescence quantum yield variability in the Southern Ocean. *Limnology and Oceanography*. https://doi.org/10.1002/lno.12295

Photosystem I Kinetics

Tommy Ryan-Keogh (SOCCO, CSIR) and C. Mark Moore (University of Southampton)

Aim & Objectives:

- Measure and quantify photosystem I kinetics from UW and CTD samples.
- Measure and quantify photosystem I kinetics from nutrient addition experiments.

Sampling protocols:

Seawater was collected and then pre-concentrated on a 0.8 µm polycarbonate filter and resuspended in 5.0 mL 0.2 µm filtered seawater to increase the signal to noise ratio for the measurements. Measurements were performed a Walz Dual-PAM 100 which can measure kinetics from both photosystem I and photosystem II.

Issues:

No successful measurements were able to be performed on the cruise for the following issues.

1. **40-50 Hz instrument interference**
    a. The instrument was originally set up in the temperature-controlled laboratory. However, there was a background repeating signal that had a frequency of 40-50 Hz. This was determined to be from the engines due to the location of the laboratory. The instrument was moved to the Met Lab and this background signal was removed.
2. **Temperature during pre-concentration**
    a. The first attempt at pre-concentration was conducted in the deck lab using vacuum filtration. Originally ~20 L of seawater was filtered onto a 0.8 µm polycarbonate filter, which took approximately 1 hour. A 1:100 dilution was prepared in 0.2 µm filtered seawater and measured on the Chelsea FRRf MKIII and it was found that the cells were dead. The filtration unit was then moved to the temperature-controlled room.
3. **Method of pre-concentration**
    a. The same method of pre-concentration was performed as in step 2; however, aliquots were collected from the resuspended filtrate in the filtration cup to be measured on the Chelsea FRRF MKIII every 15 minutes. Whilst this showed some initial promise of keeping cells alive by constantly agitating the filter when filtering, the resulting concentrate often showed signs of decline before any substantial biomass could be prepared. The filtration unit was switched from vacuum filtration to a peristaltic pump.

Additional 4.0 L bottles were added to the following experiments for the control and Fe-addition treatments:

ExL04; ExL05; ExL06; ExL07; ExL08; ExL09

From ExL07 we measured the chlorophyll-a concentration of the samples which were:
Control = 899.674 µg L^{-1}
Fe = 1098.101 µg L^{-1}

These high levels of biomass were still insufficient to measure a signal.

From CTD013S ~15.0 L was collected from the "party" depth and concentrated. The resultant chlorophyll-a concentration was:

2393.204 µg L^{-1}

This high level of biomass was also insufficient to measure a signal.

Future Suggestions:

If Photosystem I kinetic measurements are planned for future cruises, then I recommend the following:

1. Set up the instrument in a laboratory with no other electrical instruments running to reduce signal interference.
2. Perform cell pre-concentration in the temperature-controlled laboratory and ensure its temperature matches the sample's temperature.
3. Perform cell pre-concentration using a peristaltic pump, and if possible, use a multi-head pump to perform simultaneous filtrations.
4. Bring either a houseplant or high-density phytoplankton culture that produces a Photosystem I signal to ensure the instrument is fully operational.

Active Fluorescence and Pico-Second Lifetime Measurements

Heshani Pupulewatte (Rutgers University, New Brunswick)

Scientific Motivation:

Phytoplankton in the ocean account for less than 1% of the global photosynthetic biomass but contribute about 45% of the photosynthetically fixed carbon on Earth (Field et al. 1998). However, the growth of marine phytoplankton is commonly limited by the availability of one or more nutrients (Browning and Moore, 2023). A persistent biogeochemical feature of vast regions of the world's oceans is that the primary production is limited by nitrogen (N) and Iron (Fe). Theoretically, the N supply by diazotrophs needs to balance the N required to support primary production, assuming other key limiting nutrients such as phosphorus, and Fe, are available and the energy requirements are met. In practice, however, N2 fixation does not meet ecosystem-scale N demands in most cases, even when other nutrients are replete (Paerl 2018). On the other hand, most life forms are heavily dependent on iron and phytoplankton, have significantly higher Fe demands as opposed to their heterotrophs, as phytoplankton have Fe-rich photosynthetic apparatus (Raven et al. 1999). However, how nutrient stress affects the photosynthetic pathway is still to be understood fully.

Variable fluorescence measurements (specially the maximum quantum yield of photochemistry (F_v/F_m)) in photosystem II (PSII) are widely used to rapidly assess the extent of nutrient limitation in situ as they provide additional parameters and information regarding the photo-physiological state of PSII, which can be used to assess and quantify the extent of nutrient limitation. These parameters include the effective absorption cross section of PSII (σ_{PSII}), kinetics of electron transport on the acceptor side of PSII, and the maximum electron transfer rate through PSII (ETR) (Gorbunov and Falkowski 2021). Rapid responses in these parameters following the addition of the limiting nutrient have been used to support the existence of limitation (Falkowski et al. 1992; Suggett et al. 2009b; Ko et al. 2020).

Variable fluorescence measurements can be combined with simultaneous picosecond fluorescence lifetime measurements to resolve the three pathways in which phytoplankton can utilize or dissipate the absorbed solar energy; photochemistry, fluorescence emission, and non- radiative thermal dissipation Lin et al. 2016) Chlorophyll a (Chl a) fluorescence lifetimes measure the time it takes an excited state Chl-a molecule to return the ground state, which is directly proportional to the quantum yield of fluorescence emission in the photosynthetic unit. The simultaneous measurements of variable fluorescence and fluorescence lifetime provide further insight into the photo-physiological response of phytoplankton to nutrient limitation and relief (Gorbunov and Falkowski 2021).

The cruise transect of DY172 provides us with the opportunity to sample across the Southern Ocean and South Atlantic, where photosynthesis is limited by Iron and Nitrogen. The objective of this study is to assess variable fluorescence measurements that can be used to detect nutrient stress in the ocean. For this purpose, two instruments, namely, the mini-FIRe and PicoLiF, were established in the lab space of RRS DISCOVERY, to obtain and study underway, continuous-flow samples, CTD samples, and sub-samples from nutrient addition incubation experiments.

Instrumentation:

The mini-FIRE instrument measures fluorescence before and after a saturating single turnover flash (STF) from blue light-emitting diodes (450 nm +/- 30 nm half bandwidth), which cumulatively reduces all PSII RCs within ca. 80 µs. This STF protocol results in minimum and maximum fluorescence yields (F_0 and F_m). During FE curves, the sample is exposed to increasing PAR levels with an actinic blue light source (450 nm). (Gorbunov and Falkowski 2021).

The Pico-second Lifetime Fluorescence (PicoLiF) instrument uses a picosecond diode laser (BDS-640-SMN, Becker & Hickl GmbH, Berlin) with a pulse duration of ca. 100 to 150 ps. The laser excitation wavelength is 640 nm (BDS-640-SMN) The induced fluorescence photons are recorded by a Peltier cooled, ultra-fast single photon counting detector (Becker & Hickl GmbH, Berlin). The instrumental response function of the instrument has the half bandwidth of, which allows resolution of lifetime measurements down to ca. 20 ps. The PicoLiF measured fluorescence decays are deconvoluted from the instrument response function and then fitted to a sum of three exponentials with a custom TCSPFIT MATLAB package utilizing a Nelder-Meade simplex algorithm (Gorbunov and Falkowski 2023).

Active chlorophyll fluorescence measurements:

1. **Nutrient Addition Experiments**

Nutrient addition experiments conducted (procedure reported in detail elsewhere in the DY172 cruise report) were sub-sampled for FIRe and PicoLiF measurements using 250 ml dark bottles. Sample coolers were used in the transportation of sub-samples and placed in running water to maintain temperature. At the time of collection standard FIRe measurement were conducted both in the dark and at different PAR levels up to saturating PAR level depending on the sample. Following this, measurements were conducted at time points provided in the table given below. The saturation PAR level was chosen from an FE curve conducted on the control sample. Blank samples were collected from Control bottle number 2 by filtering 30 ml of sample through a pore size 0.2 µm non-sterile syringe filter.

Incubation Experiment	**Time point**	**Incubation Experiment**	**Time points**
ExS01	0,24,26,32,48	ExL01	0,48,96
ExS02	0,24,32,48	ExL02	0,48,96
ExS03	0,24,32,48	ExL03	0,48,96
ExS04	0,24,32,48	ExL04	0,48,96

ExS05	0,24,48,62	ExL05	0,48,96
ExS06	0,24,32,48	ExL06	0,48,96
ExS07	0,24,48	ExL07	0,24,48,96
ExS08	0,24,48	ExL08	0,48,96
ExS09	0,24,48	ExL09	0,24,48,96
ExS10	0,24,48	ExL10	0,48,96
ExS11	0,24,32,48	ExL11	0,24,48,96
ExVS01	0,48 (FIRe only)	ExL12	0,48,96 (FIRe only)
-	-	ExL13	0,96

2. CTD sampling and Underway sampling

At each CTD station depths samples were collected for FIRe (dark and P Vs E) measurements from the surface as well as above, in and below the deep chlorophyll maximum (DCM). CTD data collection has been summarized in the table below. Blank sample was obtained by filtering 30 ml of lowest biomass sample through a pore size 0.2 µm non-sterile syringe filter. (* indicates DCM/mixed layer and all samples 23 are from surface).

CTD No	Niskin Bottle No	CTD No	Niskin Bottle No
1	19*,21,23	13	15,17*,23
2	13,15*,23	14	-
3	15,17*,23	15	-
4	13,15*,23	16	17,19,21*,23
5	17,19*,23	17	19,21*,23
6	17,19*,23	18	13,15*,17,23
7	13,15*,23	19	11,13,15*,19,23
8	13,15*,23	20	13,15*,17,23
9	13,15*,23	21	11,13, 15*,17,23
10	15,17*,23	22	15*,17,23
11	15,17*,23	-	-

12	13,15*,17,23	-	-

Variable fluorescence and fluorescence lifetime data were collected continuously from surface waters while underway with FIRe and PicoLiF fluorometers respectively, as described by Lin et. al 2016. The two instruments utilized flow through cuvettes connected to the ship's surface water intake pump. Prior to entering the cuvette, the water passed through two de-bubblers. Every ~30 min. the water flow into the FIRe instrument was automatically paused to conduct Slow Light Curves, i.e., fluorescence-versus-irradiance (FE) curves.

Agulhas Eddy Sampling:

Photosynthetic parameters were measured on several depths from water samples collected from CTD's using the FIRe and PicoLiF instruments at consecutive time points. Samples were subjected to dark acclimation for one hour prior to measurements. Blank samples were collected from the DCM and/or surface layer, by filtering approx. 30 ml of sample through a pore size 0.2 µm non-sterile syringe filter. The data that was collected has been listed below and will be subjected to post-cruise data processing.

3.1 Samples measured at the Agulhas Eddy

Event	**Time point**	**Niskin No**	**Bottle ID**	**Notes**
CTD018_T	1	12	1	
CTD018_T	1	11	2	
CTD018_T	1	10	3	
CTD018_T	1	8	4	DCM
CTD018_T	1	7	5	
CTD018_T	1	6	6	
UW137	1	UW137	UW137	Surface
BLANK_1_1	1	UW137	UW137	Blank from surface
BLANK_1_2	1	8	4	Blank from DCM
CTD019_T	2	12	1	
CTD019_T	2	11	2	
CTD019_T	2	10	3	
CTD019_T	2	9	4	
CTD019_T	2	8	5	DCM
CTD019_T	2	6	6	
UW138	2	UW138	UW138	Surface
BLANK_2_1	2	UW138	UW138	Blank from surface
BLANK_2_2	2	8	5	Blank from DCM
CTD020_T	3	12	1	
CTD020_T	3	11	2	
CTD020_T	3	10	3	

CTD020_T	3	9	4	
CTD020_T	3	7 or 8	5	DCM
CTD020_T	3	6	6	
UW139	3	UW139	UW139	Surface
BLANK_3_1	3	UW139	UW139	Blank from surface
CTD021_T	4		1	
CTD021_T	4		2	
CTD021_T	4		3	
CTD021_T	4		4	
CTD021_T	4		5	DCM
CTD021_T	4		6	
UW140	3	UW140	UW140	Surface
BLANK_4_1	3	UW140	UW140	Blank from surface
CTD022_T	5	13	1	
CTD022_T	5	12	2	
CTD022_T	5	11	3	
CTD022_T	5	10	4	
CTD022_T	5	9	5	DCM
CTD022_T	5	6	6	
UW141	5	UW141	UW141	Surface
BLANK_5_1	5	UW141	UW141	Blank from surface
BLANK_5_2	5	9	5	Blank from DCM
CTD023_T	6	13	1	
CTD023_T	6	12	2	
CTD023_T	6	11	3	
CTD023_T	6	10	4	
CTD023_T	6	9	5	DCM
CTD023_T	6	6	6	
UW142	6	UW142	UW142	Surface
BLANK_6_1	6	UW142	UW142	Blank from surface
CTD024_T	7	13	1	
CTD024_T	7	12	2	
CTD024_T	7	11	3	
CTD024_T	7	10	4	
CTD024_T	7	9	5	DCM
CTD024_T	7	6	6	
UW143	7	UW143	UW143	Surface
BLANK_7_1	7	UW143	UW143	Blank from surface

Issues:

- Data collected in lower biomass regions were not able to be processed or analysed under normal data acquisition periods (20-60 seconds), therefore a combination of 60, 120, 240 seconds were used to collect data depending on the sample.
- From 13/01/2024, the biomass detected in the ocean, was too low for detection using the PicoLiF instrument. Therefore, the chlorophyll-a excitation filter that was used for the initial part of the cruise had to be removed, which makes data collected after this day to be incomparable with the rest of my data pool. As a solution to this, an attenuation factor will be calculated after I return to Rutgers University-New Brunswick, and results will be updated as soon as possible.

Acknowledgement:

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Nutrient Amendment Incubation Experiments

Tommy Ryan-Keogh (UCT), Joe Furby (UoS), Mark Moore (UoS)

A series of factorial nutrient addition experiments were performed during DY172 (see Figure 1) to investigate how spatial and temporal changes in nutrient (Fe, N and Mn) availability influenced phytoplankton physiology, growth and nutrient drawdown and enable the collection of samples for gene abundance and expression analysis (see report section *Nucleic acid sampling*). Two main experimental designs were run simultaneously, using similar methods to those employed previously in a number of settings including the HNLC Southern Ocean and the (Sub-)tropical Atlantic (Moore et al. 2006; Moore et al. 2007; Moore et al. 2009; Ryan-Keogh et al. 2013): 2-day (48-hour) incubations (*n* = 11) and 4-day incubations (*n* = 13). The smaller volume / short-term bioassays (denoted 'ExS') were designed to assess the rapid changes in phytoplankton physiology upon Fe and N addition and to resolve spatial variability in Fe and N stress across the natural biogeochemical gradients encountered during the cruise, whilst the larger volume / long-term experiments (denoted 'ExL') were designed to assess changes in community physiology, structure and nutrient drawdown in response to changes in nutrient addition, alongside providing the extra volume(s) required for collection of samples for subsequent molecular analysis as well as 14C derived C-fixation (*see cruise report section DY172 14C- uptake experiments*) and samples for protein and 77K fluorescence analysis (*see cruise report section Stoichiometry of Photosynthetic Catalysts*). An additional two smaller (denoted 'ExVS') 2-day (48 hour) experiments were also performed to further augment the spatial resolution of the ExS experiments (see Table X).

Strict controls were required to avoid the contamination of incubation bottles, sampled seawater water and nutrient spikes. All incubation bottles had been previously passed through a vigorous cleaning process involving a Decon and strong (1 M) HCl wash followed by Milli-Q rinsing and storage with 0.024 M HCl prior to sailing. The trace metal spikes were prepared from high purity salts prior to sailing, whilst the N spikes were prepared from high purity KNO_3 and NH_4Cl salts which were cleaned for trace metal impurities through a Chelex-100 cation exchange resin shortly after sailing. Seawater was collected using a trace metal clean 'tow-fish' through acid-cleaned tubing when the ship was sailing at a minimum of four knots. Bottle filling and all manipulation steps including spiking and sub-sampling were performed in a purpose built, Class-100 clean air container.

Water for the experiments was collected and transferred unfiltered into 2 L polycarbonate bottles (Nalgene) for the 48 h (ExS) incubation experiments, 4.5 L polycarbonate bottles (Nalgene) for the larger long-term (ExL) incubation experiments and 0.5L polycarbonate bottles for the smallest (ExVS) experiments. Incubation bottles were filled in a random order, 50 % at a time, with triplicate samples for initial (T_{zero}) measurements usually collected at the beginning, middle and end of the filling process (due to the short filling time, a single or double initial sample was only collected for the ExVS experiments). Filling bottles half-way and then topping up in random order ensured a relatively homogenous water collection representative of the surface conditions encountered during the sampling period. The duration of sampling was extended on some days due to the simultaneous filing of a series of bottles for temperature manipulation experiments (*see Section Ocean warming experiments*). Consequently, the average time between the primary initial and final initial sample for the ExS experiments was 22 minutes, while for the ExL experiments this reached 43 minutes, corresponding to distances of around 1.5 – 6 km (average 3.8km) and 2 – 10km (average 5.9km) for the ExS and ExL experiments respectively.

In addition to an unamended control, separate bottles were amended with 2 nM Fe in all experiments and then further bottles with either 1 µM KNO_3 and1 µM NH_4Cl or 2 nM Mn in a series of factorial designed experiments (see details below). Additional $NaHPO_4$ treatments were added into one of the 'ExVS' experiments. All experimental conditions were conducted as biological triplicates. Following nutrient amendment, all bottles were parafilm-sealed before transfer into a temperature controlled incubation container set to approximately local sea surface temperature (incubator temperature ranged from 6 – 22 °C across the cruise), noting that more precise temperature matching of incubator to in situ starting condition could not be achieved due to the duration of experiments and sharp temperature gradients encountered. The bottles were incubated on shelves surrounded by light banks with ~200 µmol photons m^2 s^1 irradiance flux and set to a day/night cycle of 16 and 8 h, respectively. Preliminary processed data are available within the directory: '*\\dynetapp.discovery.ad.noc.ac.uk\science_public\DY172\Nutrient addition experiments*' within the spreadsheets 'All_ExS' and 'All_ExL'.

Further method and (sub-)sampling details:

As outlined above, all incubation setups, subsampling, and incubation breakdowns took place under trace-metal clean SOPs, with unfiltered seawater from the trace-metal clean tow-fish fed into the clean-lab container on the Mezzanine Deck of the *RRS Discovery*.

Nutrient stocks used: nitrogen (NH_4Cl 10mM and 10mM KNO_3), iron (20µM $FeCl_2$), manganese (20µM $MnCl_2$), and phosphorus (1mM $NaHPO_4$). All stocks were trace-metal clean and made up so that 100µL could be added for every 1L of seawater (i.e. 400 µL for every 4L large bottle). Once spiked, bottles were fastened and sealed with clean parafilm, before being placed in the incubation container under a 16-8hr light-dark cycle (off at 18:00 UTC and on at 02:00 UTC) at 6-24ºC (adjusted to approximate SST). At the end of each incubation experiment, bottles were acid-washed, rinsed, and prepped ready for the next round of incubations. If not ran sequentially (i.e. setup immediately following the breakdown of a prior run), bottles were washed and stored overnight with dilute acid inside.

'Large/Long' Incubations (ExL) 4.5L acid-cleaned polycarbonate bottles labelled: 1-3 were kept as controls (no nutrient additions), 4-6 Fe addition, 7-9 N or Mn addition, and 10-12 N+Fe or Fe+Mn addition. N and Mn spikes were used to coincide with the large scale nutrient gradients encountered, such that north of ~47°S (where surface dissolved N sources were depleted), N was added, and to the south into Fe-limiting waters, Mn was added to test for potential (co-)limitation by this other trace-metal nutrient (Browning et al. 2017; Wyatt et al. 2023). The total number of bottles gradually increased up to 26 throughout the cruise as biomass changed along with the number of sub-sample measurements being taken, but the types of treatments did not differ beyond what was already stated. The bottles were subsampled after 48hr (T_1) for Chl-a, FRRf, and fluorescence lifetimes, and then broken down after 96hr (T_2) for Chl-a, FRRf, lifetimes, sterivex filter collection for RNA/DNA, macronutrients, PAM, Proteins, and cellular elemental analysis (see relevant Sections). Long incubation experiments were staggered on average by one day (up to a max of any two long incubation experiments running at any given time). Long experiments 7, 9, and 11, also had an additional 6 bottles (3x control and 3 x Fe+Mn/N), that were sampled and broken down after 24hr (T_{1a}) for Chl-a, FRRf, fluorescence lifetime measurements and sterivex filter collection for RNA/DNA.

'Small/Short' Incubations (ExS) 2L acid-cleaned polycarbonate bottles labelled: 1-3 were kept as controls (no nutrient additions), 4-6 Fe addition, 7-9 N addition, and 10-12 N+Fe addition. The bottles were subsampled up to a max of 4 different timepoints including: +12hr, +24hr, +26hr, +32hr, and +48hr, at which point they were broken down (note, ExS06 was effectively broken down at +32hr due to the samples at the end being mistakenly lost). Chl-a, FRRf, and fluorescence lifetime measurements were taken from each timepoint, with macronutrients, PABs, and MFL (see relevant Section) also being included for the final timepoint. Short incubation experiments were performed sequentially with only one set of 12 bottles being incubated at any one time.

'Very Small' Incubations (ExVS) For ExVS_01 (initiated in the centre of the Agulhas Ring on 15/01/2024), 18 x 500ml acid cleaned polycarbonate bottles were labelled from 1-18: 1-3 were kept as controls (no nutrient additions), 4-6 were Fe addition, 7-9 were N addition, 10-12 were N+Fe addition, 13-15 were P addition, and 16-18 were N+P addition. ExVS_02 only used bottles 1-12 with the same nutrient spikes as above for those same bottle numbers. Both very small incubation experiments were sampled and broken down at +48hr (T_1) with Chl-a and FRRf measurements taken from every bottle.

Preliminary results:

Results from the T = 96 hr timepoint from two representative experiments are shown below. As expected, south of the sub-tropical front (i.e. in waters with elevated ambient nitrate concentrations), Fe limitation was generally observed (Figure 2). In contrast, in waters north of the sub-tropical front (generally north of ~47°S), primary N limitation was observed, frequently in combination with secondary / co – limitation by Fe (Figure 3).

Experiment id	Latitude	Longitude	Sampling method	Start date	End date
ExL-01	-38.4763	-14.0427	Trace clean fish	23/12/23	27/12/23
ExL-02	-40.5777	-11.9417	Trace clean fish	24/12/22	28/12/22
ExL-03	-48.3630	-3.5200	Trace clean fish	27/12/23	31/12/23
ExL-04	-46.9333	-0.3773	Trace clean fish	28/12/23	01/01/24
ExL-05	-52.3393	-0.5327	Trace clean fish	31/12/23	04/01/24
ExL-06	-58.0643	0.0010	Trace clean fish	03/01/24	07/01/24
ExL-07	-56.5323	0.5483	Trace clean fish	04/01/24	08/01/24
ExL-08	-49.3243	4.2613	Trace clean fish	07/01/24	11/01/24
ExL-09	-48.3850	5.3247	Trace clean fish	08/01/24	12/01/24
ExL-10	-40.3463	5.0957	Trace clean fish	12/01/24	16/01/24
ExL-11	-38.3863	5.0673	Trace clean fish	13/01/24	17/01/24
ExL-12	-27.9570	2.9750	Trace clean fish	17/01/24	21/01/24
ExL-13	-28.2070	0.9593	Trace clean fish	18/01/24	22/01/24
ExS-01	-38.4763	-14.0427	Trace clean fish	23/12/23	25/12/23
ExS-02	-43.0977	-9.3343	Trace clean fish	25/12/23	27/12/23
ExS-03	-48.3630	-3.5200	Trace clean fish	27/12/23	29/12/23
ExS-04	-49.1490	0.6080	Trace clean fish	30/12/23	01/01/24

ExS-05	-55.5220	0.0497	Trace clean fish	02/01/24	04/01/24
ExS-06	-56.5323	0.5483	Trace clean fish	04/01/24	06/01/24
ExS-07	-50.4027	3.5743	Trace clean fish	06/01/24	08/01/24
ExS-08	-45.3890	5.2460	Trace clean fish	10/01/24	12/01/24
ExS-09	-35.3823	5.0520	Trace clean fish	14/01/24	16/01/24
ExS-10	-30.6647	3.9320	Trace clean fish	16/01/24	18/01/24
ExS-11	-28.8520	-2.5770	Trace clean fish	19/01/24	21/01/24
ExVS-01	-32.263	-4.440	Trace clean fish	15/01/24	17/01/24
ExVS-01	-29.466	6.228	Trace clean fish	20/01/24	22/01/24

Table 1: Average Tzero location, start and end dates of nutrient addition experiments. Details available in the DY172 cruise folder under '*science_public\DY172\Nutrient addition experiments/DY172 Incubation Metadata Final*' scanned logbook is available within the same folder.

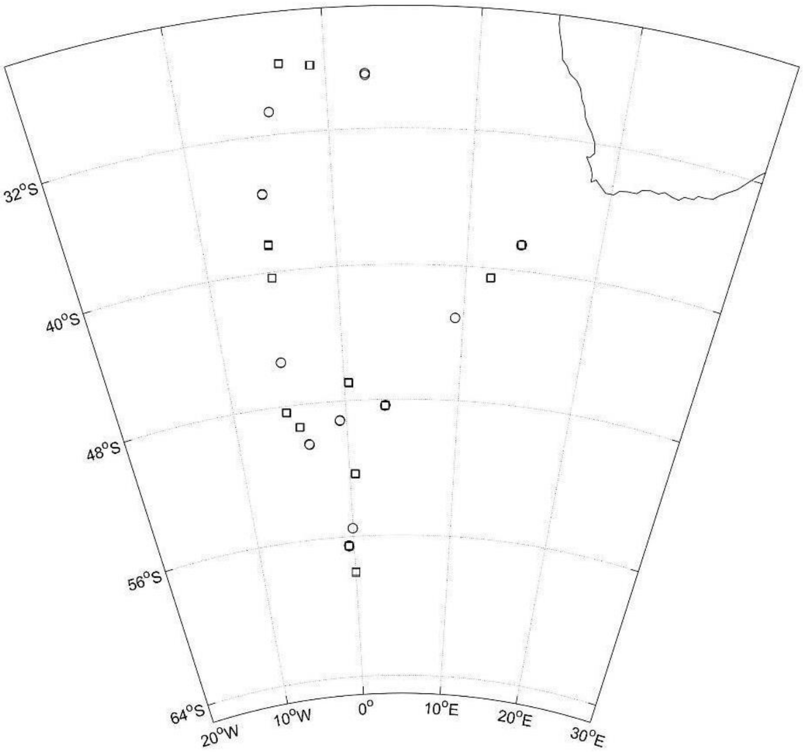


Figure 1. Locations of all ExS (circles) and ExL (squares) experiments. The locations corresponding to each of the triplicate Tzero measurements are plotted. Details available in the DY172 cruise folder under ‘*science_public\DY172\Nutrient addition experiments/DY172 Incubation Metadata Final*’

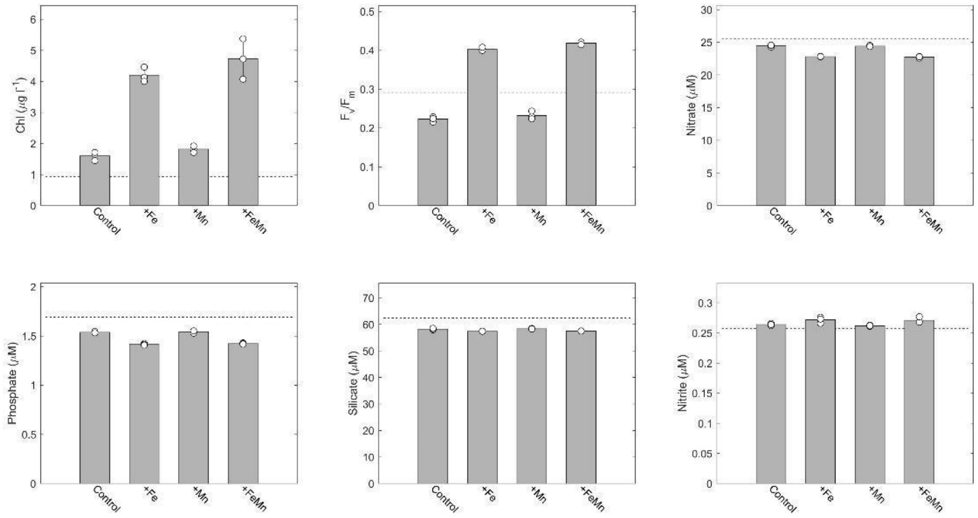


Figure 2. Example of a clear Fe limitation response from experiment ExL06

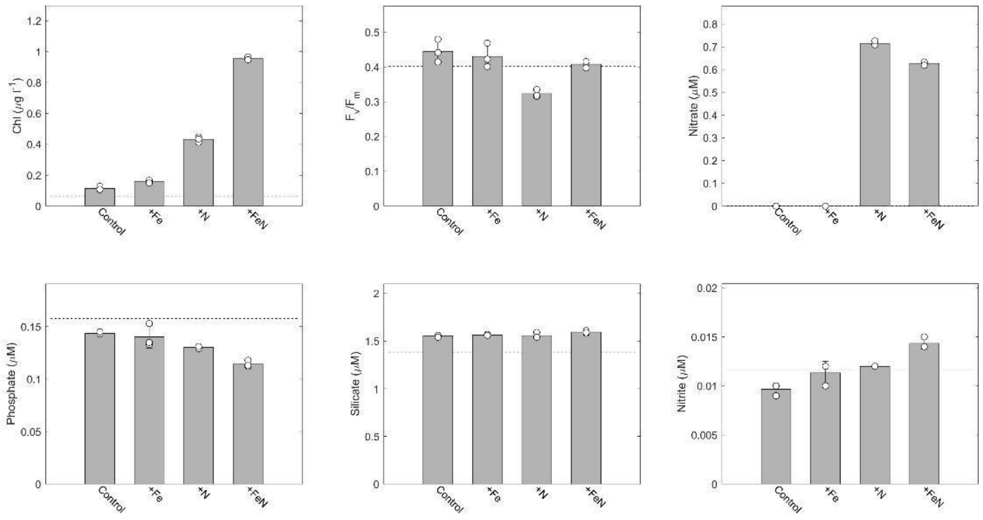


Figure 3. Example of a primary N limitation response with a secondary (serial) Fe limitation response from ExL12.

References:

Browning et al. 2017 *Nature* 551 242-246

Moore et al. 2006 *Global Change Biology* 12 626-634

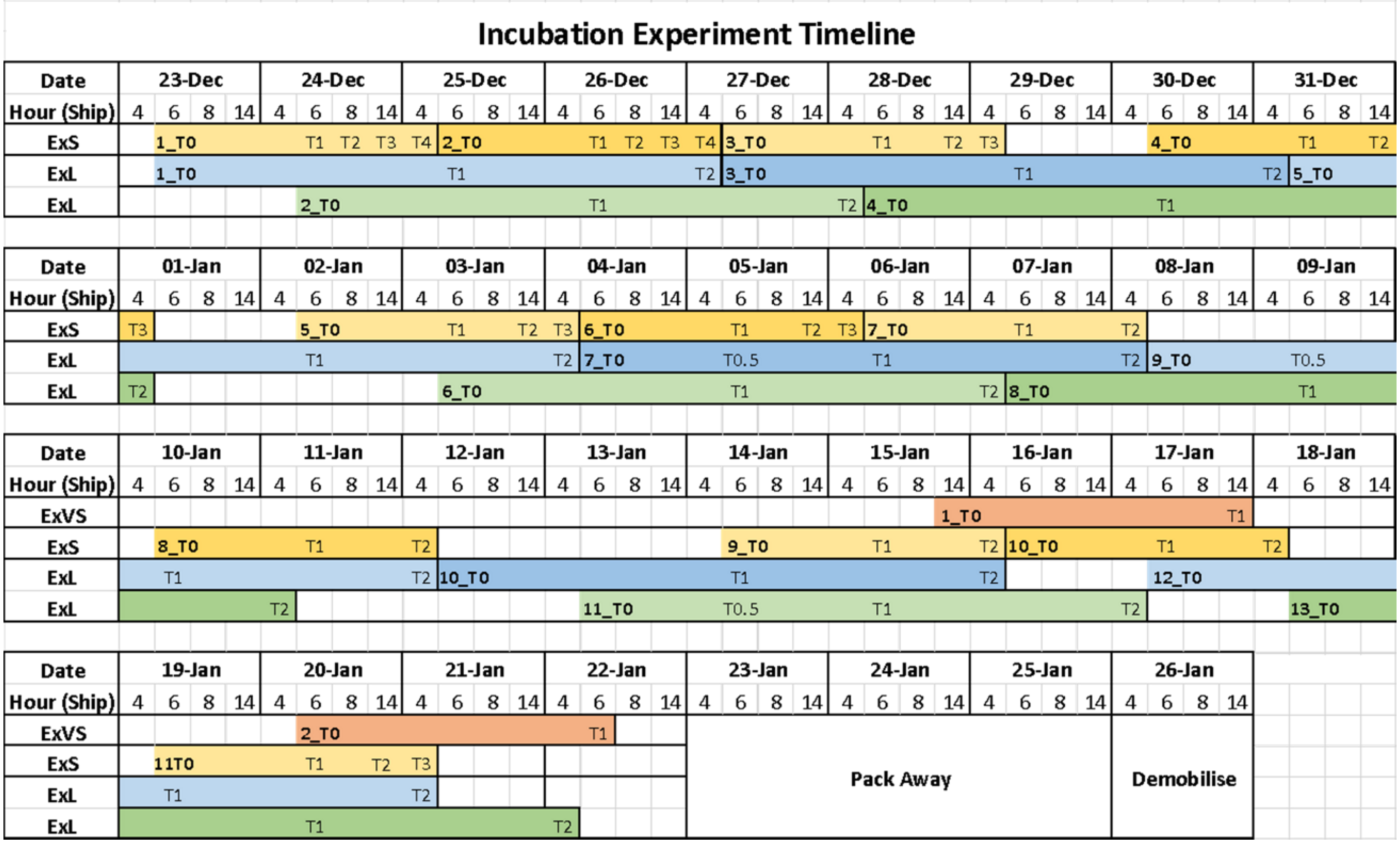
Moore et al. 2007 *Deep Sea Research II* 54 (18-20) 2045-2065

Moore et al. 2009 *Nature Geosciences* 2 867-871

Ryan-Keogh et al. 2013 *Limnology and Oceanography* 58 533-545

Wyatt et al. 2023 *PNAS* 120 28

See below Ghant Chart for a timeline of the different nutrient incubation experiments.



Incubation Experiment Timeline

Date	23-Dec				24-Dec				25-Dec				26-Dec				27-Dec				28-Dec				29-Dec				30-Dec				31-Dec			
Hour (Ship)	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14
ExS		1_T0				T1	T2	T3	T4	2_T0				T1	T2	T3	T4	3_T0				T1		T2	T3					4_T0				T1		T2
ExL		1_T0								T1							T2	3_T0								T1							T2	5_T0		
ExL						2_T0								T1							T2	4_T0								T1						

Date	01-Jan				02-Jan				03-Jan				04-Jan				05-Jan				06-Jan				07-Jan				08-Jan				09-Jan			
Hour (Ship)	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14
ExS	T3					5_T0				T1		T2	T3	6_T0				T1		T2	T3	7_T0				T1			T2							
ExL						T1							T2	7_T0				T0.5				T1							T2	9_T0				T0.5		
ExL	T2									6_T0								T1							T2	8_T0								T1		

Date	10-Jan				11-Jan				12-Jan				13-Jan				14-Jan				15-Jan				16-Jan				17-Jan				18-Jan			
Hour (Ship)	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14
ExVS																								1_T0								T1				
ExS		8_T0				T1			T2									9_T0				T1			T2	10_T0				T1			T2			
ExL		T1							T2	10_T0								T1							T2					12_T0						
ExL					T2									11_T0				T0.5				T1							T2					13_T0		

Date	19-Jan				20-Jan				21-Jan				22-Jan				23-Jan				24-Jan				25-Jan				26-Jan			
Hour (Ship)	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14	4	6	8	14
ExVS						2_T0								T1				Pack Away												Demobilise			
ExS		11T0				T1		T2	T3																								
ExL		T1							T2																								
ExL						T1							T2																				

DY172 14C- uptake experiments

Nina Schuback (Chelsea Technologies)

Single-turnover active fluorometry (STAF) measurements can be used to estimate phytoplankton photosynthetic rates at the level of charge separation in photosystem II (ETR), the 'starting point' of the photosynthetic light reaction. Tracer experiments using the radioisotope ^{14}C measure the rate of carbon fixation during the dark reaction, the opposite end of the chain of coupled reactions which make up the photosynthetic process. The two rates are linked, and ETR should provide an upper limit to how much energy is available for the fixation of carbon. However, it has been shown that under conditions of stress (supra-optimal light, nutrient stress, etc.) the ratio of ETR to carbon fixed ($\Phi_{e,C}$, e^{-} C^{-1}) increases, reflecting evolved mechanisms to optimise cellular energy allocation.

During DY172, ETR and ^{14}C-fixation were measured using a dual-incubation approach, where ^{14}C-fixation incubations were performed directly in the sampling chamber of four LabSTAF instruments. This approach minimises apparent decoupling of the two rates due to methodological bias (e.g. difference in light sources or incubation time used) which in past experiments has proven difficult to distinguish from true physiological effects.

Dual-incubation experiments were performed on LabSTAF instruments SN 19-0105-001, 19-0105-002, 21-1345-003, and 19-0105-004 at either 4 or 2 light levels, as described below.

Four light-level photosynthesis vs light (PE) curves:

Photosynthesis vs light dual-incubation experiments at 4 light levels (25, 75, 200, 400 µmol quanta m^{-2} s^{-1}) were performed on samples taken from the continuous seawater supply at underway sampling points, from the tow fish for T0 points of large nutrient addition experiments (ExpL), or from the CTD to target the deep chla maximum during sampling of the Eddie on 15-16 January (table 1).

Samples were inserted into the LabSTAF sampling chamber inside a 24 mL scintillation vial surrounded by MQ water and held in place by a custom-made sample chamber cap. In low biomass regions, a blank of 0.2µm filtrate was first run on each instrument. Then, unspiked samples were exposed to a sampling protocol to determine a photosynthetic excitation profile (PEP), dual wavelengths correction (DWC) factor, and single-turnover parameter in the dark-regulated state. After this 'dark acquisition', the samples were spiked with 20-40 µCi ^{14}C sodium bicarbonate solution and incubated with background irradiances switched on to determine single-turnover parameters in the light-regulated state. One additional vial was spiked and filtered immediately, while another additional vial was spiked and incubated in the dark. Incubation times were generally 1 h and 2 h. Carbon fixation was determined by filtering sample (10 mL) onto 25mm 0.2µm pore size PC filter and placing the filter into a scintillation vial containing 500 µL 2M HCl over night to remove inorganic carbon. Total ^{14}C spike in each incubation vial was determined from triplicate 100 µL aliquots pipetted into scintillation vials containing 500 µL 0.1M NaOH. 5 mL scintillation cocktail (Ultima Gold) were added to each scintillation vials and counts obtained by the shipboard liquid scintillation counter in disintegrations per minute (DPM).

TABLE 1: SAMPLING POINTS FOR DUAL-INCUBATION EXPERIMENT AT 4 LIGHT LEVELS DURING DY172.

date	*sample*	*sampling time (ship)*	*Lat*	*Lon*
23.déc	ExpL01	5:27	-38.475	14.044
23.déc	uw001	14:00	-39.032	13.481
24.déc	ExpL02	5:01	-40.576	11.944
24.déc	uw006	10:00	-40.719	11.800
24.déc	uw011	6:00	-43.123	9.308
25.déc	uw012	10:00	-43.611	8.787
25.déc	uw017	6:00	-46.180	5.979
26.déc	uw018	10:00	-46.397	5.730
26.déc	uw019	14:00	-46.495	5.630
26.déc	ExpL03	5:14	-48.360	3.523
27.déc	ExpL04	4:50	-46.930	3.770
28.déc	uw029	10:00	-46.859	0.345
29.déc	uw033	6:00	-46.458	-0.215
29.déc	uw034	10:00	-46.671	-0.320
29.déc	uw035	14:00	-47.211	-0.309
29.déc	uw036	18:00	-47.718	-0.338
31.déc	ExpL05	05:53	-52.341	0.534
01.janv	uw051	10:00	-55.522	-0.101
02.janv	uw056	06:00	-55.522	-0.101
02.janv	uw057	10:00	-55.517	-0.091
02.janv	uw058	14:00	-55.513	-0.082
02.janv	uw059	18:00	-56.187	0.004
03.janv	ExpL06	05:20	-58.073	-0.002
04.janv	ExpL07	04:41	-56.530	-0.548
05.janv	uw075	10:00	-53.044	-1.240
07.janv	ExpL08	06:18	-49.324	-4.258
07.janv	uw087	10:00	-49.323	-4.245
07.janv	uw088	14:00	-49.320	-4.245
07.janv	uw089	18:00	-49.329	-4.246
08.janv	ExpL09	06:28	-48.383	-5.328
09.janv	uw98	10:00	-48.362	-5.364
10.janv	uw111	10:00	-45.261	-5.244

11.janv	uw117	10:00	-41.781	-5.144
12.janv	ExpL10	05:42	-40.344	-5.094
12.janv	uw123	10:00	-40.369	-5.098
12.janv	uw124	14:00	-40.369	-5.098
12.janv	uw125	18:00	-40.369	-5.095
13.janv	ExpL11	05:55	-38.385	-5.067
13.janv	uw129	10:00	-38.331	-5.068
14.janv	uw135	10:00	-35.101	-5.048
15.janv	ctd20T	06:00	-32.868	-4.627
15.janv	ctd21T	12:00	-32.868	-4.627
16.janv	ctd24T	06:00	-32.868	-4.627
17.janv	ExpL12	05:37	-27.950	-2.972
17.janv	uw150	10:00	-27.999	-3.000
17.janv	uw151	14:00	-28.000	-3.002
17.janv	uw152	18:00	-28.000	-3.000
18.janv	ExpL13	05:11	-28.208	-0.956
19.janv	uw162	10:00	-28.852	2.762
20.janv	uw168	10:00	-29.493	6.396

Two light level dual-incubation:

At the last time-point of each of the 13 large/long nutrient addition experiments, dual-incubations were performed at 2 light levels (75 and 200 µmol quanta m^{-2} s^{-1}) from pooled triplicates of 2 treatments (table 2, all four treatments were used at ExpL12 and ExpL13). STAF measurements to derive ETR and 14C-fixation experiments were performed following the approach described above, with incubation times of 1h and 2 h or only 2 h at very low biomass conditions.

Table 2: Sampling points for dual-incubation experiment at 2 light levels during DY172.

date	***experiment***	***treatments***
27.déc	ExpL1	C & N
28.déc	ExpL2	C & Fe
31.déc	ExpL3	C & Fe
01.janv	ExpL4	C & Fe
04.janv	ExpL5	C & Fe
07.janv	ExpL6	C & Fe
08.janv	ExpL7	C & Fe
11.janv	ExpL8	C & Fe
12.janv	ExpL9	C & Fe
16.janv	ExpL10	C & Fe
17.janv	ExpL11	C & NFe

21.janv	ExpL12	C & Fe & N & NFe
22.janv	ExpL13	C & Fe & N & NFe

Single Cell Elemental Analysis of Phytoplankton Communities

Joe Furby (University of Southampton)

Scientific Motivation:

Phytoplankton play a crucial role in oceanic nutrient cycling. Alongside macronutrient elements (e.g. C, H, N, O, S, P, Ca, Si…), an extended suite of other micronutrients are also fundamental to healthy growth, forming cofactors for essential metabolic pathways (e.g. Fe, Mn, Cu, Ni, Co, Zn…) (Twining & Baines, 2013). Extended cellular ratios of these micronutrients have been shown to vary significantly with taxa and region (Twining *et al.*, 2021). Clear patterns of nutrient (co-)limitation have been found to limit primary production, although the extent to which this impacts the extended stoichiometries is uncertain (Browning & Moore, 2023). Knowledge gained through accurately quantifying the variability in extended stoichiometries of key phytoplankton assemblages will be vital in underpinning biogeochemical models and predicting the implications of future anthropogenic influences to oceanic biogeochemistry (Twining *et al.*, 2021).

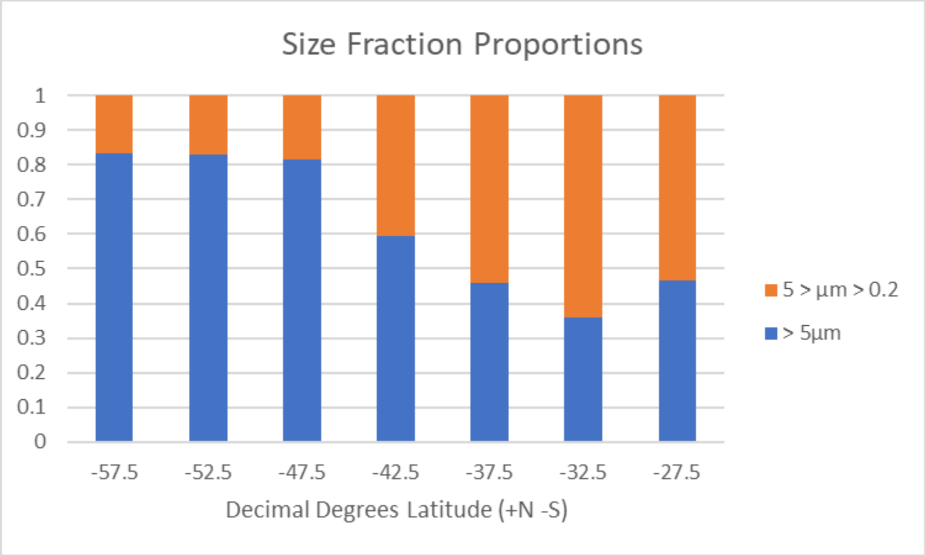
To fully capture variability in cell stoichiometries we need to step away from standard bulk-analysis techniques and look to single-cell analytical approaches (von der Au *et al.*, 2020). Inductively-Coupled-Plasma Time-of-Flight Mass Spectrometry (ICP-ToF-MS) unlocks unparalleled capacity to accurately measure a wide suite of elements in rapid succession (Chang *et al.*, 2022). Laser Ablation allows for the sample to be introduced into the ICP-ToF-MS in very small area sections (dependent on laser spot size) which allows for high resolution 2D element maps to be constructed across the ablated region of the sample filter (Gholap *et al.*, 2010).

The cruise transect will sample across a strong biogeochemical gradient carved out by a series of ocean fronts across the Southern Ocean and South Atlantic. Towards the southern end of the transect (Southern Ocean HNLC) we would expect Fe-limitation to occur and in the north of the transect (South Atlantic Gyre – oligotrophic) we would expect N-limitation to occur, with a transition across the boundary providing conditions for N|Fe co-limitation to potentially occur (Browning *et al.*, 2017).

Size-Fractionated Sampling:

The laser ablation instrument used for single cell elemental analysis is restricted to a laser spot size of 1µm^2, and so when the community-averaged cell diameter falls much below c.5µm, it becomes significantly more challenging to obtain meaningful data on the natural community elemental variability. This transition was expected to occur once we cross the Sub Antarctic Front (SAF) and head northwards into the South Atlantic Subtropical Gyre (SASG), coincident with a decline in biomass. However, the exact latitude this occurs fluctuates so to have a more concrete understanding of when this transition happens will allow for us to incorporate finer pore-sized filtration membranes and adjust filtration volumes accordingly.

From the 29th of December 2023 (Lat: -46.458ºN, Lon: -0.215ºE), for the remainder of the cruise, 300ml of seawater was subsampled, in parallel with each of the timepoints taken for single cell analysis work, for size fractionation to assess cell size changes in the natural phytoplankton communities. 150ml was filtered first through a 47mm 5µm membrane (Whatmann, Cyclopore), and then a 47mm 0.2µm membrane (Whatmann, Cyclopore) using a filtration rig under partial vacuum. The two filters were removed using forceps and placed into separate glass sample vials, before 6ml of 90% Acetone was added, and stored in the fridge for Chl-a analysis of the two size fractions (diameter>5µm and 5µm>diameter>0.2µm). In parallel, the remaining c.150ml was filtered through a 47mm 5µm membrane (Whitman, Cyclopore) using a filtration rig under partial vacuum. The cells on this filter were resuspended into 50ml of (<0.2µm filtered seawater from previous Chl-a sample run) and the filtered seawater (<5 µm cells) had active fluorescence measurements taken using a mark 3 Fast Repetition Rate fluorometer (FRRf).



Sampling for elemental analysis:

To quantify variability in cellular element stoichiometries across contrasting biogeochemical regions, samples were taken across the DY172 study area (South-East Atlantic sector of the Southern Ocean and subtropical gyre) for elemental analysis back at the University of Southampton.

Surface seawater was sampled from the trace metal clean towed-fish into acid-cleaned 2L polycarbonate bottles for each of the final T0 triplicate (T0_3) incubation experiment timepoints (approx. 06:00 Ship Time to coincide with the period prior to dawn). In addition, samples were also taken from each of the large incubation experiment T2 timepoints (T0+96hrs), including controls and nutrient addition treatments that provided the biggest growth response (sampled across triplicates). Across the higher biomass regions (lat > c.40ºS), c.500ml of sampled seawater was filtered through acid-cleaned 47m 5µm polycarbonate membranes (Whatmann, Cyclopore), using a Teflon filtration rig under partial vacuum. Filtered phytoplankton were resuspended using 50ml of filtered seawater into acid-cleaned 50ml Falcon tubes to give a preconcentration factor of c.10x. Samples were then fixed with trace metal clean 10% Glutaraldehyde (final concentration 0.4 %, add 2ml for every 50ml of filtered seawater), and were then left for 2 hours for the preservative to penetrate the cells and fix the phytoplankton in the fridge. Finally, samples were stored upright in the freezer and frozen at -80°C ready for further analysis on land. On crossing the Sub-Antarctic Front into the sub-tropical gyre boundary, the biomass declined rapidly, and the volumes filtered for each T0 sample (i.e. natural unfiltered seawater) were doubled to c.1000ml. Furthermore, size-fractionated Chl-a and active fluorescence measurements (FRRf and STAFs) revealed a coincident drop in community averaged cell size below that of the 5µm filter pore sizes, so these samples (lat < 33°S) were also filtered through 47m 0.4µm polycarbonate membranes (Whatmann, Cyclopore).

Samples Collected:

A total of 68 x 50ml samples were collected across the DY172 study area, including 34 natural samples (T0), and 34 large incubation experiment T2 (+96hr) timepoints (from 13 controls, 3 +N treatment, 10 +Fe treatments, 2 +Mn treatments, 4 +Fe +N treatments, and 2 +Fe +Mn treatments).

A summary of single cell elemental sampling is displayed below, in Table 1.

TABLE SEQ TABLE * ARABIC 1 – SINGLE CELL ELEMENTAL ANALYSIS SAMPLE METADATA.

Sample #	Experiment ID	Latitude N	Longitude E	Date (Ship)	Time (UTC)	Volume (ml)	Conc Factor	Filter Poresize (µm)
1	ExL 01 T0 3	-38.496	14.024	23/12/2023	03:50:00	500	10	5
2	ExL 01 T0 3	-38.496	14.024	23/12/2023	03:50:00	500	10	5
3	ExL 02 T0 3	-40.601	11.921	24/12/2023	03:28:00	500	10	5
4	ExS 02 T0 3	-43.104	9.328	25/12/2023	03:27:00	500	10	5
5	FISH 014	-46.213	5.944	26/12/2023	04:12:00	500	10	5
6	ExL 01 T2			27/12/2023	01:42:00	400	8	5
7	ExL 01 T2 N			27/12/2023	01:45:00	400	8	5
8	ExL 03 T0 3	-48.383	3.498	27/12/2023	03:34:00	500	10	5
9	ExL 02 T2			28/12/2023	01:15:00	400	8	5
10	ExL 02 T2 Fe			28/12/2023	01:15:00	400	8	5
11	ExL 04 T0 3	-46.909	3.44	28/12/2023	03:14:00	500	10	5
12	ExS 04 T0 3	-49.167	-0.619	30/12/2023	03:48:00	500	10	5
13	ExL 03 T2			31/12/2023	02:42:00	500	10	5
14	ExL 03 T2 Fe			31/12/2023	02:42:00	500	10	5
15	ExL 05 T0 3	-52.357	0.565	31/12/2023	04:09:00	500	10	5
16	ExL 04 T2			01/01/2024	04:20:00	350	7	5
17	ExL 04 T2 Fe			01/01/2024	04:20:00	400	8	5
18	ExS 05 T0 3	-55.522	-0.065	02/01/2024	03:37:00	500	10	5
19	ExL 06 T0 3	-58.097	-0.002	03/01/2024	03:36:00	500	10	5
20	ExL 05 T2			04/01/2024	01:22:00	300	6	5
21	ExL 05 T2 Fe			04/01/2024	01:22:00	250	5	5
22	ExL 07 T0 3	-56.508	-0.553	04/01/2024	03:02:00	500	10	5
23	CORDAY T0 2	-53.303	-1.185	05/01/2024	04:39:00	500	10	5
24	ExS 07 T0 3	-50.383	-3.593	06/01/2024	04:26:00	500	10	5
25	ExL 06 T2			07/01/2024	03:15:00	500	10	5
26	ExL 06 T2 Fe			07/01/2024	03:15:00	500	10	5
27	ExL 08 T0 3	-49.315	-4.232	07/01/2024	04:31:00	500	10	5
28	ExL 07 T2			08/01/2024	03:10:00	500	10	5
29	ExL 07 T2 Fe			08/01/2024	03:10:00	500	10	5
30	ExL 09 T0 3	-48.365	-5.348	08/01/2024	04:47:00	500	10	5
31	CORDAY T0 2	-48.335	-5.329	09/01/2024	04:27:00	500	10	5
32	ExS 08 T0 3	-45.379	-5.246	10/01/2024	03:25:00	500	10	5
33	ExL 08 T2 C			11/01/2024	02:48:00	50	1	5
34	ExL 08 T2 Fe			11/01/2024	02:48:00	50	1	5
35	ExL 08 T2 Mn			11/01/2024	02:48:00	50	1	5
36	ExL 08 T2 Fe+Mn			11/01/2024	02:48:00	50	1	5
37	CORDAY T0	-41.924	-5.15	11/01/2024	03:33:00	500	10	5
38	ExL 09 T2 C			12/01/2024	02:30:00	500	10	5
39	ExL 09 T2 Fe			12/01/2024	02:30:00	500	10	5
40	ExL 09 T2 Mn			12/01/2024	02:30:00	500	10	5
41	ExL 09 T2 Fe+Mn			12/01/2024	02:30:00	500	10	5
42	ExL 10 T0 3	-40.358	-5.095	12/01/2024	03:54:00	1000	20	5
43	ExL 11 T0 3	-38.342	-5.067	13/01/2024	04:26:00	1000	20	5
44	ExS 09 T0 3	-35.355	-5.05	14/01/2024	03:23:00	1000	20	5
45	ExVS 01 T0 2 >5	-32.251	-4.437	15/01/2024	11:39:00	1000	20	5
46	ExVS 01 T0 2>0.4	-32.251	-4.437	15/01/2024	11:39:00	1000	20	0.4
47	ExL 10 T2 C			16/01/2024	02:28:00	500	10	5
48	ExL 10 T2 Fe+N			16/01/2024	02:28:00	500	10	5
49	ExS 10 T0 3>5	-30.656	-3.929	16/01/2024	03:13:00	1000	20	5
50	ExS 10 T0 3>0.4	-30.656	-3.929	16/01/2024	03:13:00	1000	20	0.4
51	ExL 11 T2 C			17/01/2024	01:56:00	500	10	5
52	ExL 11 T2 Fe+N			17/01/2024	01:56:00	500	10	5
53	ExL 12 T0 3>5	-27.943	-2.964	17/01/2024	03:55:00	1000	20	5
54	ExL 12 T0 3>0.4	-27.943	-2.964	17/01/2024	03:55:00	1000	20	0.4
55	ExL 13 T0 3>5	-28.211	-0.923	18/01/2024	03:37:00	1000	20	5
56	ExL 13 T0 3>0.4	-28.211	-0.923	18/01/2024	03:37:00	1000	20	0.4
57	ExS 11 T0 3>5	-28.822	2.59	19/01/2024	03:35:00	1000	20	5
58	ExS 11 T0 3>0.4	-28.822	2.59	19/01/2024	03:35:00	1000	20	0.4
59	ExVS 02 T0 2>5	-29.466	6.228	20/01/2024	03:29:00	1000	20	5
60	ExVS 02 T0 2>0.4	-29.468	6.243	20/01/2024	03:29:00	1000	20	0.4
61	ExL 12 T2 C			21/01/2024		500	10	5
62	ExL 12 T2 Fe			21/01/2024		500	10	5
63	ExL 12 T2 N			21/01/2024		500	10	5
64	ExL 12 T2 Fe+N			21/01/2024		500	10	5
65	ExL 13 T2 C			22/01/2024		500	10	5
66	ExL 13 T2 Fe			22/01/2024		500	10	5
67	ExL 13 T2 N			22/01/2024		500	10	5
68	ExL 13 T2 Fe+N			22/01/2024		500	10	5

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Stoichiometry of Photosynthetic Catalysts

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Background:

Nutrient availability has a strong influence on phytoplankton physiology and growth over more than one-third of the surface oceans. DY172 was designed to sample the Southern Ocean (Iron, Fe-limited) and South Atlantic gyre (Nitrogen N-limited) ocean regions to investigate the impact of limitation on the mechanism of photosynthesis.

Photosynthesis is catalysed by two main reaction centres, light-harvesting pigment-protein complexes that convert photons into chemical potential. These are termed photosystem II (PSII) and photosystem I (PSI). PSII and PSI together represent major sinks of both nitrogen (used in the amino-acid building blocks of the protein) and iron (used to catalyse electron transfer). As such, their abundance and activity is known to be limited by the availability of both N and Fe. Further PSII and PSI do not have an equal demand for Fe and N. Notably PSI has a higher Fe requirement than PSII. As a result many laboratory and field studies have suggested the stoichiometries of PSI:PSII can change in response to nutrient limitation. This then has impacts on growth rate as in order to fix carbon PSII is required to obtain new electrons from water.

The relative abundance and rate of PSII and PSI therefore has a major impact on the transfer of electrons from water to fixed carbon - termed the electron requirement for carbon fixation $\Phi^{e,c}$ (*Nina*). $\Phi^{e,c}$ has been shown to increase with nutrient limitation, although the mechanistic basis behind this alteration in physiology is not known. Here we aimed to collect samples to quantify the abundance of PSII and PSI, in parallel with measurements of $\Phi^{e,c}$ to shed light on the mechanistic basis of physiology under contrasting nutrient-limiting regimes.

Sampling strategy:

1. *Variation spatially*: Samples were collected both from the ships non-toxic underway system (UW samples) and from Niskin bottles collected from vertical CTD casts (Table below) enabling analysis of the PSI:PSII abundances across gradients in nutrient availability. CTD sampling also enables analysis over combined light/nutrient gradients.
2. *Variation temporally*: At selected stations chosen to target the main nutrient regimes encountered 24hr diel sampling was performed from the ships UW system. This enables analysis of changes in PSI:PSII over the day/night cycle.
3. *Variation with nutrient*: A series of nutrient amendment experiments (*Moore*) were conducted to determine how phytoplankton communities respond to the addition of combinations of nutrients (Fe, N and Mn). Samples for analysis of PSI:PSII were collected from initial (To) and final (T96) timepoints.

At sea sampling:

Two methods will be attempted to measure PSI:PSII ratios. (a) Quantitative immunoblots (QI analysis) and (b) 77k fluorometry. Both analyses require filtration of phytoplankton onto GFF filters however the preservation of samples is different. For QI samples can be stored in cryo-tubes for 77k GFF filters are stored flat in petri dishes. Briefly samples are collected in 10 L Jerry cans from either the ships UW system or from Niskin bottles on the CTD. They are then quickly transferred to a filtration rig and 1-4 L are filtered onto a GFF filter (whattman). Filtration time is kept to a maximum for 1 hr to minimise changes in protein abundance during filtration. Filtration is kept to below 3000 kpa vacuum filtration. GFF filters are then placed in cryo-tube or onto petri-dish and then flash-frozen and stored at $^{-}$80° C. The volume filtered is measured with a measuring cylinder and volume recorded as it's the time of filtration.

Sampling was aligned to measurements of $\Phi^{e,c}$ (see table below).

Issues: At high biomass filters take longer to filter, potentially clogging before the volume can be filtered. In these cases a smaller volume is filtered and recorded. At low-biomass the sample collected may not be enough for analysis. In these cases a single filter is collected and preserved on peti-dish as this can be analysed for 77k or QI. Occasionally (e.g. during sampling of the Eddy and or in low-biomass incubation experiments) water volumes available were lower than expected.

Supporting data: For all 77k and QI samples there are corresponding nutrient, chlorophyll, HPLC, PABS, FCM, CytoSense measurements. As such emerging datasets can be analysed with respect to the chemistry and microbial community composition of the system.

Future analysis:

77k fluorometry is a well established technique in photosynthesis research. It is a spectroscopic technique; light at 440 nm excites in the blue peak of chlorophyll absorption is used to induce fluorescence emission that is measured between 650 nm and 750 nm (in the red). At 77k PSII has a maximum emission at 685 nm and PSI at 750 nm. The relative heights of these peaks can then be used to estimate relative abundance of PSI and PSII.

QI analysis is quantitative and relies on the western-blot technique. As all photosynthetic microbes have PSI and PSII. As such, 'Global' antibodies have been developed (available commercially) that bind to highly conserved sites in both PSI and PSII. Total proteins are then extracted from GFF filters separated on gels and exposed to these antibodies. The amount of antibody that binds is proportional to the abundance of PSI or PSII in that sample.

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Sample list:

UW PRO	UW 77K	CTD Protein	CTD 77K	EXPERIMENT
UW001 PRO	UW001 77K	CTD001 23/24	CTD001 23/24	EXL01 T0-01
UW006 PRO	UW006 77K	CTD001 19/20	CTD001 19/20	EXL01 T0-02
UW007 PRO	UW007 77K	CTD002 23/24	CTD002 23/24	EXL01 T0-03
UW011 PRO	UW011 77K	CTD002 17	CTD002 17	EXL02T0
UW012 PRO	UW012 77K	CTD002 15	CTD002 15	EXL01D13 (Control)
UW017 PRO	UW017 77K	CTD002 13	CTD002 13	EXL01D14 (FeN)
UW018 PRO	UW018 77K	CTD003 23	CTD003 23	EXL003T0
UW019 PRO	UW019 77K	CTD003 19	CTD003 19	EXL004T0
UW029 PRO	UW029 77K	CTD003 17	CTD003 17	EXL002D13 (Control)
UW033 PRO	UW033 77K	CTD003 15	CTD003 15	EXL002D14 (Fe)
UW034 PRO	UW034 77K	CTD004 23	CTD004 23	EXL003D13 (Control)
UW035 PRO	UW035 77K	CTD004 15	CTD004 15	EXL003D14 (Fe)
UW036 PRO	UW036 77K	CTD005 23	CTD005 23	EXL005T0
UW051 PRO	UW051 77K	CTD005 19	CTD005 19	EXL004D13 (Control)
UW056 PRO	UW056 77K	CTD006S 23	CTD006S 23	EXL004D14 (Fe
UW057 PRO	UW057 77K	CTD006S 19	CTD006S 19	EXL006T0
UW058 PRO	UW058 77K	CTD007S 23	CTD007S 23	EXL007T0
UW059 PRO	UW059 77K	CTD007S 15	CTD007S 15	EXL005D13 (Control)
UW060 PRO	UW060 77K	CTD008S 23	CTD008S 23	EXL005D14 (Fe)
UW061 PRO	UW061 77K	CTD008S 15	CTD008S 15	EXL006CT24 (Control)
UW062 PRO	UW062 77K	CTD009S 23	CTD009S 23	EXL006CT24 (Control)
UW063 PRO	UW063 77K	CTD009S 15	CTD009S 15	EXL006FET24 (Fe)
UW064 PRO	UW064 77K	CTD010S 23	CTD010S 23	EXL006FET24 (Fe)
UW065 PRO	UW065 77K	CTD010S 17	CTD010S 17	EXL008T0
UW075 PRO	UW075 77K	CTD011S 23	CTD011S 23	EXL006D13 (Control)
UW085 PRO	UW085 77K	CTD011S 17	CTD011S 17	EXL006D14 (Fe)
UW086 PRO	UW086 77K	CTD012S 23	CTD012S 23	EXL007D13 (Control)
UW087 PRO	UW087 77K	CTD012S 17	CTD012S 17	EXL007D14 (Fe)
UW088 PRO	UW088 77K	CTD012S 15	CTD012S 15	EXL009T0
UW089 PRO	UW089 77K	CTD012S 13	CTD012S 13	EXL009D13T24 (Control)
UW090 PRO	UW090 77K	CTD013S 23	CTD013S 23	EXL009D14T24 (Fe)
UW098 PRO	UW098 77K	CTD013S 17	CTD013S 17	EXL008D13 (Control)
UW100 PRO	UW100 77K	CTD014S 23	CTD014S 23	EXL008D14 (Fe)
UW100R PRO	UW100R 77K	CTD014S 17	CTD014S 17	EXL009D13 (Control)
UW111 PRO	UW111 77k	CTD014S 13	CTD014S 13	EXL009D14 (Fe)
UW117 PRO	UW117 77K	CTD015S 23	CTD015S 23	EXL010T0
UW120 PRO	UW120 77K	CTD015S 19	CTD015S 19	EXL011T0
UW121 PRO	UW121 77K	CTD016S 23	CTD016S 23	EXL011D13T24 (Control)
UW122 PRO	UW122 77K	CTD016S 21	CTD016S 21	EXL011D14T24 (Fe)
UW123 PRO	UW123 77K	CTD016S 17	CTD016S 17	EXL010D13 (Control)
UW124 PRO	UW124 77K	CTD017S 23	CTD017S 23	EXL010D14 (Fe)
UW125 PRO	UW125 77K	CTD017S 21	CTD017S 21	EXL010T15 (N)
UW126 PRO	UW126 77K	CTD018S 23	CTD018S 23	EXL010T16 (FeN)
UW129 PRO	UW129 77K	CTD018S 17	CTD018S 17	EXL011D13 (Control)
UW135 PRO	UW135 77K	CTD018S 15	CTD018S 15	EXL011D14 (Fe)

UW137 PRO	UW137 77K	CTD018S 13	CTD018S 13	EXL011T15 (N)
UW138 PRO	UW138 77K	UW 137	UW 137	EXL011T16 (FeN)
UW139 PRO	UW139 77K	CTD018T	CTD018T	EXL012T0
UW140 PRO	UW140 77K	UW 138	UW 138	EXL013T0
UW141 PRO	UW141 77K	CTD019T	CTD019T	EXL012D13 (Control)
UW142 PRO	UW142 77K	UW 139	UW 139	EXL012D14 (FeN)
UW143 PRO	UW143 77K	CTD020T	CTD020T	EXL013D13 (Control
UW147 PRO	UW147 77K	UW 140	UW 140	EXL013D14 (FeN)
UW148 PRO	UW148 77K	CTD021T	CTD021T	
UW149 PRO	UW149 77K	UW 141	UW 141	
UW150 PRO	UW150 77K	CTD022T	CTD022T	
UW151 PRO	UW151 77K	UW 142	UW 142	
UW152 PRO	UW152 77K	CTD023T	CTD023T	
UW153 PRO	UW153 77K	UW 143	UW 143	
UW162 PRO	UW162 77K	CTD024T	CTD024T	
UW168 PRO	UW168 77K	CTD019SS 23	CTD019SS 23	
		CTD019SS 17	CTD019SS 17	
		CTD019SS 13	CTD019SS 13	
		CTD019SS 11	CTD019SS 11	
		CTD020SS 23	CTD020SS 23	
		CTD020SS 17	CTD020SS 17	
		CTD020SS 15	CTD020SS 15	
		CTD020SS 13	CTD020SS 13	
		CTD021SS 23	CTD021SS 23	
		CTD021SS 17	CTD021SS 17	
		CTD021SS 15	CTD021SS 15	
		CTD021SS 11	CTD021SS 11	
		CTD022SS 23	CTD022SS 23	
		CTD022SS 17	CTD022SS 17	
		CTD022SS 15	CTD022SS 15	
		CTD022SS 13	CTD022SS 13	

Nucleic acid sampling

Shanhui Li (*University of Southampton)*

Background:

Oxygenic photosynthesis is initiated by water oxidised at photosystem II (PSII), a chlorophyll-binding membrane protein complex, and the derived electrons flow through a series of electron carriers including cytochrome b_6f and photosystem I (PSI). The primary products of this photosynthetic electron transport, energy (ATP) and reductant (NADPH), together represent the products of the light-dependent reactions of photosynthesis. As proposed by Behrenfeld (Behrenfeld et al., 2008; Behrenfeld & Milligan, 2013), we distinguish this 'photosynthesis' from the consumption of ATP and reductant in the Calvin cycle, as carbon fixation is only one of many processes that consume ATP and reductant.

It has been observed in mixed microbial communities in ocean systems that the number of electrons produced by PSII in the light is often in excess of the number of electrons required for carbon-fixation. This factor, here termed $\Phi^{e,c}$ - the electron requirement for carbon fixation, suggests that the capacity of the light reactions is often in excess of that required to fix carbon. Further, $\Phi^{e,c}$ has been shown to increase under nutrient limitation. The 'fate' of the extra electron produced by the light reactions is however unknown.

Here we aimed to collect samples to interrogate both the genetic diversity (DNA) of phytoplankton communities and their metatranscriptomic profiles (RNA) along natural gradients in iron (Fe) and nitrogen (N) availability, alongside measurements of $\Phi^{e,c}$. We then aimed to reveal the mechanistic basis that underpins how phytoplankton use light energy in the ocean. Three sampling strategies were adopted during DY172:

(1) Spatial (horizontal) sampling from the surface ocean using the ship's underway (non-toxic) system (see Table 1). Vertical sampling from Niskin bottles fired from the CTD sampling rosette targeting the surface (approx. 5 m) and deep-chlorophyll maximum (DCM) or bottom of mixed layer when a DCM was not present (see Table 2 and 3).

(2) Temporal (diel) sampling over light gradients achieved by sampling the ship's non-toxic system every 4 hrs while 'on-station'. This was conducted at station chosen to be representative of the main ocean regimes encountered during DY172 (Table 2).

(3) In response to nutrient by sampling the initial (T0), and end-point (T96) of incubation experiments established along the cruise track whereby factorial nutrient addition experiments were conducted (Table 4).

Complementary measurements:

In parallel to DNA and RNA samples, macronutrients, micronutrients, chlorophyll, absorption spectra, PSI:PSII ration, and photosynthetic physiology using a suit of active fluorometers, and carbon-fixation were routinely taken. While all these samples and datasets will be used to help interpret the analysis of DNA and RNA samples, it is the measurement of $\Phi^{e,c}$ that is of particular interest and as such where these samples were also collected has been highlighted in the tables below.

Issues and solutions:

Volume/biomass:

RNA and DNA sampling requires large volumes of seawater to be filtered quickly in order to sample enough biomass for analysis. The amount of material required is unknown until analysed back in the lab. As such efforts were made to sample as much volume as possible in < 40 min filtration time. 40 min is chosen to minimise any gene expression changes while being filtered. Also efforts are made to minimise the time from sampling to storage and where possible samples are maintained at close to ambient light/temperature while being processed. Whereas ample volumes are available from the non-toxic system seawater from incubations and Niskin samples are limited. DNA and RNA from Niskins were sampled from a duplicate bottle fired at the same depth as a bottle for complementary samples. Nutrients were measured on both bottles to ensure consistency.

From incubation experiments, samples were in 4.5 L bottles. In regions on low-biomass (experiments 12 and 13), duplicate bottles were sampled onto a single filter to increase the biomass filtered. Further, a parallel sample from the ship's non-toxic system was taken at the same time as the incubations were set up. As such from experiments 12 and 13 there were duplicate T0 samples: (a) those taken from the experiment set up (4 L) and (b) those taken from the ship's non-toxic system (20 L). This is noted in the sample table below.

When in regions of high-biomass, filtration became slow. When this occurs, the flow rate is reduced from 100 rpm to approx. 50 rpm to prevent the pressure increasing on cells on the filter. Despite the biomass, community composition changing dramatically during the cruise, every effort was made to standardize the sampling process.

Storage/preservation:

Given liquid nitrogen was available on the ship, samples were snap frozen and then stored at -80° C for shipment back to the lab. This method perturbs the cells to a minimal extent.

Test samples:

In order to troubleshoot extraction methods in the lab, 'additional' test samples were collected from high and low biomass regions.

Sampling methods:

Given RNA can be degraded rapidly by RNase both during sampling and during extract, efforts were made to keep sampling equipment and work areas clean. Sampling bottles were regularly washed in diluted HCl (1%, v/v), rinsed with Mili-Q water. In advance of filtration, pump tubing was cleaned with 70% EtOH, 1% HCl, RNaseZAP, and nuclease-free water. Gloves were worn to protect samples. The specific SOP for sampling is on the cruise data drive.

Samples were collected from Niskin bottles and/or the ship's non-toxic system into clean Jerry cans or subsampled from incubation bottles. Samples for RNA were first filtered using a Watson-Marlow peristaltic pump running at 100 RPM. The time of the sample was taken and the time of filtration are recorded. Samples were filtered onto Stervex filters for a maximum of 40 min and then immediately placed in a plastic bag and snap frozen in liquid nitrogen prior to storage at -80 °C. The volume filtered was measured and recorded. Volume ranged from 3.5L on incubation experiments to 7L from low biomass *in situ* samples. The same sample was then filtered onto a second Stervex filter for the DNA sample and stored in the same way. As such there are two filters per sample. The specific SOP for sampling is on the cruise data drive.

Underway sampling:

Non-toxic underway seawater samples were filtered onto 0.2 µm Sterivex filters using peristaltic pump, flash frozen using liquid nitrogen and stored in the -80 °C freezer number 4 for nucleic acid extraction at a later date. Totally, we collected 58 non-toxic underway samples for nucleic acid sampling. We successfully obtained 116 Sterivex filters ready for nucleic acid extraction, it includes 58 filters for RNA extraction and 58 filters for DNA extraction. Information relevant to these underway samples is shown in Table 1 (green and orange indicate diel and eddy samples, respectively; UW numbers marked by bold indicate samples used for Φe,c measurement).

Table 1 Non-toxic underway sample information

No.	Date	Sampling time (GMT)	Sample time (Ship)	UW Number	Type (RNA/DNA)
1	Dec-24-2023	08:00	10:00	**UW006**	RNA, DNA
2	Dec-25-2023	04:00	06:00	**UW011**	RNA, DNA
3	Dec-25-2023	08:00	10:00	**UW012**	RNA, DNA
4	Dec-26-2023	04:00	06:00	**UW017**	RNA, DNA
5	Dec-26-2023	08:00	10:00	**UW018**	RNA, DNA
6	Dec-26-2023	12:00	14:00	**UW019**	RNA, DNA
7	Dec-28-2023	08:00	10:00	**UW029**	RNA, DNA
8	Dec-29-2023	04:00	06:00	**UW033**	RNA, DNA
9	Dec-29-2023	08:00	10:00	**UW034**	RNA, DNA
10	Dec-29-2023	12:00	14:00	**UW035**	RNA, DNA
11	Dec-29-2023	16:00	18:00	**UW036**	RNA, DNA
12	Jan-01-2024	08:00	10:00	**UW051**	RNA, DNA

13	Jan-02-2024	04:00	06:00	**UW056**	RNA, DNA
14	Jan-02-2024	08:00	10:00	**UW057**	RNA, DNA
15	Jan-02-2024	12:00	14:00	**UW058**	RNA, DNA
16	Jan-02-2024	16:00	18:00	**UW059**	RNA, DNA
17	Jan-02-2024	20:00	22:00	UW060	RNA, DNA
18	Jan-03-2024	00:00	02:00	UW061	RNA, DNA
19	Jan-03-2024	04:00	06:00	UW062	RNA, DNA
20	Jan-03-2024	08:00	10:00	UW063	RNA, DNA
21	Jan-03-2024	12:00	14:00	UW064	RNA, DNA
22	Jan-03-2024	16:00	18:00	UW065	RNA, DNA
23	Jan-05-2024	08:00	10:00	**UW075**	RNA, DNA
24	Jan-07-2024	00:00	02:00	UW085	RNA, DNA
25	Jan-07-2024	04:00	06:00	UW086	RNA, DNA
26	Jan-07-2024	08:00	10:00	**UW087**	RNA, DNA
27	Jan-07-2024	12:00	14:00	**UW088**	RNA, DNA
28	Jan-07-2024	16:00	18:00	**UW089**	RNA, DNA
29	Jan-07-2024	20:00	22:00	UW090	RNA, DNA
30	Jan-09-2024	08:00	10:00	**UW098**	RNA, DNA
31	Jan-10-2024	08:00	10:00	**UW111**	RNA, DNA
32	Jan-11-2024	08:00	10:00	**UW117**	RNA, DNA
33	Jan-11-2024	20:00	22:00	UW120	RNA, DNA
34	Jan-12-2024	00:00	02:00	UW121	RNA, DNA
35	Jan-12-2024	04:00	06:00	UW122	RNA, DNA
36	Jan-12-2024	08:00	10:00	**UW123**	RNA, DNA
37	Jan-12-2024	12:00	14:00	**UW124**	RNA, DNA
38	Jan-12-2024	16:01	18:01	**UW125**	RNA, DNA
39	Jan-12-2024	20:03	22:03	UW126	RNA, DNA
40	Jan-13-2024	08:00	10:00	**UW129**	RNA, DNA
41	Jan-14-2024	08:00	10:00	**UW135**	RNA, DNA
42	Jan-14-2024	17:13	19:13	UW137	RNA, DNA
43	Jan-15-2024	23:05	01:05	UW138	RNA, DNA
44	Jan-15-2024	05:15	07:15	UW139	RNA, DNA
45	Jan-15-2024	11:15	13:15	UW140	RNA, DNA
46	Jan-15-2024	17:27	19:27	UW141	RNA, DNA
47	Jan-16-2024	23:10	01:10	UW142	RNA, DNA
48	Jan-16-2024	05:03	07:03	UW143	RNA, DNA

49	Jan-16-2024	20:07	22:07	UW147	RNA, DNA
50	Jan-17-2024	00:00	02:00	UW148	RNA, DNA
51	Jan-17-2024	03:00	05:00	UW149	RNA, DNA
52	Jan-17-2024	08:00	10:00	**UW150**	RNA, DNA
53	Jan-17-2024	12:00	14:00	**UW151**	RNA, DNA
54	Jan-17-2024	16:07	18:07	**UW152**	RNA, DNA
55	Jan-17-2024	20:04	22:04	UW153	RNA, DNA
56	Jan-18-2024	02:45	04:45	UW155	RNA, DNA
57	Jan-19-2024	08:00	10:00	**UW162**	RNA, DNA
58	Jan-20-2024	08:00	10:00	**UW168**	RNA, DNA

On station stainless steel CTD sampling:

Samples from different depths of 21 stations were collected using stainless steel CTD rosette, filtered onto 0.2 µm Sterivex filters using peristaltic pump, flash frozen using liquid nitrogen and stored in the -80 °C freezer number 4 for nucleic acid extraction at a later date. In total, we successfully obtained 84 Sterivex filters ready for nucleic acid extraction, it includes 42 filters for RNA extraction and 42 filters for DNA extraction. Sampling information is shown in Table 2.

Table 2 Stainless steel CTD sample information

Date	Sampling time (GMT)	Sampling time (Ship)	Cast Number	Depth (Niskin Number)	Type (RNA/DNA)
Dec-23-2023	07:30	09:30	CTD_002	5 m (23)	RNA, DNA
	07:30	09:30	CTD_002	50 m (15)	RNA, DNA
Dec-24-2023	07:15	09:15	CTD_003	5 m (24)	RNA, DNA
	07:15	09:15	CTD_003	41 m (18)	RNA, DNA
Dec-26-2023	07:26	09:26	CTD_004	5 m (24)	RNA, DNA
	07:26	09:26	CTD_004	71 m (16)	RNA, DNA
Dec-27-2023	07:10	09:10	CTD_005	5 m (24)	RNA, DNA
	07:10	09:10	CTD_005	30 m (20)	RNA, DNA
Dec-28-2023	07:00	09:00	CTD_006	10 m (24)	RNA, DNA
	07:00	09:00	CTD_006	35 m (20)	RNA, DNA
Jan-01-2024	06:55	08:55	CTD_007	5 m (24)	RNA, DNA
	06:55	08:55	CTD_007	75 m (16)	RNA, DNA
Jan-02-2024	06:25	08:25	CTD_008	5 m (24)	RNA, DNA
	06:25	08:25	CTD_008	90 m (16)	RNA, DNA
Jan-03-2024	08:30	06:30	CTD_009	5 m (24)	RNA, DNA

	08:30	06:30	CTD_009	75 m (16)	RNA, DNA
Jan-04-2024	08:28	06:28	CTD_010	8 m (24)	RNA, DNA
	08:28	06:28	CTD_010	80 m (18)	RNA, DNA
Jan-05-2024	08:35	06:35	CTD_011	5 m (24)	RNA, DNA
	08:35	06:35	CTD_011	50 m (18)	RNA, DNA
Jan-07-2024	06:45	08:45	CTD_012	5 m (24)	RNA, DNA
	06:45	08:45	CTD_012	50 m (17)	RNA, DNA
Jan-09-2024	06:20	08:20	CTD_013	5 m (24)	RNA, DNA
	06:20	08:20	CTD_013	50 m (18)	RNA, DNA
Jan-10-2024	07:25	09:25	CTD_014	5 m (24)	RNA, DNA
	07:25	09:25	CTD_014	40 m (18)	RNA, DNA
Jan-11-2024	07:10	09:10	CTD_015	5 m (24)	RNA, DNA
	07:10	09:10	CTD_015	37 m (20)	RNA, DNA
Jan-12-2024	06:25	08:25	CTD_016	3 m (24)	RNA, DNA
	06:25	08:25	CTD_016	15 m (22)	RNA, DNA
Jan-13-2024	07:30	09:30	CTD_017	5 m (24)	RNA, DNA
	07:30	09:30	CTD_017	20 m (22)	RNA, DNA
Jan-14-2024	07:00	09:00	CTD_018	5 m (24)	RNA, DNA
	07:00	09:00	CTD_018	67 m (15)	RNA, DNA
Jan-17-2024	06:15	08:15	CTD_019	5 m (24)	RNA, DNA
	06:15	08:15	CTD_019	120 m (16)	RNA, DNA
Jan-18-2024	07:10	09:10	CTD_020	5 m (24)	RNA, DNA
	07:10	09:10	CTD_020	93 m (16)	RNA, DNA
Jan-19-2024	07:30	09:30	CTD_021	5 m (24)	RNA, DNA
	07:30	09:30	CTD_021	100 m (16)	RNA, DNA
Jan-20-2024	06:30	08:30	CTD_022	5 m (24)	RNA, DNA
	06:30	08:30	CTD_022	85 m (16)	RNA, DNA
	06:30	08:30	CTD_022	440 m (5)	RNA, DNA

Eddy sampling:

The deep chlorophyll maximum samples from eddy were collected using titanium CTD, filtered onto 0.2 µm Sterivex filters using peristaltic pump, flash frozen using liquid nitrogen and stored in the -80 °C freezer number 4 for nucleic acid extraction at a later date. We totally collected 7 samples from eddy for nucleic acid sampling. We successfully obtained 14 Sterivex filters ready for nucleic acid extraction, it includes 7 filters for RNA extraction and 7 filters for DNA extraction. The surface seawaters from eddies were collected using ship's underway system as mentioned above. Information on these samples is shown in Table 3. The bold indicates samples used for *Φ*e,c measurement.

Table 3 Titanium CTD sample information

Date	Sampling time (GMT)	Sampling time (Ship)	Cast Number	Depth	Type (RNA/DNA)
Jan-14-2024	17:13	19:13	UW137	-	RNA, DNA
Jan-14-2024	17:05	19:05	CTD018T	80 m	RNA, DNA
Jan-15-2024	23:05	01:05	UW138	-	RNA, DNA
Jan-15-2024	22:49	00:49	CTD019T	110 m	RNA, DNA
Jan-15-2024	05:15	07:15	UW139	-	RNA, DNA
Jan-15-2024	04:50	06:50	CTD020T	121 m	RNA, DNA
Jan-15-2024	11:15	13:15	UW140	-	RNA, DNA
Jan-15-2024	10:50	12:50	CTD021T	94 m	RNA, DNA
Jan-15-2024	17:27	19:27	UW141	-	RNA, DNA
Jan-15-2024	17:12	19:12	CTD022T	95 m	RNA, DNA
Jan-16-2024	23:10	01:10	UW142	-	RNA, DNA
Jan-16-2024	23:09	01:09	CTD023T	107 m	RNA, DNA
Jan-16-2024	05:03	07:03	UW143	-	RNA, DNA
Jan-16-2024	05:01	07:01	CTD024T	85 m	RNA, DNA

Incubation experiment sampling:

Incubation experiments were performed using 4.5L bottles on board. After long-term (4 days) incubations, all the bottles for the control (C) and treatments were subsampled and filtered onto 0.2 µm Sterivex filters using peristaltic pump, flash frozen using liquid nitrogen and stored in the -80 °C freezer number 4 for nucleic acid extraction at a later date. Totally, we performed 13 long/large incubation experiments with different nutrient amendments in triplicates. For each experiment, we subsampled T0 samples in triplicate, and the replicates of all treatments in T96 for RNA and DNA sampling. *Φ*e,c measurement was performed using T0 samples, and pooled triplicates of two treatments in T96 (Bold indicates the treatments used for *Φ*e,c measurement). We also subsampled T24 samples from two treatments only for RNA sampling. Information on those samples is shown in Table 4. In total, we obtained 402 Sterivex filters ready for nucleic acid extraction, it includes 210 filters for RNA extraction and 192 filters for DNA extraction.

Table 4 Incubation experiment sample information

Date	Ex. No.	T0				Nutrient addition	T24		T96	
		Time (GMT)	Time (Ship)	UW No.	Sampling type		Trt.	Sampling type	Trt.	Sampling type
Dec-23-2023	ExL-01	03:06	05:06	-	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	-	-	**C**, Fe, **N**, FeN	RNA, DNA
Dec-24-2023	ExL-02	02:40	04:40	-	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	-	-	**C**, Fe, N, **FeN**	RNA, DNA
Dec-27-2023	ExL-03	02:58	04:58	-	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	-	-	**C, Fe**, Mn, FeMn	RNA, DNA
Dec-28-2023	ExL-04	02:24	04:24	-	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	-	-	**C, Fe**, Mn, FeMn	RNA, DNA
Dec-31-2023	ExL-05	03:34	05:34	-	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	-	-	**C, Fe**, Mn, FeMn	RNA, DNA
Jan-03-2024	ExL-06	03:02	05:02	UW062	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	-	-	**C, Fe**, Mn, FeMn	RNA, DNA
Jan-04-2024	ExL-07	02:17	04:17	-	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	C, Fe	RNA	**C, Fe**, Mn, FeMn	RNA, DNA
Jan-07-2024	ExL-08	04:02	06:02	UW086	RNA, DNA, *Φ*e,c	C, Fe, N, FeMn	-	-	**C, Fe**, N, FeMn	RNA, DNA
Jan-08-2024	ExL-09	04:04	06:04	-	RNA, DNA, *Φ*e,c	C, Fe, Mn, FeMn	C, Fe	RNA	**C, Fe**, Mn, FeMn	RNA, DNA
Jan-12-2024	ExL-10	03:27	05:27	UW122	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	-	-	**C, Fe**, N, FeN	RNA, DNA
Jan-13-2024	ExL-11	03:20	05:20	-	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	C, FeN	RNA	**C**, Fe, N, **FeN**	RNA, DNA
Jan-17-2024	ExL-12	03:13	05:13	UW149	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	-	-	**C, Fe, N, FeN**	RNA, DNA
Jan-18-2024	ExL-13	02:37	04:37	UW155	RNA, DNA, *Φ*e,c	C, Fe, N, FeN	-	-	**C, Fe, N, FeN**	RNA, DNA

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Sample collection Marine Carbonate System

Ben Cala (NIOZ)

The main focus was the collection of carbonate system samples at the station of the mooring (Station 31) where pH and total alkalinity (TA) samples were collected. Further TA samples were taken at full-depth metal-free CTD stations as well as the Eddy stations (see log sheets).

Additionally, small DIC samples were taken from the underway (once a day) and at the end of some of the Long Incubation Experiments to compliment Nina Schubeck's C14 measurements as well as the end of the ocean warming experiments by Corday Seldon.

All analysis of the samples will be done after the cruise, at NIOZ.

TA and pH sampling:

Samples for pH and total alkalinity (TA) were collected following the best-practice recommendations (Dickson et al., 2007). The samples were collected with Tygon tubing into 250 ml borosilicate glass bottles. The bottles were rinsed three times, filled from the bottom smoothly and overflown for two times the bottle volume and sealed with ground glass stoppers. Sampling from the metal-free CTD was done by Maeve Lohan.

Once in the laboratory, a headspace in the TA samples was created by removing 1% of the volume to allow the expansion of the seawater, then the samples were poisoned with a saturated mercury (II) chloride solution (50 µl) to prevent any biological activity. The samples were sealed with greased glass stoppers to prevent gas exchange and secured with tape. Samples were then thoroughly mixed and stored in the dark at temperatures below 25°C.

DIC samples:

DIC samples were collected in small gas-tight vials either the volume of 5 mL or 22mL. The vials were pre-poised with 15 µL saturated mercury (II) chloride solution. Seawater was added form a convex meniscus at the rim to avoid air bubbles in the vial upon closure. Samples were subsequently stored in the dark at temperatures below 25°C.

Samples from the Long Incubation Experiments L11 and L12 were not added to the pre-poisoned vial (instead just left in the original gas-tight vial) due to work time constraints.

Citations:

Dickson, A. G., Sabine, C. L., and Christian, J. R. (Eds.): SOP 1: Water sampling for the parameters of the oceanic carbon dioxide system, in: Guide to Best Practices for Ocean CO2 Measurements, PICES Special Publication 3, North Pacific Marine Science Organization, Sidney, BC, Canada, 1–6, 2007.

Ocean warming experiments

Corday Selden (Rutgers University, New Brunswick NJ USA)

This work was highly collaborative and included significant contributions from Mark Moore (trace metal-clean water collection), Tommy Ryan-Keogh (trace metal-clean water collection), Joe Furby (trace metal-clean water collection), Karel Bakker (macronutrients), Ben Cala (DIC), Heshani Pupulewatte (MiniFIRe, PicoLiF), and Sarah Le Besque (CytoSense). The protocols for these contributions are detailed elsewhere in this report.

Scientific Motivation:

Though the Southern Ocean (SO) represents only ~20% of the world's ocean, it is a major conduit through which the ocean absorbs carbon dioxide (CO_2) (DeVries 2014). This vital function is driven by the biological carbon (C) pump—the sum set of ecosystem processes which transfer CO_2 counter-gradient to the deep sea, where it can remain out of contact with the atmosphere for decades to centuries. Phytoplankton mediate the first step of the biological C pump—the photosynthetic conversion of CO_2 to organic C—and thus set an upper limit on biological C sequestration. Without the biological pump, the SO would be a net source of CO_2 to the atmosphere (Huang et al. 2023).

As biological function depends on growth environment, biological C sequestration in the SO is subject to shift under climate change, with reciprocal consequences for global climate (i.e., feedbacks). The Metabolic Theory of Ecology (Brown et al. 2004) predicts that increasing temperature increases metabolic rates, and consequently growth, due to the direct effects of temperature on enzymatic efficiency (Arroyo et al. 2022). Ocean warming might thus be expected to increase rates of primary production if not for pervasive nutrient limitation. Indeed, growth limitation due to deprivation of nitrogen—an essential building block required protein, pigment, and nucleic acid construction—is observed to dampen the effects of warming on phytoplankton metabolism (Marañón et al. 2018). Yet, unlike most of the surface ocean, SO phytoplankton are not limited by nitrogen availability but rather by iron (Fe), which is chiefly used as a co-factor in oxidoreductases (enzymes that mediate oxidation-reduction reactions). Thus, cellular demand for N and Fe under may vary with warming, affecting the extent to which limitation by these nutrients alters microbial response to warming.

Here, we examine the effects of warming on N uptake and C fixation across Fe- and N-limited waters of the Southern Ocean and South Atlantic. Samples for additional N uptake rates and urea and ammonium concentrations were also collected in surface waters across the transect at stations where ocean warming experiments were not performed to provide experimental context.

Brief Research Summary:

Natural seawater (Table 1) was collected ~0300 UTC from the Tow-FISH and incubated at ambient, +2°C, and +4°C for 72 hours, after which time the following measurements were made in duplicate: ^{15}N compound (nitrate, ammonium urea) uptake rates, ^{13}C fixation, POC/PON concentration and natural C/N isotope abundance, nutrient concentrations (nitrate, ammonium, urea, nitrite, phosphate, silicate), Fv/Fm, dissolved inorganic C (DIC), and flow cytometry. These and additional measurements (including dFe and size-fractionated chlorophyll a analyses) were also made upon sample collection. Additional ^{15}N uptake rate measurements were made in surface waters (collected as above) across the transect (Table 2).

Materials and Methods:

Experimental design

Water was collected from the Tow-FISH using the trace-metal clean approach detailed in this report for nutrient additions experiments (Moore, Ryan-Keogh). In brief, 24 2.4 L square polycarbonate bottles (Nalgene), three 4 L round polycarbonate bottles (Nalgene), and a 4 L LDPE Cubitainer^{TM} (Qorpak) were thrice rinsed and filled with natural surface seawater before sunrise (typically ~0400 UTC). The polycarbonate bottles were immediately transferred to standing incubators (Minus40): nine (either 2.4 L and 1 4 L) to an incubator set to the ambient temperature, nine to another set 2°C above ambient temperature, and nine to a third set 4°C above ambient temperature. Samples to determine initial concentrations of particulate organic C and N (POC/PON), and ammonium and urea concentrations were collected from the 4 L Cubitainer^{TM} in the wet lab as described below. Initial concentrations of other nutrients (nitrate, nitrite, phosphate, silicate, iron) and chlorophyll *a* were collected immediately before and after bottle-filling in the clean lab. Samples for MiniFIRE and PicoLiF analysis (Pupulewatte) were collected either in parallel with these samples (when temperature experiments were set-up on the same day as nutrient additions experiments), or from the 4 L Cubitainers (if not).

Seawater was incubated for 72 hours, during which time the bottles were never opened. Incubator temperatures were monitored continuously throughout incubations using HOBO Data Loggers (Onset). Incubator lights were on for 15 h per day (0330 – 1830 UTC) at a PAR level ~200 μmol photons m^{-2} s^{-1}. After ~72 hours and at the beginning of the light period (at 0330 UTC), ammonium uptake rate measurement incubations were initiated with the addition of a ^{15}N-ammonium tracer (see below) in two replicate 2.4 L polycarbonate bottles in each incubator (six bottles total). Collection of RNA/DNA samples subsequently commenced from the 4 L polycarbonate bottles (one sample per treatment; additional 2 L polycarbonate bottles were incubated in tandem to increase biomass on filter for experiments conducted in highly oligotrophic waters). An hour later (~0430 UTC) urea uptake rates were initiated, again in duplicate per treatment. Samples for MiniFIRE/PicoLiF (Pupulewatte), CytoSense (Le Besque), general macronutrients (Bakker), and urea, ammonium, and POC/PON analysis (below) were then collected from unamended 2.4 L in duplicate per treatment. The remaining 2.4 L bottles (duplicate/treatment) were spiked with both ^{15}N-nitrate and ^{13}C-bicarbonate at ~0530 UTC. Immediately prior to this spike, bottles were subsampled for DIC concentration (Cala). We note that natural abundance POC/PON was the last measurement to be collected from the non-tracer bottles and was not typically completed until after the nitrate/bicarbonate uptake bottles were spiked. During DIC subsampling and ^{15}N-nitrate/^{13}C-bicarbonate spiking, natural abundance bottles were returned to the incubators.

On days when ocean warming experiments were neither being set up nor ended, ammonium, nitrate, and urea uptake rates were measured in surface seawater collected as described above, with the exception that round 2.3 L polycarbonate (Nalgene) bottles were used. Tracer spikes were added immediately upon sample collection, and ammonium, urea, and POC/PON samples were collected from a Cubitainer immediately afterwards. Additional samples, including macronutrients, were collected in the trace metal clean van during bottle filling.

Particulate organic nitrogen (N) and carbon (C) sample collection:

Samples for PON/POC concentration and determination of the natural abundance of N and C isotopes ($^{15/14}$N and $^{13/12}$C) were collected by (gentle) vacuum pump filtration on combusted (2 hours at 450°C) GF/F filters (0.7 µm; Whatman). Samples were held either in 2.4 L polycarbonate incubation bottles (t=final) or 4 L Cubitainers^{TM} (t=initial) and transferred to a 200 ml filter tower (which was washed profusely with ultrapure water between uses) using a 500 ml (+/- 5 ml) graduated cylinder. Sample containers were gently inverted prior to volume measurements to prevent settling of large particles. Filters were collected using clean forceps and transferred to microcentrifuge tubes. Samples were stored onboard at -20°C. We note that there was an unfortunate freezer malfunction, which led to the defrosting of samples. The freezer failed no earlier than 1400 UTC on 15/1/24; samples were transferred to a second freezer at 0730 UTC on 16/1/24. All samples will nonetheless be transported frozen to Rutgers University by plane in a hard cooler (YETI) built for long (5-7 d) frozen storage. At Rutgers, they will be stored at -20°C until drying (50°C for 1-5 days), pelletization, and analysis on an EA-IRMS. Samples for natural isotope abundances were processed on a different filter rig than that used in isotope tracer experiments (detailed below) and stored separately at every stage of processing.

^{15}N and ^{13}C uptake rate incubations:

Ammonium, nitrate, urea, and bicarbonate uptake rates were measured via short-term (6 hour) tracer experiments conducted in duplicate. Bottles were amended with tracer amounts (~10% of ambient pool) of ^{15}N-labelled ammonium, nitrate, and urea, respectively. ^{13}C-bicarbonate was added to the same bottles as nitrate. Nitrate concentrations determined onboard were used to adjust the isotope addition to ensure that the tracer was never added in potentially stimulating quantities (>10%). Ammonium concentrations were similarly monitored, but never rose above ~500 nM. Consequently, the minimum addition (50 nM, final concentration) was added in all experiments for ammonium and urea. Where nitrate concentrations were <500 nM, a spike of 50 nM was used as well. Assuming an ambient DIC concentration of approximately 2000 µM, a spike of 200 µM was used for ^{13}C fixation rate measurements in all experiments. After the tracer addition, bottles were incubated under approximate ambient light and temperature conditions for ~6 hours, then filtered and stored as described above for (but physically separated from) natural abundance POC/PON. See note above regarding freezer malfunction.

Ammonium sample collection and analysis:

Filtered seawater for ammonium and urea analysis was collected using acid-washed 60 ml syringes and 0.22 μm Sterivex filters. Both syringes and filters were reused, with ultrapure water and sample rinses between samples. Samples for shipboard ammonium analysis were collected in pre-treated (7% OPA working reagent, 1+ d) 15 ml centrifuge tubes (Corning), which were thrice rinsed with ultrapure water and sample prior to sample collection. Samples were stored at 4°C until onboard analysis for no more than three days; no significant change in ammonium concentration could be observed during storage following this protocol (unpaired t-test, n=6, p=0.58).

Ammonium concentrations were determined onboard via the OPA method (Holmes et al. 1999) using a Trilogy Fluorometer (Turner). Briefly, 0.8 ml of OPA working reagent was added to 10 ml aliquots of sample or standard. These vials were incubated in the dark for 3-4 hours. Fresh secondary standards were made daily in ultrapure water from a 10 mM ammonium stock solution. A 200 nM standard was analyzed at intervals of 5-8 samples to assess and correct for drift. To correct for matrix effects, aliquots of filtered surface seawater and ultrapure water were analyzed immediately following addition of OPA working reagent. Detection limits were calculated per run as three times the standard deviation of seven replicate blanks, and were typically in the range of 2-7 nM.

Urea sample collection:

Samples for urea analysis were collected by syringe filtration as described above for ammonium. Filtrate was collected in 15 ml centrifuge tubes (Falcon), leaving at least 3 ml of headspace remaining at the top. Filtrate was frozen at -20°C. Please see note regarding onboard freezer malfunction in POC/PON section above. Transport plan for urea samples is as written for POC/PON samples. Urea samples will be analyzed via standard colorimetric method (Strickland and Parsons 1972) promptly at Rutgers University.

RNA/DNA sample collection:

Samples for RNA/DNA were collected on sterile 0.45 μm Sterivex^{TM} (Sigma Aldrich) filters from 4 L polycarbonate bottles using a peristaltic pump. (Size chosen to correspond with GF/Fs). Prior to sample collection, tubing was washed with 10% HCl followed by profuse ultrapure water and a brief sample rinse. Samples collection lasted no more than 1 hour, or until the full bottle volume was filtered. Remaining water was evacuated from the filters by pushing air through using an acid-washed (10% HCl, 2 d) 60 ml syringe (reused across samples). RNALater was immediately added to filters using a 1 ml pipette. Filters were then held at 4°C for 1-3 hours. The RNALater was subsequently expelled using the same syringe as above. Sterivex^{TM} were then sealed using a fitted cap on the Luer-lock end and Hemato-seal (Fisherbrand) at the other end. Filters were bagged individually and immediately placed in the -80°C freezer on the ship. They will be stored here until the ship returns to Southampton, at which time they will be shipped to Corday Selden at Rutgers.

Table 1. Sampling locations for ocean warming experiments.

Experiment ID	**Date**	**Initial SST (°C)**	**Associated station (approx.)**	**Associated nut. add. expts.**	**Notes**
1	24/12/23	12.5	2	ExL02	No RNA/DNA collected; no C fixation rate collected
2	28/12/23	7.2	6	ExL04	
3	1/1/24	3.2	7	N/A	
4	5/1/24	2.1	11	N/A	Ended at 48 hours instead of 72
5	9/1/24	5.9	14	N/A	
6	13/1/24	16.5	18	ExL11	
7	18/1/24	23.9	28	ExL13	Additional volume added for natural abundance POC/PON and RNA/DNA

Table 2. Sampling locations for surface ammonium, urea, and nitrate uptake rates (at approximate ambient temperature/light and without additional acclimation period), and additional ammonium/urea concentrations.

Date	**Associated station (approx.)**	**Associated nutrient addition experiments**	**Notes**
23/12/23	2	N/A	Uptakes also measured at DCM (Ti CTD)
26/12/23	4	N/A	
30/12/23	N/A	ExS04	
31/12/23	N/A	ExL05	
2/1/24	8	ExS05	
3/1/24	9	N/A	
6/1/24	N/A	ExS07	
8/1/24	14	ExL09	
10/1/24	15	ExS08	
11/1/24	16	N/A	
14/1/24	19	ExS09	
17/1/24	27	ExL12	
19/1/24	29	ExS11	
20/1/24	30	Exvs02	

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Mooring recovery

Ben Cala (NIOZ)

Short overview:

The main purpose of the mooring is in situ calcium carbonate dissolution measurements. Various types of calcium carbonate minerals were attached to the mooring line at regular depth intervals and deployed on the BEYΩND cruise (64PE513) on February 14th, 2023 at 30°01.53 S, 9°31.65 E. In total, 460 samples were attached to the line at 46 different depths (10 samples at every depth level). The mooring also includes a sediment trap at 4500 m and instruments that aid the interpretation of the mooring line's movements in the water. A schematic of the mooring in shown in Figure 1.

A more thorough description of the mooring (samples and instruments) can be found in the BEYΩND cruise report.

Sample recovery:

All samples were successfully recovered (sample tubes 101-146 and 151-196, Figure 2) and were found at their intended position on the mooring line. An exception are the samples below the sediment trap, which have moved up the cable, presumably during deployment. Right below the sediment trap, the first 2 sample tubes were placed as expected but at the next depth, three instead of 2 sample tubes were found (141,142,192) and at the next depth all remaining sample tubes were right after one another (143-146 and 192-196). This position has been marked with a blue tape, which will aid in determining how far below the sediment trap the samples accumulated.

Instrument recovery:

Upon recovery, MicroCATs were rinsed with freshwater and left soaking in fresh water until the data collection was manually stopped around 3h after recovery. The clock shift was noted.

The Aquadopp was rinsed in freshwater, data acquisition was stopped and data was saved and converted.

The pressure and tilt sensors were rinsed with freshwater, data acquisition was stopped, the clock shift noted, and data on the SD card was saved. The sensors were disconnected from power supply. Datalogger #25 had a broken o-ring but the inside was dry and the data collected seems sensible.

The acoustic releases and the iridium beacon were rinsed in freshwater and the batteries of the beacon were removed.

Rinsing and storage of sample tubes:

Immediately upon recovery of each sample tube was rinsed for 5 s in a bucket of MilliQ to get rid of the majority of the salt water. The MilliQ in the bucket was changed after every 8 sample tubes. Afterwards, the tubes were dunked for 30 s in 30 L of pH-buffered MilliQ (30L were buffered with 6 ml of 22% NH_4OH, resulting in a pH of 10). Sample tubes were rudimentarily dried with paper towels before put into a plastic sheet-lined aluminium box. After recovery and rinsing of all samples, the boxes were put outside in the sun to dry. The lids of the boxes were left slightly ajar to allow air circulation while minimizing contamination with dust from the ship. Subsequently, the sample boxes were stored in the climate control room (low humidity). For transport in the container to Southampton, 2kg of Silica Gel filled in nylon tights were added to every box to minimize a build-up of high humidity inside the boxes.

Sediment trap:

The sediment trap was kept upright through the entire recovery to avoid leakage of the $HgCl_2$ -poisoned sediment trap bottles. Appropriate PPE was worn for retrieving the individual sediment trap bottles and the deck was thoroughly rinsed afterwards. Upon recovery, it was found that that the sediment trap motor did not sit properly in the carousel (see Figure 3). The position of the carousel was still at 0 and since the sediment trap bottles are entirely empty, the dislodging of the motor most likely happened during deployment. The contents of the sediment trap bottles were therefore declared hazardous waste.

Other activities:

Carbonate system sampling

At the site of the mooring recovery (Station 31) two CTD casts were deployed, CTD029_T (full-depth) and CTD023_SS (2500m).

CTD029_T: 24 bottles were closed at 24 depths (bottle 23 misfired). TA, pH and macronutrient samples taken from all bottles with 2 duplicates each for TA and pH.

CTD023_SS: 16 bottles were closed at 8 depths. TA, pH and macronutrient samples were taken at all 8 depths with one duplicate each.

Calibration of instruments

Of all the instruments, only the MicroCATs were calibrated (the pressure/tilt sensors were calibrated on the BEYΩOND cruise and no large drifts in pressure measurements are expected. Additionally, one of those sensors had a broken o-ring and it would not have been possible to deploy that instrument safely. The MicrOCATs were attached to the steel CTD on a cast to 2500 m (CTD023_SS). A logging interval of 10 s was chosen. Data was downloaded and the calibration will be done after the cruise.

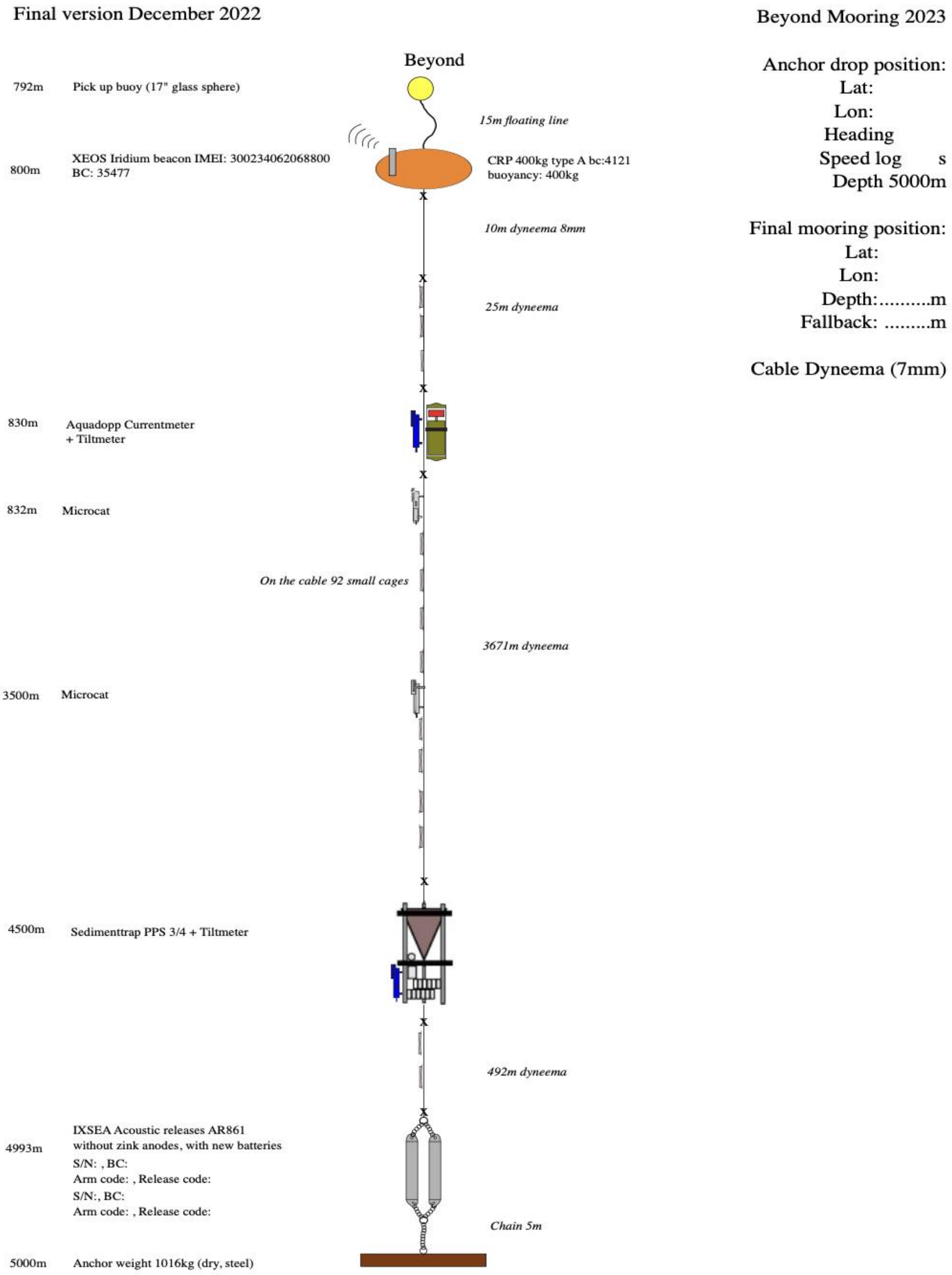

Figure 1: Schematic of the recovered mooring.

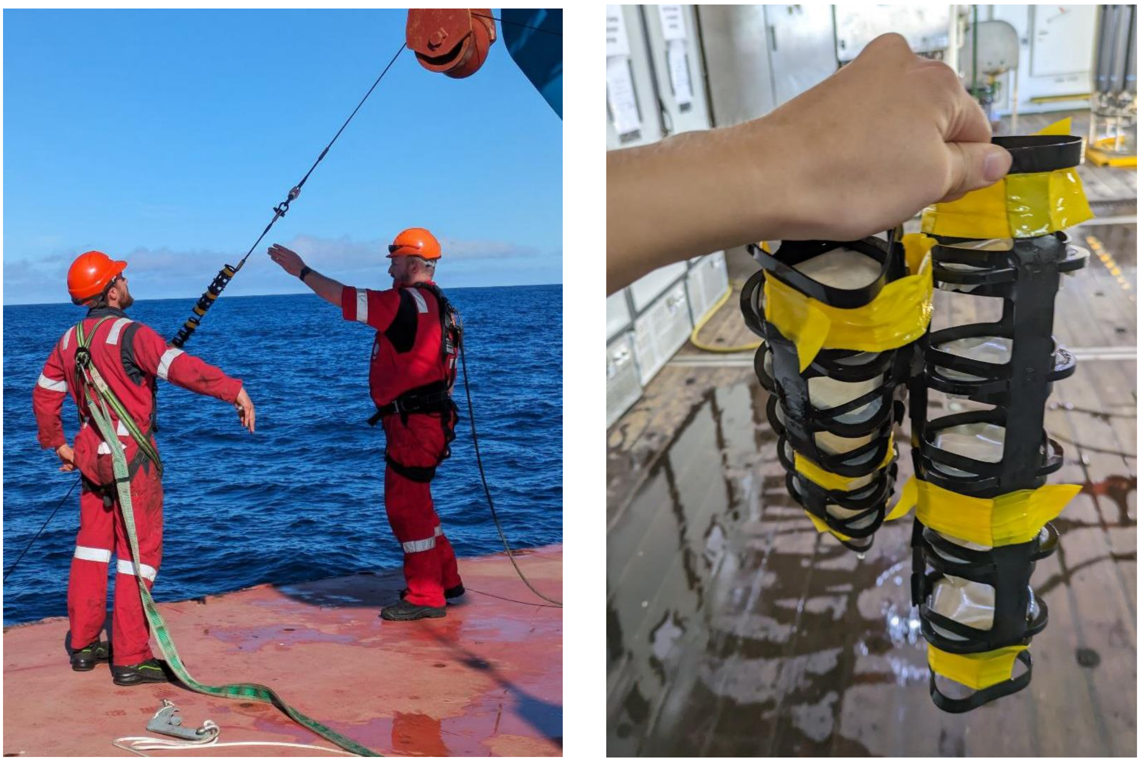

Figure 2: (a) Dissolution sample tubes attached to the mooring line. (b) Sample tubes on their way to getting rinsed.

Figure 3: Recreation of how the sediment trap motor was found inside the sediment trap, instead of being placed in the hole on the right of the trap.

Argo deployments

(deployed by crew on request of Fiona Carse, Met Office)

Locations and serial numbers of Argo floats deployed in international waters:

Lat	Long	Serial Number
-43.112	9.319	Argo Float 1 (9627)
-46.387	5.743	Argo Float 2 (9614)
-50.702	0.06	Argo Float 3 (10061)
-58.136	-0.005	Argo Float 4 (10060)
-58.136	-0.005	Argo Float 5 (10059)

Appendix 1 – Sensor Information Sheets

SENSOR INFORMATION SHEET – Stainless-Steel CTD with LADCPs

Instrument / Sensor	Manufacturer/ Model	Serial Number	Channel	Casts Used
Stainless steel 24-way frame	NOCS	CTD 6	N/A	All stainless casts
EM Swivel	MDS ST6003-2E2-Ti	1267-2 (No. 6)	N/A	All stainless casts
Primary CTD deck unit	SBE 11plus	11P-24680-0588	N/A	All casts
CTD Underwater Unit	SBE 9plus	09P-34173-0758	N/A	All stainless casts
24-way Carousel	SBE 32	32-60380-0805	N/A	All stainless casts
Primary Temperature Sensor	SBE 3P	3P-5785	F0	All stainless casts
Primary Conductivity Sensor	SBE 4C	4C-3054	F1	All stainless casts
Digiquartz Pressure sensor	Paroscientific	90074	F2	All stainless casts
Secondary Temperature Sensor	SBE 3P	3P-5835	F3	All stainless casts
Secondary Conductivity Sensor	SBE 4C	4C-3698	F4	All stainless casts
Primary Pump	SBE 5T	5T-3090	N/A	All stainless casts
Secondary Pump	SBE 5T	5T-3086	N/A	All stainless casts
Primary Dissolved Oxygen Sensor	SBE 43	43-0862	V0	All stainless casts
Free	N/A	N/A	V1	N/A
Altimeter	Valeport VA500	81629	V2	All stainless casts
Light Scattering Sensor	WETLabs BBRTD	1055	V3	All stainless casts
Cosine PAR Up-looking DWIRR	Biospherical QCP2350-HP	70510	V4	All stainless casts
Cosine PAR Down-looking UWIRR	Biospherical QCP2350-HP	70520	V5	All stainless casts
Fluorometer	CTG Aquatracka MKIII	88-2960-163	V6	All stainless casts
Transmissometer	WET Labs C-star	CST-1797TR	V7	All stainless casts
20L Water Samplers	OTE	SMOTE20L01-SMOTE20L24	N/A	All stainless casts

SENSOR INFORMATION SHEET – Metal Free (Titanium) CTD

Instrument / Sensor	Manufacturer/ Model	Serial Number	Channel	Casts Used
Titanium 24-way frame	NOCS	CTD TITA1	N/A	All titanium casts
Primary CTD deck unit	SBE 11plus	11p-24680-0588	N/A	All casts
CTD Underwater Unit	SBE 9plus	09p-24680-0637	N/A	All titanium casts
24-way Carousel	SBE 32	32-1376	N/A	All titanium casts
Primary Temperature Sensor	SBE 3P	3p-4380	F0	All titanium casts
Primary Conductivity Sensor	SBE 4C	4c-2571	F1	All titanium casts
Digiquartz Pressure sensor	Paroscientific	79501	F2	All titanium casts
Secondary Temperature Sensor	SBE 3P	3p-4381	F3	All titanium casts
Secondary Conductivity Sensor	SBE 4C	4c-3768	F4	All titanium casts
Primary Pump	SBE 5T	5T-3088	n/a	All titanium casts
Secondary Pump	SBE 5T	5T-7371	n/a	All titanium casts
Primary Dissolved Oxygen Sensor	SBE 43	43-1624	V0	All titanium casts
pH Sensor	AMT deep pH	349	V1	Casts 001T - 008T
pH Sensor	AMT deep pH	348	V1	Casts 009T - 015T
pH Sensor	AMT deep pH	347	V1	Casts 016T - 012T
Altimeter	Valeport VA500	81630	V2	All titanium casts
Light Scattering Sensor	WETLabs BBRTD	758R	V3	All titanium casts
Cosine PAR Up-looking DWIRR	Biospherical QCP2350-HP	70510	V4	Casts 019T – 024T
Free	N/A	N/A	V5	N/A
Fluorometer	CTG Aquatracka MKIII	088244	V6	All titanium casts
Transmissometer	WET Labs C-star	CST-1720TR	V7	All titanium casts
TMF 10L Water Samplers	OTE	1-24	n/a	All titanium casts

Appendix 2 – SeaSave Setup Files

Stainless-Steel CTD Setup

```
PSA file: C:\Users\sandm\Documents\Cruises\DY172\Data\Seasave Setup Files\DY172_SS_0758_nmea.psa

Date: 01/25/2024

Instrument configuration file: C:\Users\sandm\Documents\Cruises\DY172\Data\Seasave Setup Files\DY172_SS_0758_nmea.xmlcon

Configuration report for SBE 911plus/917plus CTD
------------------------------------------------

Frequency channels suppressed : 0
Voltage words suppressed      : 0
Computer interface            : RS-232C
Deck unit                     : SBE11plus Firmware Version >= 5.0
Scans to average              : 1
NMEA position data added      : Yes
NMEA depth data added         : No
NMEA time added               : Yes
NMEA device connected to      : PC
Surface PAR voltage added     : No
Scan time added               : Yes

1) Frequency 0, Temperature

   Serial number : 03P-5785
   Calibrated on : 27 September-2022
   G            : 4.33658211e-003
   H            : 6.27646682e-004
   I            : 1.93672006e-005
   J            : 1.40268085e-006
   F0           : 1000.000
   Slope        : 1.00000000
   Offset       : 0.0000

2) Frequency 1, Conductivity

   Serial number : 04C-3054
   Calibrated on : 20-October-2022
   G            : -9.81413427e+000
   H            : 1.42429882e+000
   I            : -6.71449422e-004
   J            : 1.24452309e-004
   CTcor        : 3.2500e-006
   CPcor        : -9.57000000e-008
   Slope        : 1.00000000
   Offset       : 0.00000

3) Frequency 2, Pressure, Digiquartz with TC

   Serial number : 90074
   Calibrated on : 23-September-2022
   C1           : -6.571123e+004
   C2           : 2.050504e-001
   C3           : 1.612220e-002
   D1           : 2.883800e-002
   D2           : 0.000000e+000
```

```
   T1          : 2.986693e+001
   T2          : -2.678465e-004
   T3          : 3.986390e-006
   T4          : 7.472100e-010
   T5          : 0.000000e+000
   Slope        : 1.00012000
   Offset       : 0.01710
   AD590M       : 1.283700e-002
   AD590B       : -8.642460e+000

4) Frequency 3, Temperature, 2

   Serial number : 03P-5835
   Calibrated on : 27-September-2022
   G           : 4.37858164e-003
   H           : 6.72798884e-004
   I          : 2.73204304e-005
   J          : 2.04986555e-006
   F0          : 1000.000
   Slope        : 1.00000000
   Offset       : 0.0000

5) Frequency 4, Conductivity, 2

   Serial number : 04C-3698
   Calibrated on : 5-January-2023
   G           : -1.01605852e+001
   H           : 1.44047851e+000
   I          : -3.61508073e-003
   J          : 3.52128282e-004
   CTcor        : 3.2500e-006
   CPcor        : -9.57000000e-008
   Slope        : 1.00000000
   Offset       : 0.00000

6) A/D voltage 0, Oxygen, SBE 43

   Serial number : 43-0862
   Calibrated on : 18-October-2022
   Equation      : Sea-Bird
   Soc          : 4.93900e-001
   Offset       : -4.94900e-001
   A           : -3.54540e-003
   B           : 1.19980e-004
   C           : -1.87350e-006
   E           : 3.60000e-002
   Tau20        : 9.90000e-001
   D1          : 1.92634e-004
   D2          : -4.64803e-002
   H1          : -3.30000e-002
   H2          : 5.00000e+003
   H3          : 1.45000e+003

7) A/D voltage 1, pH

   Serial number : N/A
   Calibrated on : N/A
   pH slope      : 0.0000
   pH offset     : 0.0000

8) A/D voltage 2, Altimeter
```

```
   Serial number : 81629
   Calibrated on : 21-June-2022
   Scale factor  : 15.000
   Offset        : 0.000

9) A/D voltage 3, OBS, WET Labs, ECO-BB

   Serial number : 1055
   Calibrated on : 07-June-2022
   ScaleFactor   : 0.003302
   Dark output   : 0.058000

10) A/D voltage 4, PAR/Irradiance, Biospherical/Licor

    Serial number       : 70510
    Calibrated on       : 13-August-2021
    M                   : 1.00000000
    B                   : 0.00000000
    Calibration constant : 16666666700.00000000
    Conversion units    : umol photons/m^2/sec
    Multiplier          : 1.00000000
    Offset              : -0.06110141

11) A/D voltage 5, PAR/Irradiance, Biospherical/Licor, 2

    Serial number       : 70520
    Calibrated on       : 13-August-2021
    M                   : 1.00000000
    B                   : 0.00000000
    Calibration constant : 15384615400.00000000
    Conversion units    : umol photons/m^2/sec
    Multiplier          : 1.00000000
    Offset              : -0.06666738

12) A/D voltage 6, Fluorometer, Chelsea Aqua 3

    Serial number : 88-2960-163
    Calibrated on : 20-April
    VB            : 0.099110
    V1            : 1.905480
    Vacetone      : 0.471530
    Scale factor  : 1.000000
    Slope         : 1.000000
    Offset        : 0.000000

13) A/D voltage 7, Transmissometer, WET Labs C-Star

    Serial number : 1797TR
    Calibrated on : 18-April-2023
    M             : 21.5613
    B             : -0.1315
    Path length   : 0.250

Scan length             : 45
---------------------------------------------
Pump Control
   This setting is only applicable to a custom build of the SBE 9plus.
   Enable pump on / pump off commands: NO
---------------------------------------------
Data Acquisition:
   Archive data:            YES
   Delay archiving:          NO
```

```
  Data archive:               C:\Users\sandm\Documents\Cruises\DY172\Data\SS CTD Raw Data\DY172_CTD_023S.hex
  Timeout (seconds) at startup:    60
  Timeout (seconds) between scans: 10
---------------------------------------------
Instrument port configuration:
  Port      = COM1
  Baud rate = 19200
  Parity    = N
  Data bits = 8
  Stop bits = 1
---------------------------------------------
Water Sampler Data:
  Water Sampler Type:   SBE Carousel
  Number of bottles:    32
  Port:                COM5
  Enable remote firing: NO
  Firing sequence:      User input
  Tone for bottle fire confirmation uses PC sound card.
---------------------------------------------
Header information:
  Header Choice = Prompt for Header Information
    prompt 0 = Ship / Cruise: RRS DISCOVERY / DY172
    prompt 1 = Event:
    prompt 2 = Cast:
    prompt 3 = Station:
    prompt 4 = Julian Day:
    prompt 5 = Date:
    prompt 6 = Time (UTC):
    prompt 7 = Latitude:
    prompt 8 = Longitude:
    prompt 9 = Depth (uncorrected m)
    prompt 10 = Principal Scientist: Thomas Bibby
    prompt 11 = Operator:
---------------------------------------------
TCP/IP - port numbers:
  Data acquisition:
    Data port:           49163
    Status port:         49165
    Command port:        49164
  Remote bottle firing:
    Command port:        49167
    Status port:         49168
  Remote data publishing:
    Converted data port:  49161
    Raw data port:       49160
---------------------------------------------
Miscellaneous data for calculations
  Depth, Average Sound Velocity, and TEOS-10
    Latitude when NMEA is not available:  0.000
    Longitude when NMEA is not available: 0.000
  Average Sound Velocity
    Minimum pressure [db]:              20.000
    Minimum salinity [psu]:             20.000
    Pressure window size [db]:          20.000
    Time window size [s]:               60.000
  Descent and Acceleration
    Window size [s]:                     2.000
  Plume Anomaly
    Theta-B:                          0.000
    Salinity-B                        0.000
    Theta-Z / Salinity-Z              0.000
    Reference pressure [db]           0.000
```

```
  Oxygen
    Window size [s]:                2.000
    Apply hysteresis correction:        1
    Apply Tau correction:             1
  Potential Temperature Anomaly
    A0:                        0.000
    A1:                        0.000
    A1 Multiplier:                  Salinity
---------------------------------------------
Serial Data Output:
  Output data to serial port: NO
---------------------------------------------
Mark Variables:
  Variables:
  Digits   Variable Name [units]
  ------   ---------------------
  0       Scan Count
  4       Depth [salt water, m]
  7       Conductivity [S/m]
  5       Salinity, Practical [PSU]
---------------------------------------------
Shared File Output:
  Output data to shared file: NO
---------------------------------------------
TCP/IP Output:
  Raw data:
    Output raw data to socket:           NO
    XML wrapper and settings:            NO
    Seconds between raw data updates:      0.000
  Converted data:
    Output converted data to socket:       NO
    XML format:                        NO
---------------------------------------------
SBE 11plus Deck Unit Alarms
  Enable minimum pressure alarm:    NO
  Enable maximum pressure alarm:    NO
  Enable altimeter alarm:          NO
---------------------------------------------
SBE 14 Remote Display
  Enable SBE 14 Remote Display:     NO
---------------------------------------------
PC Alarms
  Enable minimum pressure alarm:    NO
  Enable maximum pressure alarm:    NO
  Enable altimeter alarm:          NO
  Enable bottom contact alarm:     NO
  Alarm uses PC sound card.
---------------------------------------------
Options:
  Prompt to save program setup changes: YES
  Automatically save program setup changes on exit: NO
  Confirm instrument configuration change: YES
  Confirm display setup changes: YES
  Confirm output file overwrite: YES
  Check scan length: NO
  Compare serial numbers: NO
  Maximized plot may cover Seasave: NO
```

Metal Free (Titanium) CTD Setup

```
PSA file: C:\Users\sandm\Documents\Cruises\DY172\Data\Seasave Setup Files\DY172_Ti_0637_nmea.psa

Date: 01/25/2024

Instrument configuration file: C:\Users\sandm\Documents\Cruises\DY172\Data\Seasave Setup Files\DY172_Ti_0637_nmea.xmlcon

Configuration report for SBE 911plus/917plus CTD
-------------------------------------------------

Frequency channels suppressed : 0
Voltage words suppressed      : 0
Computer interface            : RS-232C
Deck unit                     : SBE11plus Firmware Version >= 5.0
Scans to average              : 1
NMEA position data added      : Yes
NMEA depth data added         : No
NMEA time added               : Yes
NMEA device connected to      : PC
Surface PAR voltage added     : No
Scan time added               : Yes

1) Frequency 0, Temperature

   Serial number : 03P-4380
   Calibrated on : 15-June-2023
   G             : 4.37185884e-003
   H             : 6.54541956e-004
   I             : 2.34432141e-005
   J             : 1.79787545e-006
   F0            : 1000.000
   Slope         : 1.00000000
   Offset        : 0.0000

2) Frequency 1, Conductivity

   Serial number : 04C-2571
   Calibrated on : 20-October-2022
   G             : -9.93071893e+000
   H             : 1.53932079e+000
   I             : 7.22598124e-004
   J             : 5.64322127e-005
   CTcor         : 3.2500e-006
   CPcor         : -9.57000000e-008
   Slope         : 1.00000000
   Offset        : 0.00000

3) Frequency 2, Pressure, Digiquartz with TC

   Serial number : 79501
   Calibrated on : 11-January-2023
   C1            : -6.052595e+004
   C2            : -1.619787e+000
   C3            : 1.743190e-002
   D1            : 2.819600e-002
   D2            : 0.000000e+000
   T1            : 3.011561e+001
   T2            : -5.788717e-004
   T3            : 3.417040e-006
   T4            : 4.126500e-009
```

T5 : 0.000000e+000
Slope : 0.99991000
Offset : -1.54590
AD590M : 1.293660e-002
AD590B : -9.522570e+000

4) Frequency 3, Temperature, 2

Serial number : 03P-4381
Calibrated on : 15-June-2023
G : 4.42335019e-003
H : 6.44515215e-004
I : 2.24226094e-005
J : 1.92778223e-006
F0 : 1000.000
Slope : 1.00000000
Offset : 0.0000

5) Frequency 4, Conductivity, 2

Serial number : 04C-3768
Calibrated on : 20-October-2022
G : -1.02169859e+001
H : 1.49445288e+000
I : -1.59468470e-004
J : 1.08012380e-004
CTcor : 3.2500e-006
CPcor : -9.57000000e-008
Slope : 1.00000000
Offset : 0.00000

6) A/D voltage 0, Oxygen, SBE 43

Serial number : 43-1624
Calibrated on : 13-May-23
Equation : Sea-Bird
Soc : 3.51200e-001
Offset : -6.99300e-001
A : -3.90860e-003
B : 1.65510e-004
C : -2.81980e-006
E : 3.60000e-002
Tau20 : 1.19000e+000
D1 : 1.92634e-004
D2 : -4.64803e-002
H1 : -3.30000e-002
H2 : 5.00000e+003
H3 : 1.45000e+003

7) A/D voltage 1, pH

Serial number : AMT DEEP pH 347
Calibrated on : 22 August 2023
pH slope : 5.9694
pH offset : 2.4329

8) A/D voltage 2, Altimeter

Serial number : 81630
Calibrated on : 09-June-2022
Scale factor : 15.000
Offset : 0.000

```
9) A/D voltage 3, OBS, WET Labs, ECO-BB

  Serial number : 758R
  Calibrated on : 21-September-2022
  ScaleFactor   : 0.003461
  Dark output   : 0.073000

10) A/D voltage 4, PAR/Irradiance, Biospherical/Licor

   Serial number       : N/A
   Calibrated on       : N/A
   M                   : 0.00000000
   B                   : 0.00000000
   Calibration constant : 0.00000000
   Multiplier          : 1.00000000
   Offset              : 0.00000000

11) A/D voltage 5, PAR/Irradiance, Biospherical/Licor, 2

   Serial number       : N/A
   Calibrated on       : N/A
   M                   : 0.00000000
   B                   : 0.00000000
   Calibration constant : 0.00000000
   Multiplier          : 0.00000000
   Offset              : 0.00000000

12) A/D voltage 6, Fluorometer, Chelsea Aqua 3

   Serial number : 088-244
   Calibrated on : 29-November-2022
   VB           : 0.358220
   V1           : 2.123120
   Vacetone     : 0.550570
   Scale factor  : 1.000000
   Slope        : 1.000000
   Offset       : 0.000000

13) A/D voltage 7, Transmissometer, WET Labs C-Star

   Serial number : CST-1720TR
   Calibrated on : 7-October-2021
   M            : 21.4787
   B            : -0.2105
   Path length   : 0.250

Scan length                : 45
---------------------------------------------
Pump Control
  This setting is only applicable to a custom build of the SBE 9plus.
  Enable pump on / pump off commands: NO
---------------------------------------------
Data Acquisition:
  Archive data:                 YES
  Delay archiving:               NO
  Data archive:                 C:\Users\sandm\Documents\Cruises\DY172\Data\Ti CTD Raw Data\DY172_CTD_029T.hex
  Timeout (seconds) at startup:    60
  Timeout (seconds) between scans: 10
---------------------------------------------
Instrument port configuration:
  Port      = COM1
```

```
  Baud rate = 19200
  Parity    = N
  Data bits = 8
  Stop bits = 1
---------------------------------------------
Water Sampler Data:
  Water Sampler Type:   SBE Carousel
  Number of bottles:    32
  Port:                 COM5
  Enable remote firing: NO
  Firing sequence:      User input
  Tone for bottle fire confirmation uses PC sound card.
---------------------------------------------
Header information:
  Header Choice = Prompt for Header Information
    prompt 0 = Ship / Cruise: RRS DISCOVERY / DY172
    prompt 1 = Event:
    prompt 2 = Cast:
    prompt 3 = Station:
    prompt 4 = Julian Day:
    prompt 5 = Date:
    prompt 6 = Time (UTC):
    prompt 7 = Latitude:
    prompt 8 = Longitude:
    prompt 9 = Depth (uncorrected m)
    prompt 10 = Principal Scientist: Thomas Bibby
    prompt 11 = Operator:
---------------------------------------------
TCP/IP - port numbers:
  Data acquisition:
    Data port:          49163
    Status port:        49165
    Command port:       49164
  Remote bottle firing:
    Command port:       49167
    Status port:        49168
  Remote data publishing:
    Converted data port:  49161
    Raw data port:      49160
---------------------------------------------
Miscellaneous data for calculations
  Depth, Average Sound Velocity, and TEOS-10
    Latitude when NMEA is not available:  0.000
    Longitude when NMEA is not available: 0.000
  Average Sound Velocity
    Minimum pressure [db]:             20.000
    Minimum salinity [psu]:            20.000
    Pressure window size [db]:         20.000
    Time window size [s]:              60.000
  Descent and Acceleration
    Window size [s]:                   2.000
  Plume Anomaly
    Theta-B:                           0.000
    Salinity-B                         0.000
    Theta-Z / Salinity-Z               0.000
    Reference pressure [db]            0.000
  Oxygen
    Window size [s]:                   2.000
    Apply hysteresis correction:       1
    Apply Tau correction:              1
  Potential Temperature Anomaly
    A0:                                0.000
```

```
    A1:                       0.000
    A1 Multiplier:                  Salinity
--------------------------------------------
Serial Data Output:
  Output data to serial port: NO
--------------------------------------------
Mark Variables:
  Variables:
  Digits   Variable Name [units]
  ------   --------------------
  0       Scan Count
  4       Depth [salt water, m]
  7       Conductivity [S/m]
  5       Salinity, Practical [PSU]
--------------------------------------------
Shared File Output:
  Output data to shared file: NO
--------------------------------------------
TCP/IP Output:
  Raw data:
    Output raw data to socket:            NO
    XML wrapper and settings:             NO
    Seconds between raw data updates:       0.000
  Converted data:
    Output converted data to socket:       NO
    XML format:                        NO
--------------------------------------------
SBE 11plus Deck Unit Alarms
  Enable minimum pressure alarm:    NO
  Enable maximum pressure alarm:    NO
  Enable altimeter alarm:          NO
--------------------------------------------
SBE 14 Remote Display
  Enable SBE 14 Remote Display:     NO
--------------------------------------------
PC Alarms
  Enable minimum pressure alarm:    NO
  Enable maximum pressure alarm:    NO
  Enable altimeter alarm:          NO
  Enable bottom contact alarm:      NO
  Alarm uses PC sound card.
--------------------------------------------
Options:
  Prompt to save program setup changes: YES
  Automatically save program setup changes on exit: NO
  Confirm instrument configuration change: YES
  Confirm display setup changes: YES
  Confirm output file overwrite: YES
  Check scan length: NO
  Compare serial numbers: NO
  Maximized plot may cover Seasave: NO
```

0

Appendix 3 Trace Metal Clean Log:

time	entry#	event	comment	Latitude (°)	Longitude (°)
23/12/2023 12:02	1	Fish001	New Filter	-39.029	13.484
23/12/2023 20:00	2	Fish002	-	-39.751	12.775
24/12/2023 00:00	3	Fish003	-	-40.243	12.278
24/12/2023 08:00	4	Fish004	-	-40.718	11.801
24/12/2023 12:00	5	Fish005	-	-41.228	11.268
24/12/2023 16:00	6	Fish006	-	-41.721	10.767
24/12/2023 20:00	7	Fish007	-	-42.211	10.257
25/12/2023 00:09	8	Fish008	-	-42.72	9.738
25/12/2023 08:00	9	Fish009	-	-43.61	8.788
25/12/2023 12:00	10	Fish010	-	-44.122	8.264
25/12/2023 16:00	11	Fish011	-	-44.621	7.699
25/12/2023 20:00	12	Fish012	-	-45.146	7.122
26/12/2023 00:00	13	Fish013	-	-45.682	6.553
26/12/2023 04:00	14	Fish014	-	-46.188	5.971
26/12/2023 12:00	15	Fish015	-	-46.496	5.629
26/12/2023 16:00	16	Fish016	-	-46.998	5.072
26/12/2023 20:00	17	Fish017	-	-47.48	4.528
27/12/2023 00:00	18	Fish018	-	-48.001	3.958
27/12/2023 13:00	19	Fish019	New Filter	-48.37	2.617
27/12/2023 16:00	20	Fish020	-	-48.205	2.096
27/12/2023 20:00	21	Fish021	-	-47.713	1.46
28/12/2023 00:04	22	Fish022	-	-47.207	0.808
29/12/2023 08:00	23	Fish023	-	-46.672	-0.319
29/12/2023 12:08	24	Fish024	-	-47.203	-0.307
29/12/2023 16:02	25	Fish025	-	-47.722	-0.335
29/12/2023 20:00	26	Fish026	-	-48.229	-0.124
29/12/2023 23:58	27	Fish027	-	-48.709	-0.317
30/12/2023 08:00	28	Fish028	-	-49.601	-0.222
30/12/2023 12:00	29	Fish029	-	-50.056	0.327
30/12/2023 16:00	30	Fish030	-	-50.685	0.058
30/12/2023 20:00	31	Fish031	-	-51.289	-0.275
31/12/2023 00:00	32	Fish032	-	-51.882	-0.285
31/12/2023 08:08	33	Fish033	New Filter	-52.399	0.615
01/01/2024 08:00	34	Fish034	-	-52.261	-0.032
01/01/2024 12:03	35	Fish035	-	-52.935	-0.014
01/01/2024 16:00	36	Fish036	-	-53.607	-0.008
01/01/2024 20:00	37	Fish037	-	-54.295	0.001
02/01/2024 11:57	38	Fish038	-	-55.513	-0.081

02/01/2024 16:00	39	Fish039	-	-56.188	0.005
02/01/2024 20:00	40	Fish040	-	-56.905	0.002
03/01/2024 00:07	41	Fish041	-	-57.592	0.002
03/01/2024 16:00	42	Fish042	-	-57.613	-0.261
03/01/2024 20:00	43	Fish043	-	-57.256	-0.386
04/01/2024 00:00	44	Fish044	-	-56.835	-0.492
04/01/2024 08:00	45	Fish045	-	-56.369	-0.578
04/01/2024 12:00	46	Fish046	-	-55.807	-0.672
04/01/2024 16:00	47	Fish047	-	-55.22	-0.804
04/01/2024 20:00	48	Fish048	-	-54.6	-0.937
04/01/2024 23:57	49	Fish049	-	-53.973	-1.046
05/01/2024 12:00	50	Fish050	-	-52.363	-1.375
05/01/2024 15:00	51	Fish051	no fish sample actually happened, there is no Fish051	-52.174	-1.428
05/01/2024 16:00	52	Fish052	-	-52.084	-1.645
05/01/2024 20:00	53	Fish053	-	-51.552	-2.321
06/01/2024 00:00	54	Fish054	-	-50.997	-2.958
06/01/2024 08:00	55	Fish055	-	-49.932	-3.964
06/01/2024 12:00	56	Fish056	-	-49.356	-4.24
07/01/2024 20:00	57	Fish057	-	-48.937	-4.69
07/01/2024 23:58	58	Fish058	New Filter	-48.655	-5.031
09/01/2024 00:02	59	Fish059	-	-48.351	-5.365
09/01/2024 13:38	60	Fish060	-	-47.657	-5.32
09/01/2024 13:54	61	Fish061	Iceberg	-47.623	-5.306
09/01/2024 14:00	62	Fish062	Iceberg	-47.633	-5.287
09/01/2024 14:05	63	Fish063	Iceberg	-47.641	-5.272
09/01/2024 14:10	64	Fish064	Iceberg	-47.652	-5.267
09/01/2024 14:18	65	Fish065	Iceberg	-47.66	-5.287
09/01/2024 14:25	66	Fish066	Iceberg	-47.65	-5.294
09/01/2024 14:33	67	Fish067	Iceberg	-47.634	-5.282
09/01/2024 14:37	68	Fish068	Iceberg	-47.624	-5.283
09/01/2024 16:02	69	Fish069	-	-47.374	-5.314
09/01/2024 20:00	70	Fish070	-	-46.654	-5.3
10/01/2024 00:16	71	Fish071	-	-45.893	-5.278
10/01/2024 08:00	72	Fish072	-	-45.261	-5.244
10/01/2024 12:00	73	Fish073	-	-44.593	-5.232
10/01/2024 16:00	74	Fish074	-	-43.879	-5.209
10/01/2024 20:00	75	Fish075	-	-43.211	-5.186
11/01/2024 00:12	76	Fish076	-	-42.495	-5.172
11/01/2024 08:00	77	Fish077	-	-41.782	-5.144

11/01/2024 12:00	78	Fish078	-	-41.16	-5.118
11/01/2024 14:36	79	UW079	-	-40.765	-5.111
12/01/2024 20:00	80	Fish080	-	-39.706	-5.079
13/01/2024 00:13	81	Fish081	-	-38.957	-5.068
13/01/2024 12:00	82	Fish082	New Filter	-37.896	-5.066
13/01/2024 16:00	83	Fish083	-	-37.396	-5.059
13/01/2024 20:00	84	Fish084	-	-36.719	-5.05
14/01/2024 00:00	85	Fish085	-	-35.984	-5.072
14/01/2024 08:00	86	Fish086	-	-35.101	-5.048
14/01/2024 12:00	87	Fish087	-	-34.491	-5.012
14/01/2024 17:25	88	Fish088	Eddy 1	-33.992	-4.995
14/01/2024 23:20	89	Fish089	Eddy 2	-33.424	-4.814
15/01/2024 07:20	90	Fish090	Eddy 3	-32.518	-4.519
15/01/2024 11:24	91	Fish091	Eddy 4	-32.289	-4.448
15/01/2024 17:37	92	Fish092	Eddy 5	-31.709	-4.277
15/01/2024 23:20	93	Fish093	Eddy 6	-31.169	-4.088
16/01/2024 08:00	94	Fish094	Eddy 7	-30.163	-3.722
16/01/2024 12:00	95	Fish095	-	-29.482	-3.536
16/01/2024 16:00	96	Fish096	-	-28.849	-3.266
16/01/2024 20:00	97	Fish097	-	-28.191	-3.086
17/01/2024 20:00	98	Fish098	-	-28.074	-2.292
18/01/2024 00:09	99	Fish099	-	-28.151	-1.473
18/01/2024 08:00	100	Fish100	-	-28.233	-0.79
18/01/2024 16:00	101	Fish101	-	-28.471	0.548
18/01/2024 20:00	102	Fish102	-	-28.587	1.284
19/01/2024 00:02	103	Fish103	-	-28.727	2.027
19/01/2024 12:00	104	Fish104	-	-28.989	3.484
19/01/2024 16:00	105	Fish105	-	-29.105	4.186
19/01/2024 20:00	106	Fish106	-	-29.238	4.929
20/01/2024 00:01	107	Fish107	-	-29.367	5.667
20/01/2024 08:00	108	Fish108	-	-29.493	6.398
20/01/2024 12:00	109	Fish109	-	-29.605	7.088
20/01/2024 16:00	110	Fish110	-	-29.733	7.806
20/01/2024 20:00	111	Fish111	-	-29.844	8.523
21/01/2024 00:09	112	Fish112	-	-29.974	9.241
21/01/2024 04:00	113	Fish113	Last sample	-30.022	9.505

Appendix 4 Underway Sampling Log:

time	entry#	event	Latitude (°)	Longitude (°)
23/12/2023 12:04	1	UW001	-39.032	13.481
23/12/2023 16:00	2	UW002	-39.425	13.089
23/12/2023 20:00	3	UW003	-39.75	12.775
24/12/2023 00:17	4	UW004	-40.274	12.247
24/12/2023 03:57	5	UW005	-40.625	11.895
24/12/2023 08:00	6	UW006	-40.719	11.8
24/12/2023 12:00	7	UW007	-41.226	11.27
24/12/2023 16:00	8	UW008	-41.721	10.767
24/12/2023 20:00	9	UW009	-42.21	10.258
25/12/2023 00:00	10	UW010	-42.703	9.756
25/12/2023 04:00	11	UW011	-43.123	9.308
25/12/2023 08:00	12	UW012	-43.611	8.787
25/12/2023 12:00	13	UW013	-44.122	8.264
25/12/2023 16:00	14	UW014	-44.621	7.698
25/12/2023 20:00	15	UW015	-45.148	7.121
26/12/2023 00:00	16	UW016	-45.682	6.553
26/12/2023 03:57	17	UW017	-46.18	5.979
26/12/2023 08:00	18	UW018	-46.397	5.73
26/12/2023 12:00	19	UW019	-46.495	5.63
26/12/2023 16:00	20	UW020	-46.997	5.072
26/12/2023 20:00	21	UW021	-47.48	4.527
27/12/2023 00:00	22	UW022	-48.002	3.957
27/12/2023 04:00	23	UW023	-48.404	3.476
27/12/2023 08:00	24	UW024	-48.373	3.515
27/12/2023 16:00	25	UW025	-48.204	2.095
27/12/2023 20:00	26	UW026	-47.712	1.458
28/12/2023 00:00	27	UW027	-47.215	0.819
28/12/2023 04:00	28	UW028	-46.889	0.325
28/12/2023 08:00	29	UW029	-46.859	0.345
28/12/2023 12:00	30	UW030	-46.716	0.396
28/12/2023 16:00	31	UW031	-46.56	0.38
28/12/2023 20:00	32	UW032	-46.439	0.271
29/12/2023 04:00	33	UW033	-46.458	-0.215
29/12/2023 08:00	34	UW034	-46.671	-0.32
29/12/2023 12:11	35	UW035	-47.211	-0.309
29/12/2023 16:00	36	UW036	-47.718	-0.338
29/12/2023 20:00	37	UW037	-48.23	-0.124
30/12/2023 00:04	38	UW038	-48.72	-0.325

30/12/2023 04:00	39	UW039	-49.18	-0.626
30/12/2023 08:00	40	UW040	-49.6	-0.224
30/12/2023 12:00	41	UW041	-50.055	0.327
30/12/2023 16:00	42	UW042	-50.683	0.058
30/12/2023 20:00	43	UW043	-51.287	-0.274
31/12/2023 00:05	44	UW044	-51.892	-0.266
31/12/2023 04:00	45	UW045	-52.349	0.547
31/12/2023 08:00	46	UW046	-52.406	0.628
31/12/2023 12:00	47	UW047	-52.388	0.387
31/12/2023 16:00	48	UW048	-52.395	0.237
31/12/2023 20:00	49	UW049	-52.347	0.117
01/01/2024 04:00	50	UW050	-52.097	-0.15
01/01/2024 08:00	51	UW051	-52.26	-0.032
01/01/2024 12:00	52	UW052	-52.925	-0.013
01/01/2024 16:00	53	UW053	-53.607	-0.008
01/01/2024 20:00	54	UW054	-54.295	0.001
02/01/2024 00:02	55	UW055	-54.989	-0.022
02/01/2024 04:00	56	UW056	-55.522	-0.101
02/01/2024 08:00	57	UW057	-55.517	-0.091
02/01/2024 12:00	58	UW058	-55.513	-0.082
02/01/2024 16:00	59	UW059	-56.187	0.004
02/01/2024 20:00	60	UW060	-56.903	0.002
03/01/2024 00:00	61	UW061	-57.573	0.002
03/01/2024 04:00	62	UW062	-58.132	0
03/01/2024 08:00	63	UW063	-58.136	-0.004
03/01/2024 12:00	64	UW064	-58.082	-0.028
03/01/2024 16:00	65	UW065	-57.613	-0.261
03/01/2024 20:00	66	UW066	-57.257	-0.385
04/01/2024 00:00	67	UW067	-56.834	-0.492
04/01/2024 04:00	68	UW068	-56.443	-0.564
04/01/2024 08:00	69	UW069	-56.369	-0.578
04/01/2024 12:00	70	UW070	-55.806	-0.673
04/01/2024 16:00	71	UW071	-55.221	-0.804
04/01/2024 20:00	72	UW072	-54.6	-0.937
05/01/2024 00:04	73	UW073	-53.953	-1.051
05/01/2024 04:00	74	UW074	-53.346	-1.173
05/01/2024 08:00	75	UW075	-53.044	-1.24
05/01/2024 12:00	76	UW076	-52.363	-1.375
05/01/2024 16:00	77	UW077	-52.084	-1.645
05/01/2024 20:00	78	UW078	-51.552	-2.322

06/01/2024 00:05	79	UW079	-50.987	-2.969
06/01/2024 04:00	80	UW080	-50.443	-3.537
06/01/2024 08:00	81	UW081	-49.931	-3.965
06/01/2024 12:10	82	UW082	-49.332	-4.252
06/01/2024 16:00	83	UW083	-49.318	-4.253
06/01/2024 20:00	84	UW084	-49.319	-4.253
07/01/2024 00:00	85	UW085	-49.323	-4.247
07/01/2024 04:00	86	UW086	-49.336	-4.299
07/01/2024 08:00	87	UW087	-49.323	-4.245
07/01/2024 12:00	88	UW088	-49.32	-4.245
07/01/2024 16:00	89	UW089	-49.329	-4.246
07/01/2024 20:00	90	UW090	-48.937	-4.691
08/01/2024 00:08	91	UW091	-48.644	-5.045
08/01/2024 04:02	92	UW092	-48.409	-5.296
08/01/2024 08:00	93	UW093	-48.285	-5.46
08/01/2024 12:00	94	UW094	-48.297	-5.667
08/01/2024 16:00	95	UW095	-48.337	-5.809
08/01/2024 20:00	96	UW096	-48.331	-5.482
09/01/2024 04:00	97	UW097	-48.351	-5.367
09/01/2024 08:00	98	UW098	-48.362	-5.364
09/01/2024 11:59	99	UW099	-47.933	-5.367
09/01/2024 14:18	100	UW100	-47.661	-5.288
09/01/2024 14:22	101	UW101	-47.656	-5.297
09/01/2024 14:24	102	UW102	-47.653	-5.296
09/01/2024 14:25	103	UW103	-47.652	-5.296
09/01/2024 14:26	104	UW104	-47.649	-5.293
09/01/2024 14:28	105	UW105	-47.645	-5.29
09/01/2024 14:30	106	UW106	-47.64	-5.286
09/01/2024 16:00	107	UW107	-47.382	-5.313
09/01/2024 20:00	108	UW108	-46.654	-5.3
10/01/2024 00:08	109	UW109	-45.917	-5.279
10/01/2024 04:02	110	UW110	-45.346	-5.248
10/01/2024 08:00	111	UW111	-45.261	-5.244
10/01/2024 12:11	112	UW112	-44.563	-5.229
10/01/2024 16:01	113	UW113	-43.877	-5.209
10/01/2024 20:00	114	UW114	-43.211	-5.186
11/01/2024 00:06	115	UW115	-42.513	-5.172
11/01/2024 04:00	116	UW116	-41.882	-5.149
11/01/2024 08:00	117	UW117	-41.781	-5.144
11/01/2024 12:00	118	UW118	-41.159	-5.118

11/01/2024 16:00	119	UW119	-40.728	-5.107
11/01/2024 20:00	120	UW120	-40.367	-5.101
12/01/2024 00:00	121	UW121	-40.369	-5.096
12/01/2024 04:00	122	UW122	-40.365	-5.096
12/01/2024 08:00	123	UW123	-40.369	-5.098
12/01/2024 12:00	124	UW124	-40.369	-5.098
12/01/2024 16:00	125	UW125	-40.369	-5.095
12/01/2024 20:00	126	UW126	-39.705	-5.079
13/01/2024 00:02	127	UW127	-38.989	-5.07
13/01/2024 04:00	128	UW128	-38.379	-5.067
13/01/2024 08:00	129	UW129	-38.331	-5.068
13/01/2024 12:00	130	UW130	-37.895	-5.066
13/01/2024 16:00	131	UW131	-37.397	-5.059
13/01/2024 20:00	132	UW132	-36.72	-5.05
14/01/2024 00:05	133	UW133	-35.969	-5.071
14/01/2024 04:00	134	UW135	-35.26	-5.049
14/01/2024 08:00	135	UW135	-35.101	-5.048
14/01/2024 12:00	136	UW136	-34.489	-5.011
14/01/2024 17:15	137	UW137	-34.001	-4.997
14/01/2024 22:50	138	UW138	-33.431	-4.816
15/01/2024 06:50	139	UW139	-32.6	-4.545
15/01/2024 10:58	140	UW140	-32.3	-4.448
15/01/2024 17:14	141	UW141	-31.723	-4.278
15/01/2024 23:10	142	UW142	-31.169	-4.088
16/01/2024 05:10	143	UW143	-30.6	-3.901
16/01/2024 08:00	144	UW144	-30.164	-3.723
16/01/2024 12:00	145	UW145	-29.481	-3.536
16/01/2024 16:00	146	UW146	-28.85	-3.266
16/01/2024 20:00	147	UW147	-28.189	-3.086
17/01/2024 00:00	148	UW148	-27.999	-3
17/01/2024 04:00	149	UW149	-27.949	-2.966
17/01/2024 10:02	150	UW150	-27.999	-3
17/01/2024 12:00	151	UW151	-28	-3.002
17/01/2024 16:02	152	UW152	-28	-3
17/01/2024 20:00	153	UW153	-28.074	-2.292
18/01/2024 00:01	154	UW154	-28.148	-1.5
18/01/2024 04:00	155	UW155	-28.213	-0.907
18/01/2024 08:00	156	UW156	-28.234	-0.789
18/01/2024 12:00	157	UW157	-28.352	-0.167
18/01/2024 16:00	158	UW158	-28.471	0.547

18/01/2024 20:00	159	UW159	-28.587	1.283
19/01/2024 00:04	160	UW160	-28.727	2.03
19/01/2024 04:00	161	UW161	-28.836	2.655
19/01/2024 08:00	162	UW162	-28.852	2.762
19/01/2024 13:05	163	UW164	-29.019	3.687
19/01/2024 16:00	164	UW164	-29.105	4.186
19/01/2024 20:00	165	UW165	-29.238	4.931
20/01/2024 00:04	166	UW166	-29.368	5.675
20/01/2024 04:02	167	UW167	-29.473	6.273
20/01/2024 08:00	168	UW168	-29.493	6.396
20/01/2024 12:00	169	UW169	-29.606	7.091
20/01/2024 16:00	170	UW170	-29.733	7.805
20/01/2024 20:00	171	UW171	-29.844	8.52
21/01/2024 00:00	172	UW172	-29.97	9.218
21/01/2024 04:05	173	UW173	-30.023	9.511
22/01/2024 08:00	174	UW174	-29.988	9.5
22/01/2024 12:10	175	UW175	-29.401	8.974
22/01/2024 16:00	176	UW176	-28.781	9.043
22/01/2024 22:00	177	UW177	-27.797	9.279
23/01/2024 00:00	178	UW178	-27.493	9.408
23/01/2024 04:03	179	UW179	-26.824	9.537
23/01/2024 08:00	180	UW180	-26.194	9.646
23/01/2024 12:00	181	UW181	-25.548	9.812
23/01/2024 16:00	182	UW182	-24.891	10.025
23/01/2024 20:00	183	UW183	-24.184	10.201
24/01/2024 00:00	184	UW184	-23.484	10.387
24/01/2024 04:00	185	UW185	-22.999	10.5
24/01/2024 08:00	186	UW186	-22.999	10.501
24/01/2024 12:00	187	UW187	-22.999	10.499

Appendix 5 Aerosol Sampling Log:

time	entry#	event	Latitude (°)	Longitude (°)
24/12/2023 14:40	1	TM001-start	-41.564	10.941
27/12/2023 13:19	2	TM001-end	-48.371	2.554
28/12/2023 13:42	3	TM002-start	-46.642	0.416
30/12/2023 11:32	4	TM002-end	-49.985	0.362
30/12/2023 12:05	5	TM003-start	-50.069	0.322
01/01/2024 14:14	6	TM003-end	-53.303	-0.015
01/01/2024 14:35	7	TM004-start	-53.365	-0.015
03/01/2024 11:40	8	TM004-end	-58.131	-0.01
03/01/2024 12:00	9	TM005-start	-58.082	-0.028
05/01/2024 13:08	10	TM005-end	-52.328	-1.381
05/01/2024 13:43	11	TM006-start	-52.328	-1.381
07/01/2024 13:11	12	TM006-end	-49.324	-4.246
07/01/2024 13:31	13	TM007-start	-49.326	-4.246
09/01/2024 13:05	14	TM007-end	-47.755	-5.339
09/01/2024 13:46	15	TM008-start	-47.634	-5.317
11/01/2024 13:35	16	TM008-end	-40.869	-5.118
11/01/2024 14:08	17	TM009-start	-40.78	-5.114
13/01/2024 13:46	18	TM009-end	-37.677	-5.05
13/01/2024 14:06	19	TM010-start	-37.637	-5.051
15/01/2024 12:59	20	TM010-end	-32.034	-4.376
15/01/2024 13:26	21	TM011-start	-31.96	-4.356
17/01/2024 13:09	22	TM011-end	-27.999	-3
17/01/2024 13:35	23	TM012-start	-27.998	-2.999
19/01/2024 07:55	24	TM012-end	-28.849	2.748
19/01/2024 08:07	25	TM013-start	-28.856	2.782
21/01/2024 14:33	26	TM013-end	-30.018	9.521
21/01/2024 14:55	27	TM014-start	-30.018	9.521
23/01/2024 15:29	28	TM014-end	-24.98	10.001
23/01/2024 15:52	29	TM-exposure-blank-start	-24.914	10.019
25/01/2024 09:15	30	TM-exposure-blank-end	-22.937	12.222

Appendix 6 Vertically resolved discrete chlorophyll profiles:

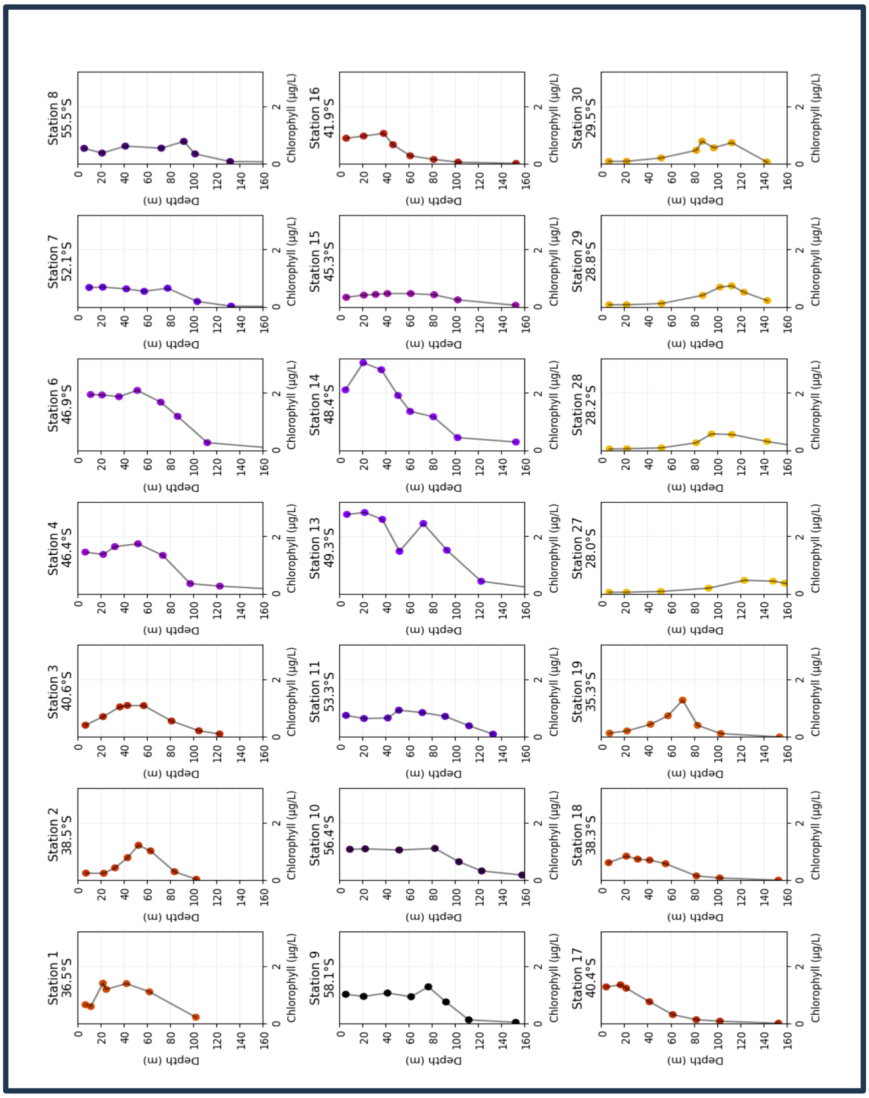

Figure A6 Thumbnails of vertical profiles of measured extracted chlorophyll

Appendix 7 CTD profiles:

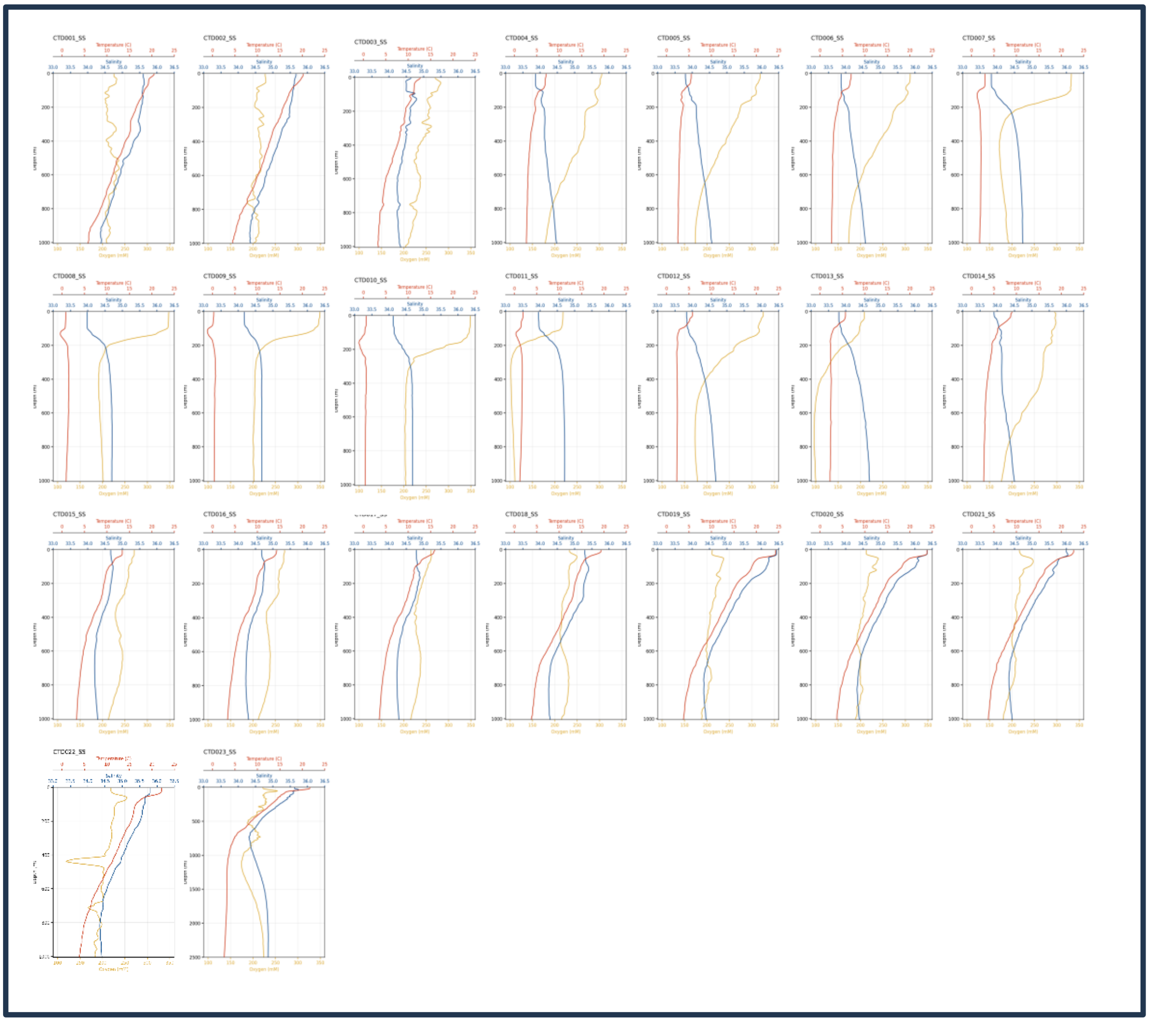

Figure A71 Thumbnails of physical data from SS_CTD casts.

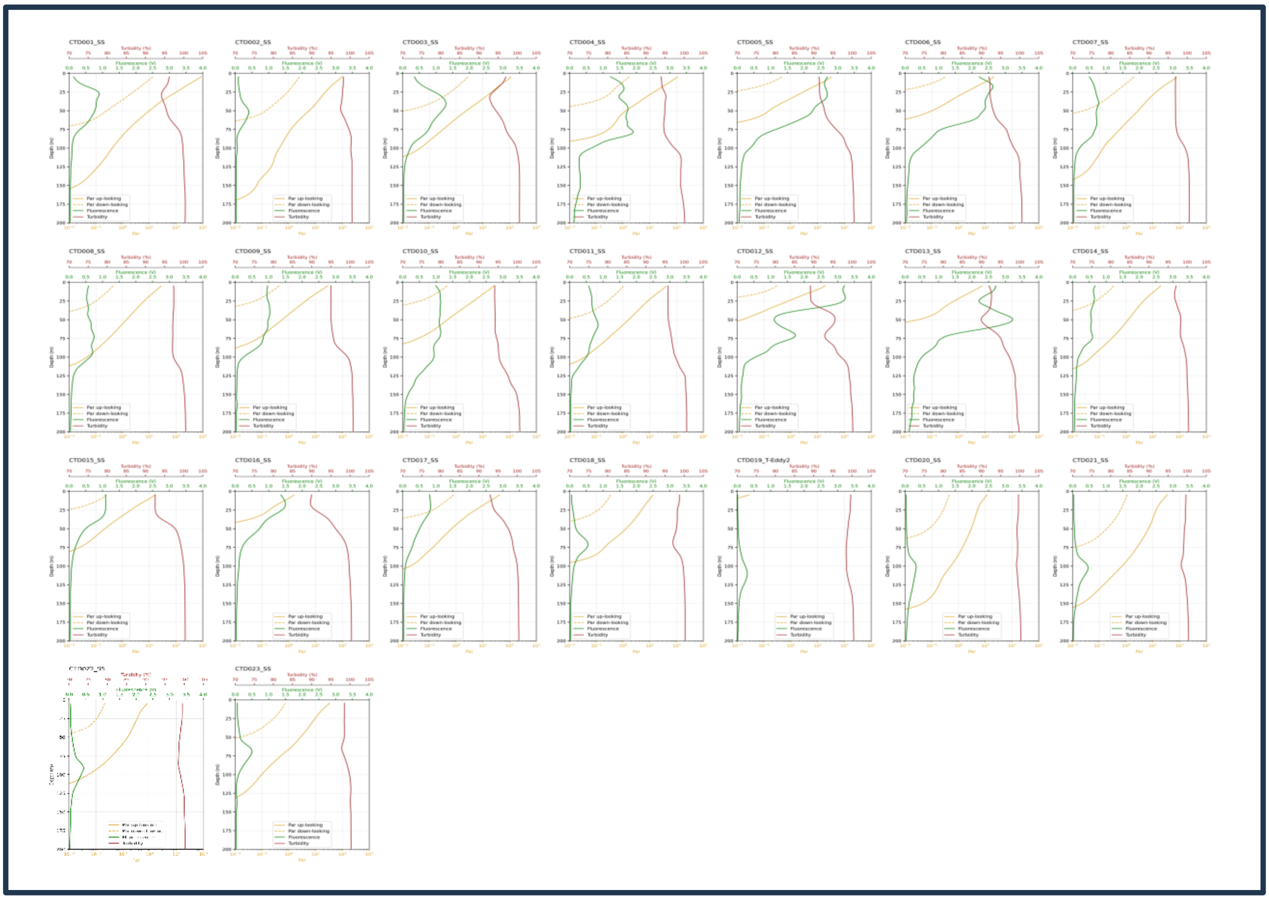

Figure A72 Thumbnails of biological data from SS_CTD casts.

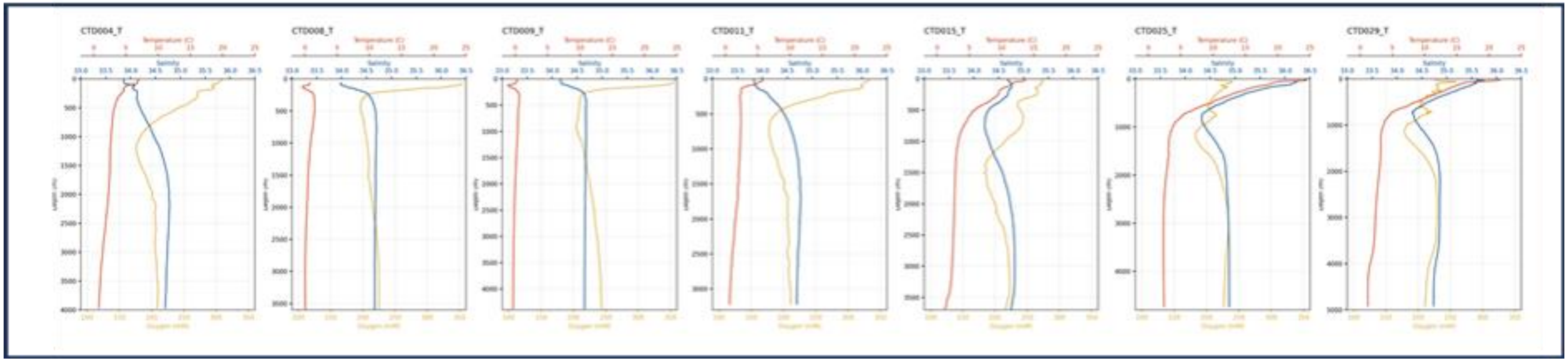

Figure A73 Thumbnails of physical data from deep Ti-CTD casts