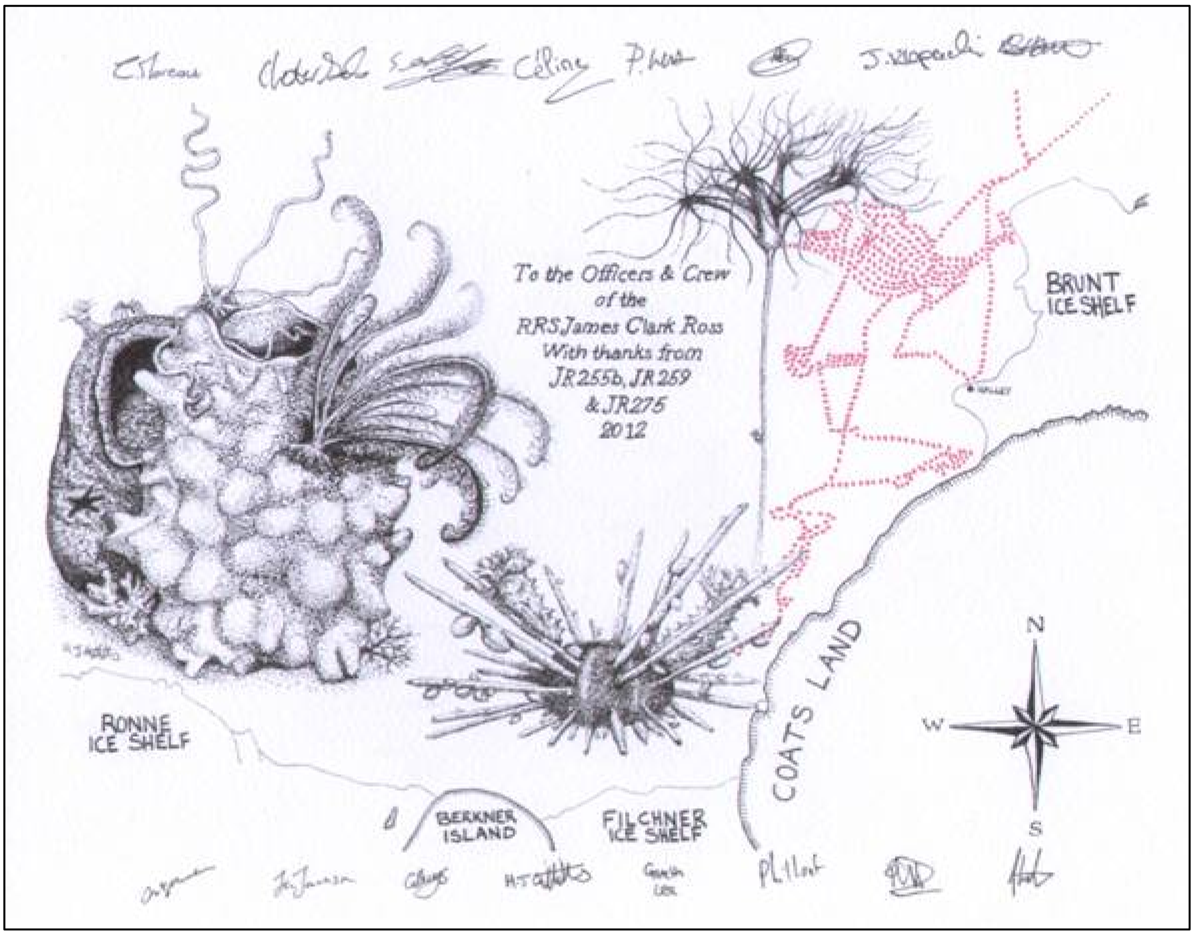
RRS James Clark Ross

JR275 Cruise Report



Benthic Biology of the Weddell Sea

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H. J. Griffiths, R. V. Downey, D. S. Hamilton, C. Heuzé, J. Jackson, M. Mackenzie, C. Moreau, A. Reed & C. J. Sands

British Antarctic Survey Cruise Report

Falkland Islands – South Orkney Islands – South Sandwich Islands – Weddell Sea – Halley – Signy – Falkland Islands

Report of *RRS James Clark Ross* cruise JR75, February-March 2012

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Summary

RRS James Clark Ross cruise JR275 took place from 7th February 2012 to 22nd March 2012. It was a BAS Nation Capability cruise for the Evolutionary History Workpackage. It was joined with cruise JR259 (Geological LTMS, specifically Swath Bathymetry and rock dredging, which belonged to the same BAS Science Programme) with which the main objectives of JR275 were closely integrated. It was also joined with JR255B (retrieval of oceanographic gliders from the Powell Basin/South Orkney shelf area) and logistics visits to both Halley and Signy. The cruise track is shown in Figure 1. Four days were lost at the start of the cruise due to late arrival of the ship in the Falklands and engineering works. Work in the South Sandwich arc was abandoned because of bad weather and shortage of time. Work in the south-eastern Weddell Sea was largely dictated by the movements and formation of the sea-ice and icebergs which are major features of this region.

Work in the Weddell Sea was primarily for cruise JR275, and consisted of bethic biology sampling on the Weddell Sea continental margin and continental slope using Agassiz trawls and an Epibenthic Sledge fitted with a camera. This sampling was very successful. During this time, JR259 provided swath bathymetry coverage of sites to be sampled by the biologists, and provided data and assessments of the suitability of sites. This combination was very effective at identifying sites for the biology sampling. A swath bathymetry survey of the continental slope from the shelf break to locally over 2000 m was completed for the eastern Weddell Sea, as far as ice conditions and time would allow. After personnel uplift from Halley, and transect of the Weddell Sea, the retrieval of oceanographic gliders for JR255B was attempted.

Objectives

Our main aim was to sample large macro-and mega- size fractions of seabed dwelling (benthic) animals in the Weddell Sea. Our sampling regime was designed to investigate patterns of biodiversity, and once compared to other sources of material, biogeography and phylogeography in the benthos of this region of the Southern Ocean. We planned for several sites to be situated in the Filchner Trough region of the Weddell Sea. Due to sea-ice conditions we were unable to work in all of our original target areas. We deployed an Agassiz trawl in 56 locations at depths of between 400 and 2,000 m to investigate variability of taxa presence from continental shelf to continental slope depths, and within two basins on the continental shelf. We also conducted a single Agassiz and two Epibenthic Sledge deployments east of the South Orkney Islands in order to test the new Deep Water Camera System and CTD. Two additional targets, calderas at the South Sandwich Islands, had to be abandoned due to poor weather conditions.

Funding

Cruise JR275 was part of the EvolHist Workpackage of the Environmental Change and Evolution Programme (BAS).

Summary narrative for JR275/JR255B/JR259/

February to March 2012

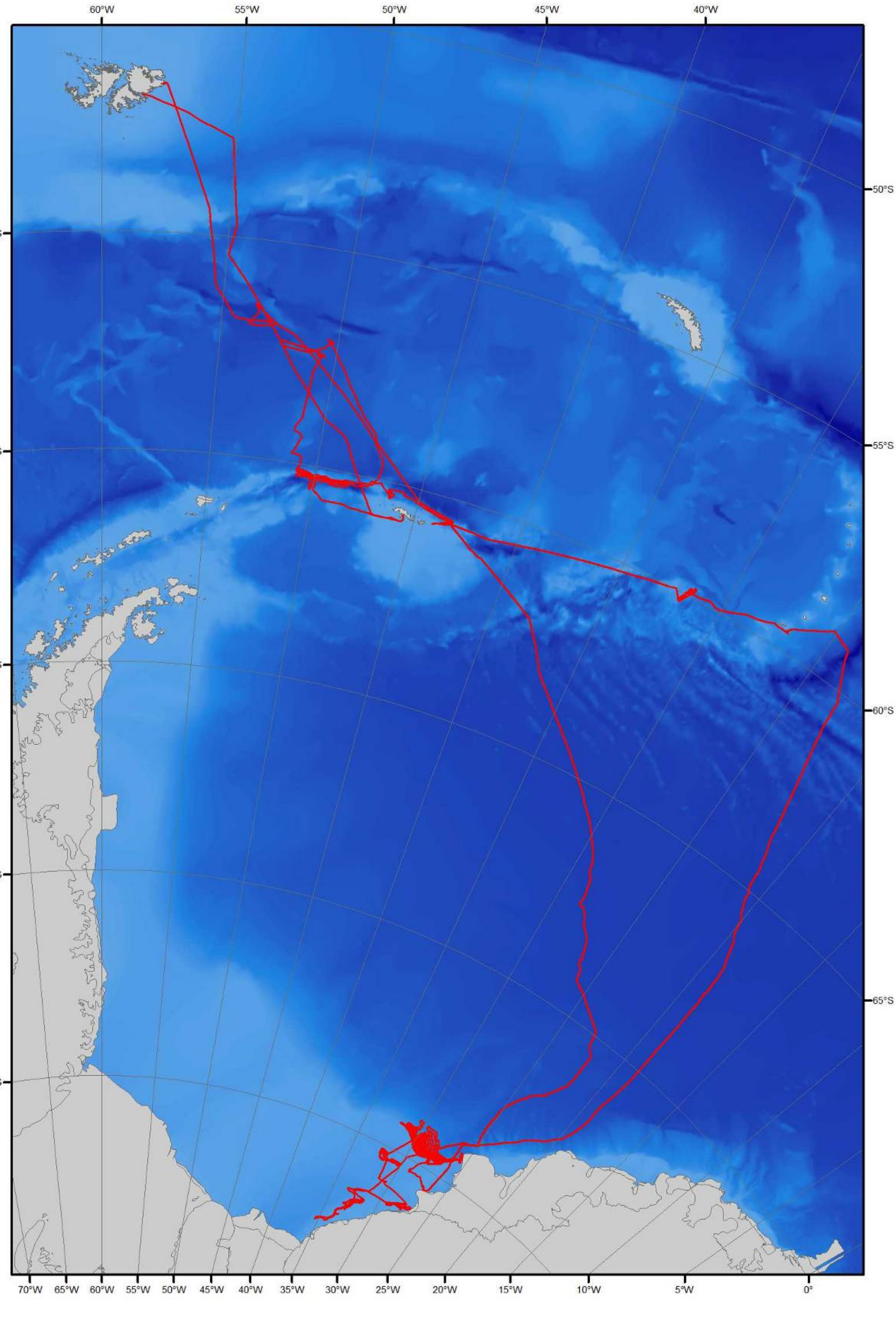
The cruise track is shown in Figure 1. Note that the cruise took place at the same time and in collaboration with JR255, a LTMS/Geology cruise. Cruise JR255B, which involved the recovery of gliders in the Powell Basin area, also took place during the same leg. The leg was also used to uplift personnel from Halley and Signy.

Arrival in the South Sandwich Islands coincided with the arrival of anticyclonic weather, and forecasts suggested that this would strongly affect waves in the area for several days. As we had already lost critical days from the cruise, and the number of days available for work in the South Sandwich area was already limited, this forced us to a decision. The decision was made to move immediately to the Weddell Sea, as waiting in the South Sandwich Islands area would have left us short of science time in both areas.

Work in the Weddell Sea progressed very well, with JR275 achieving a good coverage of benthic biology sites. Part of the eastern continental slope in the Weddell Sea was mapped using swath bathymetry.

During attempts to recover the gliders for JR255B, swath mapping was carried out along the South Scotia Ridge between the South Orkney Block and Clarence Island at the north tip of the Antarctic Peninsula.

Figure 1: Cruise Track for JR255B/259/275



Timetable of Events

Date	Julian Day	Notes
4.2.12	35	Scientific Party joined ship at Mare Harbour, Transit to FIPASS
5.2.12	36	Mobilisation/Engineering works at FIPASS
6.2.12	37	Mobilisation/Engineering works at FIPASS
7.2.12	38	Sailed from FIPASS, Falkland Islands 16.35 (Z). Lifeboat deployment and training.
8.2.12	39	Passage to West Scotia Sea. Arrived at W5 segment of West Scotia Ridge
9.2.12	40	Overnight swath survey and two rock dredges. Started passage to South Orkney shelf.
10.2.12	41	Passage to South Orkney shelf.
11.2.12	42	Site on South Orkney shelf. CTD, Agassiz trawl and two deployments of EBS (Epibenthic Sledge) with trials of deep water camera system (Deployments 7-10) 300m (4 hours used out of allocated 12).
12.2.12	43	Passage toward Herdman Bank/southern South Sandwich Islands.
13.2.12	44	Overnight swath of eastern flank of Herdman Bank followed by a rock dredge (DR205). Start passage to southern South Sandwich Islands (heading for Kemp and Adventure caldera).
14.2.12	45	Large storm. Hove to in morning. Decided to abandon work in South Sandwich area. Started passage to Weddell Sea.
15.2.12	46	Passage across Weddell Sea.
16.2.12	47	Passage across Weddell Sea.
17.2.12	48	Passage across Weddell Sea.
18.2.12	49	Transect to southern part of Weddell Sea. Attempted to cut through ice to Filchner Trough in southern Weddell Sea. Abandoned attempt. Overnight swath survey on east flank of middle Filchner Trough.
19.2.12	50	Southeast Filchner Trough. Deployments 19-23 (1xCTD, 3xAGT, 1xEBS) 600m.
20.2.12	51	Southeast Filchner Trough. Deployments 24-27 (1xCTD, 3xAGT) 800m. Deployments 28-31 (1xCTD, 3xAGT) 600m. (Too rocky for EBS deployment)

21.2.12	52	Coastal fjords sites. Deployments 32-36 (1xCTD, 3xAGT, 1xEBS) 600m. Deployments 37-40 (1xCTD, 2xAGT, 1xEBS) 550m.
22.2.12	53	Central east Filchner sites. Deployments 41-45 (1xCTD, 3xAGT, 1xEBS) 400m. Deployments 46-50 (1xCTD, 3xAGT, 1xEBS) 600m.
23.2.12	54	Northeast Filchner sites. Deployments 51-56 (1xCTD, 4xAGT, 1xEBS) 400m. Deployments 57-61 (1xCTD, 3xAGT, 1xEBS) 600m.
24.2.12	55	Northeast Filchner sites. Deployments 62-66 (1xCTD, 3xAGT, 1xEBS) 600m. Deployments 67-71 (1xCTD, 3xAGT, 1xEBS) 600m.
25.2.12	56	Swath survey of Continental slope.
26.2.12	57	Deep sea biology site at 2000 m on Continental slope at east edge of Crary Fan. Deployments 74-78 (1xCTD, 3xAGT, 1xEBS) 2,000m.
27.2.12	58	Swath survey of Continental slope.
28.2.12	59	Biology site at 1500 m on Continental slope at east edge of Crary Fan. Deployments 79-83 (1xCTD, 3xAGT, 1xEBS) 1,500m.
29.2.12	60	Biology sites at 600 and 400 m on Continental slope at east edge of Crary Fan. Deployments 84-89 (1xCTD, 3xAGT, 1xEBS) 600m. Deployments 90-94 (1xCTD, 3xAGT, 1xEBS) 400m.
1.3.12	61	Biology site at 1000 m on Continental slope at east edge of Crary Fan. Deployments 95-99 (1xCTD, 3xAGT, 1xEBS) 1,000m.
2.3.12	62	Swath survey of Continental slope from east edge of Crary Fan to Brunt iceshelf.
3.3.12	63	Swath survey of Continental slope from east edge of Crary Fan to Brunt iceshelf.
4.3.12	64	Biology sites at 400 m on Continental shelf. Deployments 100-109 (2xCTD, 6xAGT, 2xEBS) 400m.
5.3.12	65	Halley last call. Personnel uplift at Creek 3. Started passage north across Weddell Sea.
6.3.12	66	Passage north across Weddell Sea.
7.3.12	67	Passage north across Weddell Sea. CTD at 4700 m to correct cable spooling.
8.3.12	68	Passage north across Weddell Sea.
9.3.12	69	Passage north across Weddell Sea.
10.3.12	70	Passage along to South Orkney continental shelf edge to first glider site north of South Orkney Islands.

11.3.12	71	Failed attempt to recover glider SG546 for JR255B north of Inaccessible Islands, South Orkney Islands.
12.3.12	72	Glider SG539 recovered for JR255B
13.3.12	73	Transit north and failed search for glider SG522 for JR255B.
14.3.12	74	Failed search for glider SG522 for JR255B. Started transect back to South Scotia Ridge.
15.3.12	75	Completed transit south from glider site to South Scotia Ridge. Started swath survey on South Scotia Ridge at ca. 16.00 (Z).
16.3.12	76	Swath survey on South Scotia Ridge.
17.3.12	77	Continued swath survey on South Scotia Ridge. Transit to Signy and stand by for last call.
18.3.12	78	Last call and personnel uplift, Signy.
19.3.12	79	Transit to Falkland Islands
20.3.12	80	Transit to Falkland Islands
21.3.12	81	Arrival at Falklands
22.3.12	82	Demobilisation

Personnel

Officers and crew for JR275

BURGAN, Michael JS	Master
PAGE, Timothy S	Chief Officer
O'DONNELL, Wendy A	2nd Officer
BARRATT, Thomas R	3rd Officer
GLOISTEIN, Michael EP	ETO Comms
PECK, David J	DO Sci Ops
ANDERSON, Duncan E	Ch Engineer
PICKARD, Colin S	2nd Engineer
SLATER, Bobby L	3rd Engineer
COUPER, Robert JJ	4th Engineer
WALE, Gareth M	Deck Eng
GOIER, Gerald F	ETO
TURNER, Richard J	Purser
BOWEN, Albert Martin	Bosun
RAPER, Ian	Bosun's Mate
PHILLIPS David A	SG1
MACNEIL, Seamus	SG1
SHEARER, James S	SG1
WALLEY, Mark S	SG1
HERNANDEZ, Francisco J	SG1
HERBERT, Ian B	MG1
PATTERSON, Mark	MG1
HUNTLEY, Ashley Alan	Chief Cook
LEE, Jamie Edward	2nd Cook
JONES, Lee J	Sr Steward
GREENWOOD, Nicholas R	Steward
RAWORTH, Graham	Steward
HENRY, Glyndor, N	Steward
RUDD, James	Doctor

Scientific Party

GRIFFITHS, Huw J	BAS Marine Biologist (PSO)
SANDS, Chester J	BAS Molecular Ecologist
JACKSON, Jennifer	BAS Molecular Geneticist
DOWNEY, Rachel V	BAS Marine Biologist
MACKENZIE, Melanie	Museum Victoria, Collection Manager / Holothuroid taxonomist
MOREAU, Camille	MSc student, Institut Universitaire Européen de la Mer - Brest
REED, Adam	PhD student, University of Southampton & BAS
HEUZÉ, Celine	UEA PhD Student
HAMILTON, Douglas S	UEA Chemical Oceanographer
THOMAS, Seth J	BAS (Antarctic and Marine Engineering)
KLEPACKI, Julian ZB	BAS (Antarctic and Marine Engineering)
LENS, Peter C D	BAS (IT Support)

Cruise participants at Creek 3, near Halley, Brunt Iceshelf, 5th March 2012.Photograph by Richard Turner.

Back row: Gareth Lee, Seth Thomas, Philip Leat, Chester Sands, Camille Moreau, Mark Patterson, Bobby Slater, James Shearer, Alex Tate, Jennifer Jackson, Adam Reed

Front Row: Celine Heuze, Huw Griffiths, Peter Lens, Douglas Hamilton, Rachel Downey, Melanie Mackenzie, Gwen Buys, Gerald Goier

Project Reports:

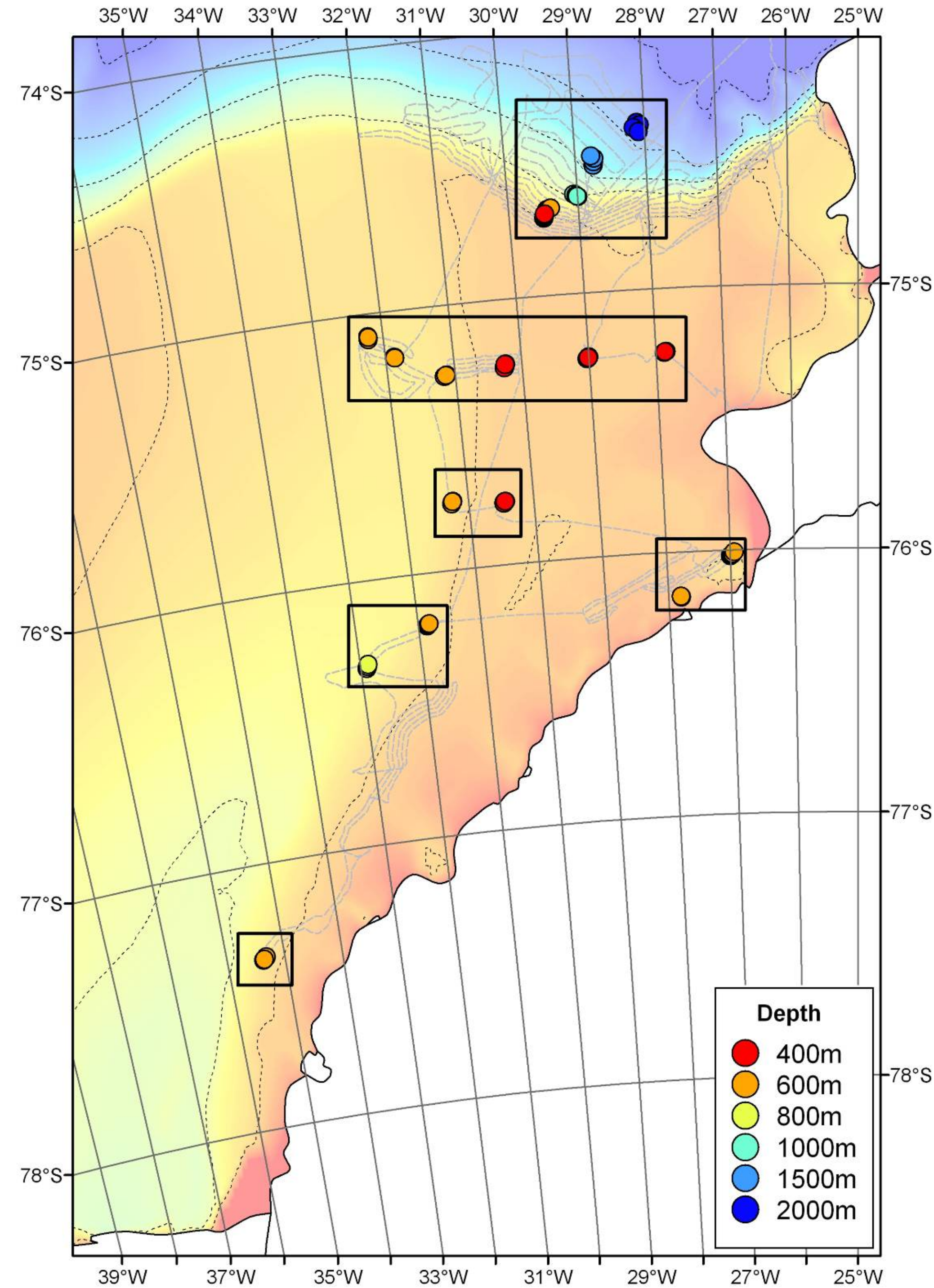


Figure 2: Sampling locations for JR275.

Agassiz Trawl (AGT)

Huw Griffiths, Rachel Downey, Jennifer Jackson, Melanie Mackenzie, Camille Moreau, Adam Reed & Chester Sands

Our apparatus, an Agassiz trawl (AGT), was used to sample animals approximately 1 cm and larger in length, which comprise the larger macro- and megafauna, but did capture some smaller animals as well. Each of the stations comprised of three replicate trawls.

Our Agassiz trawl used a mesh size of 1 cm and had a mouth width of 2 m. At each station the seabed topography was examined prior to trawl deployment using multibeam sonar (swath). The deployment protocol was standardised. While the AGT was lowered, the ship had to compensate for the wire lowering speed of 45 m.min-1 by steaming at 0.3 knots until the AGT reached the seabed and at 0.5 knots until the full trawling wire length was put out. The full trawling cable length we used was 1.5 times the water depth. The net was then trawled at 1 knot for between 2 and 10 minutes (depending on depth, seabed type and the condition of the animals in the initial trawl). With the ship stationary, the AGT was hauled at 30 m.min-1 in order to avoid damaging the gear. When the AGT had left the seafloor, the hauling speed was increased to 45 m.min-1 and the ship speed to 0.3 knots.

Once on board, the samples were photographed as total catch and then hand-sorted into groups varying from Phylum to species level collections. Representatives of many taxa were photographed in detail. The wet-mass (biomass) of the different taxa was assessed by using calibrated scales (with accuracy and resolution of 0.001 kg). Samples were taken from a selection of specimens for DNA analysis. Depending on the taxon, animals were either preserved in 96% ethanol, 4% buffered formalin or frozen at –20ºC.

There were a total of 55 AGT deployments in the Weddell Sea (fig. 3 & appendix 1) and a single test deployment off the South Orkney Islands.

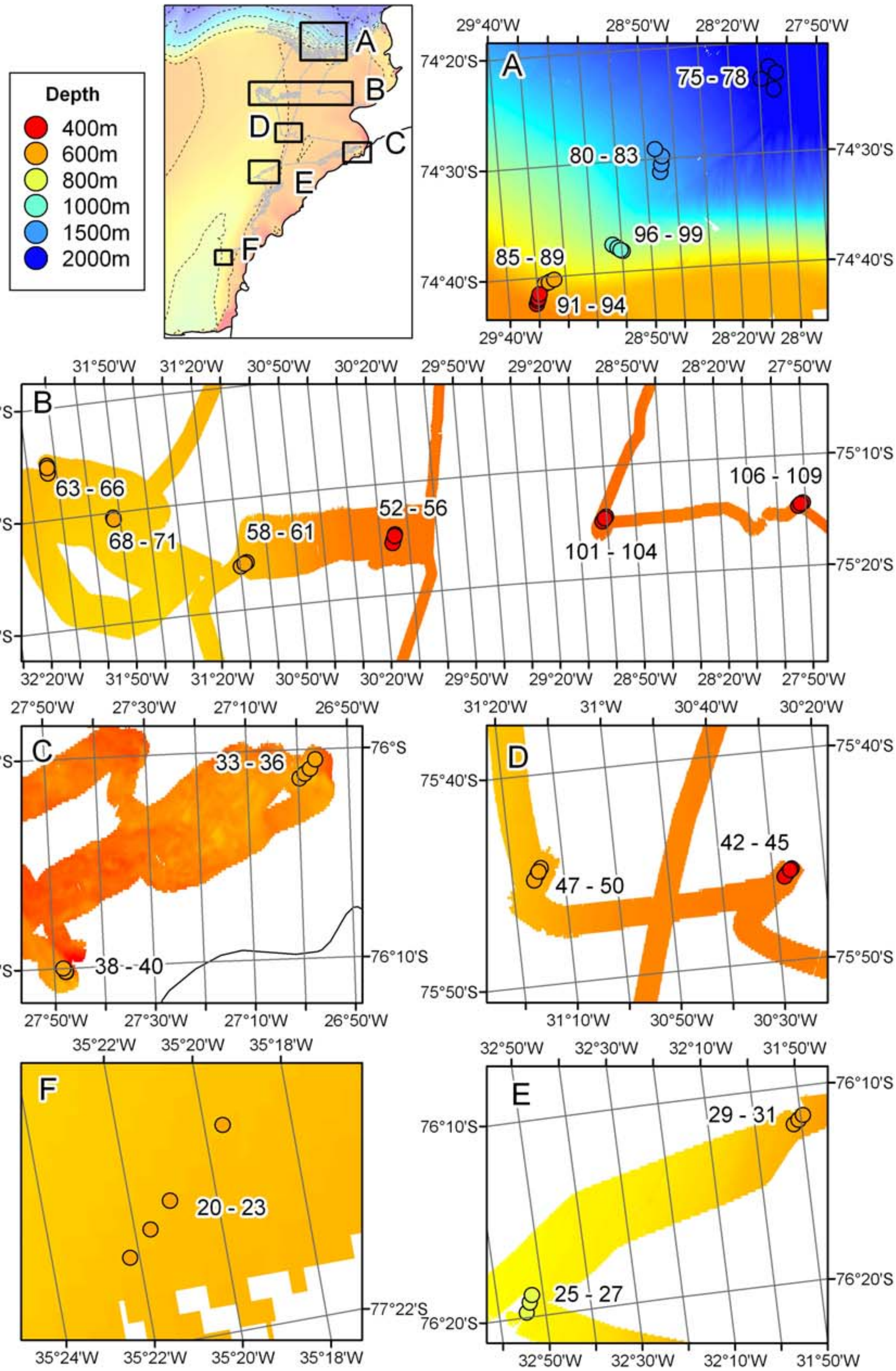


Figure 3: Regional sampling maps for JR275

Preliminary Results

One of the first analyses done after the AGT catches were sorted and fixed was to count the number of phyla present in the catch, to assess the richness at Phylum level of the trawled area (Table 1). Total numbers of phyla varied between 1 in event 87 (the result of a failed deployment and twisted net) and 14 at event 33 in the first of the two coastal fjords. Thirty-three of the trawls contained ten or more phyla .

The only phylum to be found in all 55 catches was the crustacean (including event 87 – failed trawl). Echinoderms were found in all catches except for event 87. Eight phyla were found in 5 or fewer trawls.

The most numerous class of animals caught were the brittlestars (Echinodermata, Ophiuroidea) with 3,981 individual animals caught. Both the annelid worms and holothurians (sea cucumbers) had over 1,000 individual specimens recorded. The bivalve molluscs totalled over 700 individuals, although this total is likely to increase when the epifaunal animals found on the sea urchins are counted in detail. The sponges accounted for around 90 kg of wet mass and were the highest mass of animals recorded. The holothurians totalled 13.5 kg of wet weight.

The region with the highest total number of individual animals (when averaged over the total number of trawls in that region, corrected to a 1000 m trawl length) was the 600 m station on Continental slope, at the east edge of Crary Fan (trawls 85 to 88) (see figure 4). This coincided with the highest total wet weight of animals (mostly sponges) from the same region (see figure 5). In general, the deeper stations had lower total numbers of animals although this did not always equate to a low total biomass. Two stations which stood out as having particularly low total numbers of individuals were the 600 m Northeast Filchner sites closest to the middle of the Filchner Trough (trawls 63 – 65 & 68 – 70). From the swath it was possible to observe large amounts of iceberg scour in these areas. This iceberg scour is a probable explanation of the low numbers of animals and relatively low total biomasses recorded at these sites. The majority of the biomass recorded from most stations could be attributed to the sponges (if present) and the echinoderms (always present). For numbers of individuals, echinoderms overwhelmingly dominated every station except for the 2,000 m station at east edge of Crary Fan (trawls 75 – 77), which was dominated by sea spiders (Chelicerata).

Table 1: Distribution of phyla caught by AGT.

Trawl	Foraminifera	Sipuncula	Anthozoa	Priapula	Echiura	Ctenophora	Hemichordata	Urochordata	Platyhelminthes	Cnideria	Nemertea	Brachiopoda	Bryozoa	Chordata	Mollusca	Porifera	Chelicerata	Cnidaria	Annelida	Echinodermata	Crustacea	Number of Phyla Per Trawl
20									X			X		X	X		X	X	X	X	X	9
21										X				X	X	X	X	X	X	X	X	9
22	X									X								X		X	X	5
25				X							X	X	X	X	X	X	X	X	X	X	X	12
26												X	X	X	X	X		X	X	X	X	9
27												X	X		X	X		X	X	X	X	8
29										X	X		X	X	X	X	X	X	X	X	X	11
30									X		X	X		X	X	X	X	X	X	X	X	11
31												X			X		X	X	X	X	X	7
33							X	X	X		X	X	X	X	X	X	X	X	X	X	X	14
34												X	X	X	X	X	X	X	X	X	X	10
35					X						X	X	X	X	X	X	X	X	X	X	X	12
38												X	X	X	X	X	X	X	X	X	X	10
39												X	X	X	X	X	X	X	X	X	X	10
42			X						X			X	X	X	X	X	X	X	X	X	X	12
43											X	X	X	X	X	X	X	X	X	X	X	11
44											X		X	X	X	X	X	X	X	X	X	10
47		X								X	X	X	X	X	X	X	X	X	X	X	X	13
48										X			X	X	X	X	X	X	X	X	X	10
49														X	X		X	X	X	X	X	7
52											X	X	X	X	X	X	X	X	X	X	X	11
53								X		X		X		X				X	X	X	X	8
54										X	X			X		X	X	X	X	X	X	9
55											X		X	X	X	X	X	X	X	X	X	10
58			X					X			X		X	X	X	X	X	X	X	X	X	12
59									X			X	X	X		X	X	X	X	X	X	10
60								X		X		X	X	X	X	X	X	X	X	X	X	12
63										X				X	X		X		X	X	X	7
64								X		X			X		X		X	X		X	X	8
65													X						X	X	X	4
68										X	X		X		X	X		X	X	X	X	9
69									X		X	X	X	X	X	X		X	X	X	X	11
70											X		X	X		X		X	X	X	X	8
75					X					X			X	X	X	X	X	X	X	X	X	11
76							X			X						X	X	X	X	X	X	8
77											X				X	X	X	X	X	X	X	8
80						X				X			X				X		X	X	X	7
81						X	X			X				X	X	X	X		X	X	X	10
82						X	X			X				X	X	X	X		X	X	X	10
85												X	X	X	X	X	X	X	X	X	X	10
86												X	X	X	X	X	X	X	X	X	X	10
87																					X	1
88						X							X	X	X	X	X	X	X	X	X	10
91					X							X	X	X	X	X	X	X	X	X	X	11
92													X	X	X	X	X	X	X	X	X	9
93							X							X	X	X	X	X	X	X	X	9
96										X			X		X	X	X	X	X	X	X	9
97						X				X			X	X	X	X	X	X	X	X	X	11
98									X	X			X	X		X	X	X	X	X	X	10
101											X		X	X	X	X	X	X	X	X	X	10
102											X	X	X	X	X	X	X	X	X	X	X	11
103											X	X	X	X	X	X	X	X	X	X	X	11
106									X		X		X	X	X	X	X	X	X	X	X	11
107													X	X	X	X	X	X	X	X	X	9
108				X							X		X	X	X	X	X	X	X	X	X	11
Total Trawls Per Phylum	1	1	2	2	3	5	5	5	8	19	21	24	40	44	45	45	46	49	52	54	55	

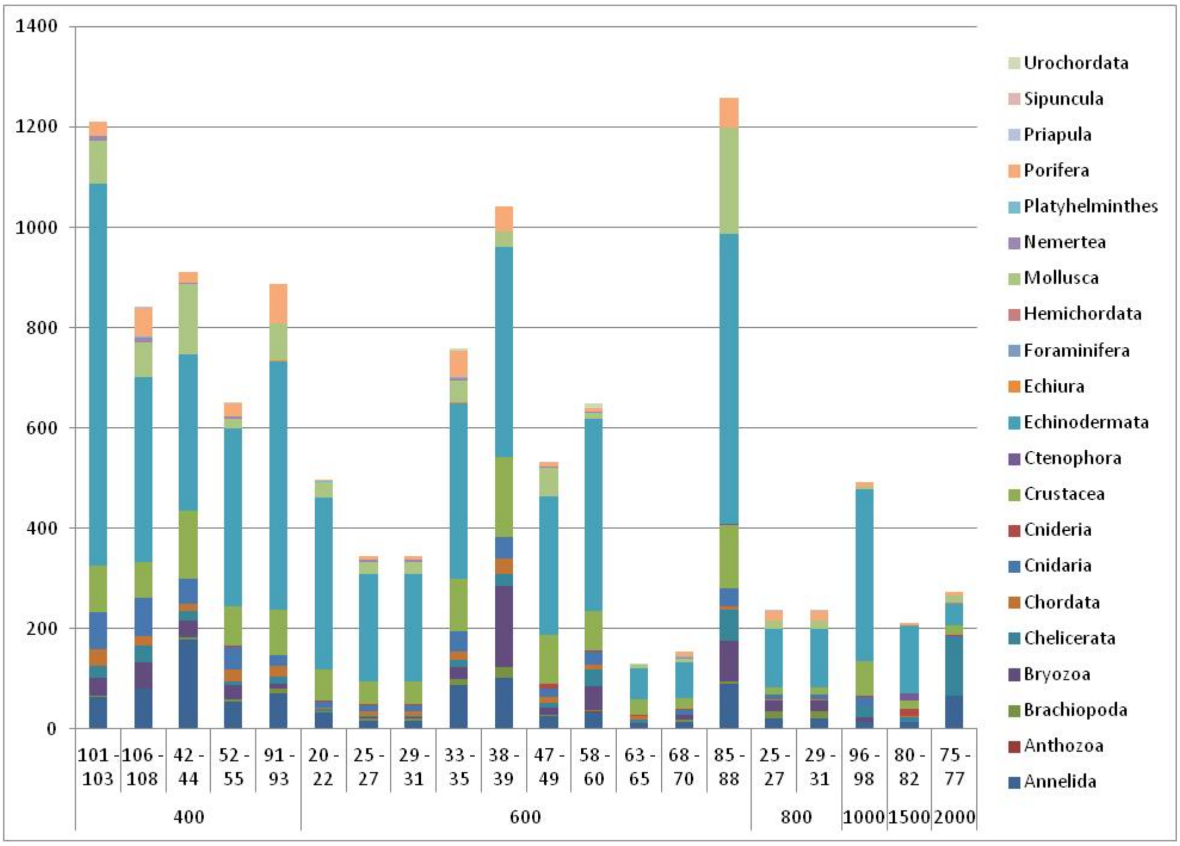


Figure 4. Mean numbers of animals from each location (numbers corrected to a 1000 m long trawl).

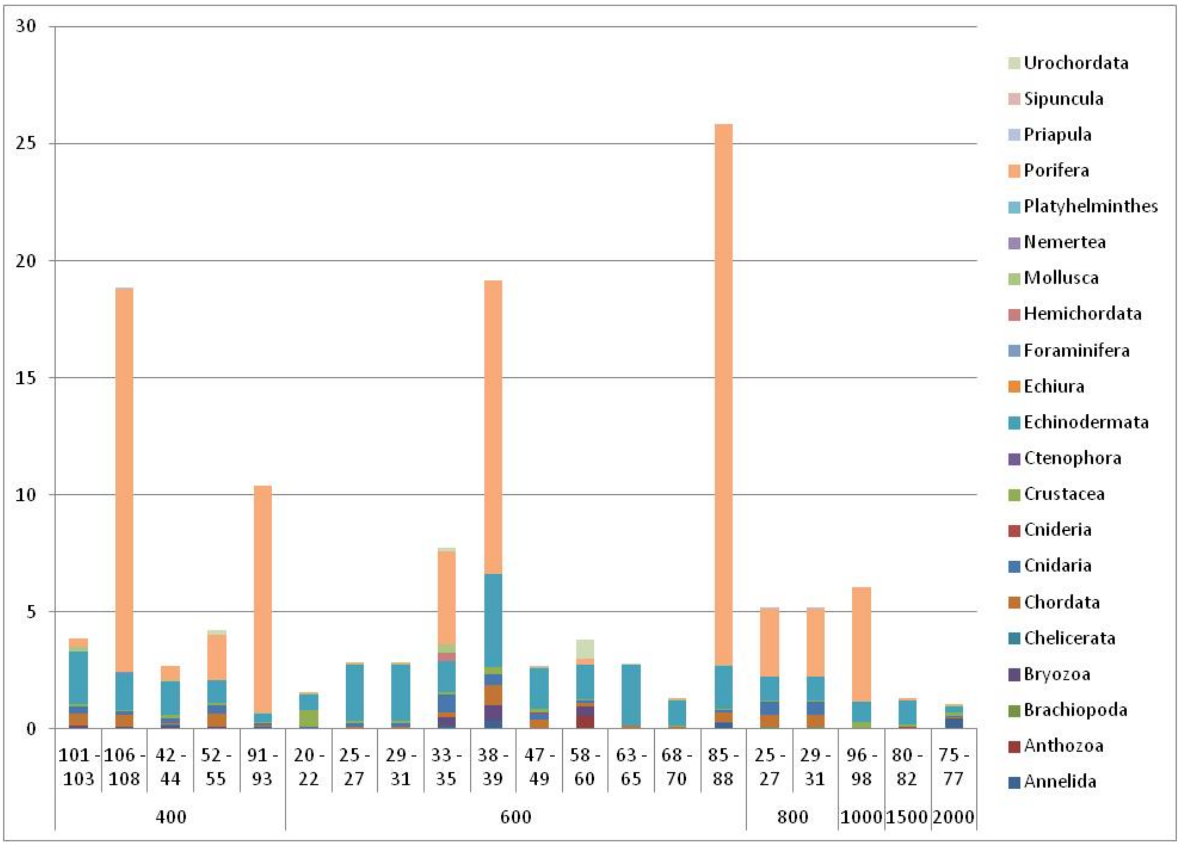


Figure 5. Mean wet weight (kg) of animals from each location (numbers corrected to a 1000 m long trawl).

Epibenthic Sledge (EBS)

Huw Griffiths, Rachel Downey, Jennifer Jackson, Melanie Mackenzie, Camille Moreau, Adam Reed & Chester Sands

A single Epi-Benthic Sledge (EBS) was deployed at each station. In addition to the usual collection nets, the EBS was fitted with a Deep Water Camera System (DWCS), a mini CTD, and 2 lasers for measurement (although the latter proved to be unusable).

Samples were collected by means of a modified epibenthic sledge (Brenke, 2005) in the southern Weddell Sea. Sampling consisted of a total of 2 test deployments off the South Orkney Islands and 16 deployments in different areas of the Weddell Sea and across 6 different depth horizons (400 – 2,000 m, Fig. 13).

The EBS (EBS, Fig. 20) is a proven apparatus for sampling small benthic macrofauna. The sledge is equipped with an epi-net (below) and a supra-net (above). The mesh size of the nets is 500 µm. The cod ends are equipped with net-buckets containing a 300 µm mesh window (Brenke, 2005). The EBS was trawled for 10 min. on the sea bed on each occasion (except for event 36 due to a problem with deployment and event 40 due to a limited sampling area). In total, the operation time of each deployment ranged between 0.5 to 2.5 hours.

Samples were sieved with cold sea water, and immediately fixed in 96% pre-cooled ethanol and kept for 48 hours in -20 °C for later DNA extraction.

The EBS is securely fastened on the aft deck of JCR where the AME staff disconnect the power supply from the DWCS camera and lights whilst scientists recover the samples in the cod ends of the epi- and supra-nets (left). These are then filtered to remove most of the sediment before storing in pre-cooled ethanol and eventual microscopy examination. Once the samples were safely removed, the EBS was placed horizontally on the deck and the power module of the DWCS and the CTD were removed for data download and battery charging.

As the samples were not examined at sea during JR275 there are no preliminary results to report.

Deep Water Camera System (DWCS)

Huw Griffiths & Seth Thomas

The Deep Water Camera System (DWCS) was used for the first time on JR275. It worked well and the quality of the video footage obtained was very good. Although the video system functioned well, the lasers (designed to be used for measuring animals on the video) did not function at all and had to be removed for testing. It was not possible to get the lasers functioning correctly during this cruise.

The DWCS was used during every EBS deployment. It captured video of a range of habitat types from flat mud to three-dimensional habitats made up of bryozoans, sponges and soft-corals (examples in figure 6). Detailed analysis of the video footage will be undertaken at a later date.

Figure 6: Example images obtained by the Deep Water Camera System attached to the EBS.

Molecular work at sea

Jennifer Jackson

We collected small tissue samples from 74 jellyfish, 183 holothurians, 10 anenomes, 13 octopus, one sponge and one non-shelled gastropod and stored these in 95% ethanol at -20°C in 1.5 ml Eppendorf tubes. Genomic DNA was extracted from 70 jellyfish, 8 holothurians and one sponge in 5 extraction events (Table 2). Tissue samples were lysed over 7-11 hours at 65°C on a heated plate, shaking at >500rpm. Qiaquick spin columns were used to wash and elute genomic DNA into 200 microlitres of TE buffer. All tissue samples and DNA extracts were stored at -20°C for further downstream analysis at the BAS ANGEL laboratory (with the exception of cephalopods which will be sent to Dr Jan Strugnell of La Trobe University, Melbourne). Additional tissue samples were collected from all holothurians for DNA barcoding at the BOLD facility. Tissue samples collected from holothurians for DNA extraction at BAS are given in Appendix 2.

Table 2. Tissue samples and DNA extracts of JR275 specimens by taxonomic group, event number, geographic location and depth.

Deployment number	lat-long	Max depth	Specimen ID	DNA extracts?
Scyphozoa				
21	77.35S 35.34W	652.8	148,149	Yes
22	77.35S 35.32W	654.2	151	Yes
29	76.20S 31.86W	579.0	262,263	Yes
34	76.02S 26.97W	613.0	425,426	Not 425
47	75.74S 31.24W	584.9	670-673,686	Yes
48	75.75S 31.25W	590.8	695-700	Not 699
53	75.25S 30.25W	417.8	785	Yes
54	75.25S 30.26W	419.1	816	Yes
60	75.27S 31.17W	616.5	935	Yes
63	75.09S 32.22W	612.3	949	Yes
64	75.09S 32.22W	611.8	968	Yes
68	75.18S 31.87W	676.1	989,990	Yes

75	74.37S 28.10W	2053.9	1077-1079	Yes
76	74.38S 28.06W	2058.2	1082,1083,1094	Not 1083
80	74.52S 28.75W	1546.0	1140-1142, 1148,1149	Yes
81	74.51S 28.74W	1570.1	1152,1153,1155-1163,1181,1183-1186,1188-1197	Yes
82	74.49S 28.74W	1595.5	1222-1226	Yes
88	74.67S 29.43W	602.3	1326	Yes
96	74.63S 29.04W	1028.5	1416	No
97	74.63S 29.02W	1010.6	1441-1444	Yes
98	74.64S 28.99W	971.1	1462	Yes
Anthozoa				
21	77.35S 35.34W	652.8	118	No
30	76.19S 31.84W	578.9	342,343	No
33	76.02S 26.99W	610.0	394,395	No
35	76.02S 26.96W	613.0	464,465	No
49	75.75S 31.27W	584.9	748	No
68	75.18S 31.87W	676.1	992	No
91	74.71S 29.51W	410.0	1355	No
Cephalopoda				
8	60.68S 44.01W	281.6	6	No
21	77.35S 35.34W	652.8	117	No
52	75.24S 30.25W	419.2	782	No
63	75.09S 32.22W	612.3	960	No
69	75.18S 31.87W	657.5	1018	No
86	74.68S 29.45W	581.0	1297	No
102	75.25S 29.02W	396.8	1505-1507	No
106	75.24S 27.85W	415.7	1581	No
109*	75.24S 27.87W	415.7	1620-1622	No
Holothuroida				
8	60.68S 44.01W	281.6	27-34	Yes
Non shelled Gastropoda				
35	76.02S 26.96W	613.0	442	No
Porifera				
8	60.68S 44.01W	281.6	35	Yes

* collected via epibenthic sledge

DNA bar-coding of benthic fauna

Rachel Downey, Melanie Mackenzie, Camille Moreau & Chester Sands

Objectives

DNA bar-coding is a method that uses a short gene sequence from a standardised region of the genome (cytochrome *c* oxidase sub-unit I [COI] in this study), as a diagnostic 'biomarker' for determining species units. Using this methodology, sequences deposited in the Barcode of Life Database (BOLD) may be used to ascertain identification of species. Sequences that do not match those deposited in the database may be species as yet not submitted to the database, some of which may be undescribed species.

JR275 bar-coding will be utilised in the phylum Echinodermata (classes Ophiuroidea, Holothuroidea, and Asteroidea) to support morphotaxonomic identification, and to better determine the diversity of benthic fauna in the Weddell Sea. Identifications were undertaken within some of these classes, however, due to time constraints and lack of taxonomic expertise the identifications must be treated as preliminary. It is hoped that by combining our DNA bar-coding results with classical morphological-based taxonomy and more detailed genomic analyses, that research into Antarctic benthic diversity, ecology and evolution will be advanced.

Methods

Three classes of the phylum Echinodermata were targeted for DNA bar-coding in this study: Ophiuroidea, Holothuroidea, and Asteroidea. These three classes were targeted as they contribute to our existing research, specimens were typically present at most stations, tissue sampling was relatively straightforward, and previous DNA bar-coding in these groups has been successful. Tissue samples were taken using clean tweezers and scalpels (cleaned with 95% ethanol between specimens to reduce contamination). Only a small tissue sample was required (2 mm diameter), and tissue samples were placed in uniquely numbered microplates. After the initial trawl sorting process, 5-10 individuals from each morphotype were sampled, and each specimen was given an individual identifier.

Tissue was dissected differently for each class in order to maximise successful DNA extraction. In the Ophiuroidea, a small amount of tissue was removed from the arm of each specimen, or if unavailable, a small section of the outer disc was removed. In the class Asteroidea, tissue was usually extracted from tube feet. However, if the specimen was small, a section of their arm was removed instead. In the class Holothuroidea, tentacles and tube feet were targeted for tissue sampling. If the specimen was small tissue was taken from the body wall.

Photographs of each specimen were taken before tissue was extracted in order to give an accurate portrayal of each specimen caught. In both the Ophiuroidea and Asteroidea, dorsal and ventral photographs were taken of each specimen in order to comply with BOLD protocols and for later reference. In the Holothuroidea class, extra photographs of the specimen were taken, which included lateral views and close-ups of tentacles.

Preliminary results:

Ophiuroidea

This initial study comprised of a 10% sub-sample of all ophiuroids caught during the cruise. In total, 380 individual ophiuroids, from 12 identified genera, collected at 28 stations, had tissue samples taken for BOLD DNA bar-coding (Appendix 3). Further sub-sampling for DNA bar-coding and morphological analysis will take place at the British Antarctic Survey. In as many cases as possible, specimens were identified to genus-level, however, due to the poor condition of some specimens and lack of taxonomic expertise on board, identification was not always possible at sea. All specimens will be identified by a taxonomist upon return to BAS.

Preliminary analysis indicates the abundance of the genera *Ophiacantha*, *Ophiocten*, and *Ophioplinthus* from the southern Weddell Sea region. Highlights have been the possible finding of a previously unknown morphotype (3 specimens) of currently unidentified genus, found at a 2000 m station (AGT 77). One specimen of *Ophioplinthus sp.* also found at a 2000 m station (AGT 75) has characters we were unfamiliar with. Three specimens of an *Astrohamma*-type species were found on the South Orkney Shelf (AGT 8), which could also be potential new species and/or new genus for this region.

Holothurioidea

During the cruise, 190 specimens of holothurians were tissue sampled (Appendix 4). This equates to 15% of all holothurian specimens collected during the cruise. Holothurians specimens were tissue sampled from 50 AGT stations. Further sub-sampling for DNA bar-coding and morphological analysis will take place at Museum Victoria (Melbourne, Australia).

Asteroidea

Asteroid specimens were tissue sampled from 40 AGT stations (Appendix 5). During the cruise, 190 specimens of asteroids were tissue sampled. This equates to 69% of all asteroid specimens collected during the cruise. Further tissue sampling for DNA bar-coding and morphological identification will take place at the British Antarctic Survey.

Holothuroids collected during JR 275

Melanie Mackenzie

Aims

In joining the JR 275 EVOLHIST team as a holothuroid (sea cucumber) taxonomist my chief aims were: to sample freshly collected Weddell Sea specimens for molecular studies, collect smaller specimens by employing collecting equipment not used on previous cruises, and make preliminary identifications of specimens post-catch, with the ultimate goal to identify all holothuroid specimens for lodgement as voucher specimens at BAS /Natural History Museum and Museum Victoria following a more thorough taxonomic examination of material back in Australia. Secondary goals included photographing live and preserved specimens for more accurate field and laboratory identification, and having a cursory look at relative abundance of species. With 37 holothuroid species previously recorded from shelf and slope depths, the Weddell Sea is to date the most species rich region for holothuroids in Antarctica (O'Loughlin et.al. 2010) and it is likely that material from this cruise will yield additional undescribed species that are new to science.

Collection Methods

Holothuroids were collected using two benthic sampling methods – the Agassiz Trawl (AGT) and epibenthic sledge (EBS). The AGT net yielded larger holothuroids (as expected) such as Aspidochirotids, Elasipods and larger Dendrochirotids. The gelatinous elasipod specimens were often found draped over the outer green net, and the dendrochirotids came up at times attached to rocks, sponges or urchins. The EBS has a much smaller mesh size of only 500µm. While these samples are yet to be examined, as this is a more appropriate apparatus for sampling small benthic macrofauna, it is expected to have collected more of the smaller Dendrochirotids and Apodids, and will therefore be more likely to yield undescribed species.

The addition of a UW video attached to the EBS yielded some interesting examples of holothuroid abundance and habitats which warrant further examination. A still shot from some of this footage can be seen below with ‘cities’ of holothuroids and other animals who appear to bury themselves in the sand leaving their tentacles free to feed (Fig 7).

Figure 7: Soft substrate provides good burrowing material for holothuroids. Footage from deployment 23.

Figure 8: Footage from Deployment 66 showing the ‘sea pig’ holothuroid *Protelpidia murrayi* (Theel, 1879) – relatively abundant in the trawl area.

Preliminary Processing

Once in the lab holothuroids were split by order, family or species where time allowed and live colour photos taken when practicable. Samples were fixed in 95% ethanol for DNA and further morphological analysis, though concentration is expected to drop to 75-85% during transit due to the water content of specimens. Post-collecting, many of the specimens were examined under a field microscope and notes taken on identifying features such as tube foot and tentacle arrangements, dimensions, colour, skin texture etc. DNA tissue samples (chiefly tentacle or tube foot samples) were taken from a subsample of specimens both for BOLD bar-coding and for more thorough genetic analysis back at BAS by Dr Jennifer Jackson. Samples were taken by Melanie Mackenzie and Stuart MacMillan and DNA extractions of some of these specimens were also taken on board by Dr. Jennifer Jackson with assistance from Melanie Mackenzie using a QIAGEN DNeasy Blood & Tissue molecular kit.

Figure 9: A selection of holothuroids from one site found clinging to rocks, bryozoans etc. Photo: Melanie Mackenzie – Museum Victoria

Photography

The majority of live-colour photographs of specimens were taken on board by Camille Moreau with some also taken by Adam Reed and Melanie Mackenzie. Remaining holothuroid samples were briefly examined and photographed post-fixation during return transit to the Falkland Islands, again chiefly by Camille Moreau with some preserved images also taken by Dr Jim Rudd of BAS (the *James Clark Ross* doctor) with assistance from Douglas Hamilton (chemist - University of East Anglia) and Melanie Mackenzie. Photographs were taken using a digital SLR Nikon D3X with 60 mm lens and flash rigging and Nikon D700 with 60 mm lens. Previous field identification of Antarctic holothuroids has proved problematic as live specimen colour and morphology can look quite different from the preserved specimens usually seen by taxonomists in museums and research institutions.

Figure 10: Live colour photos of *Protelpidia murrayi* from Catch 47. Photo: Camille Moreau

Figure 11: Live colour photos of a juvenile psolid sea cucumber on an urchin spine. Photo: Camille Moreau

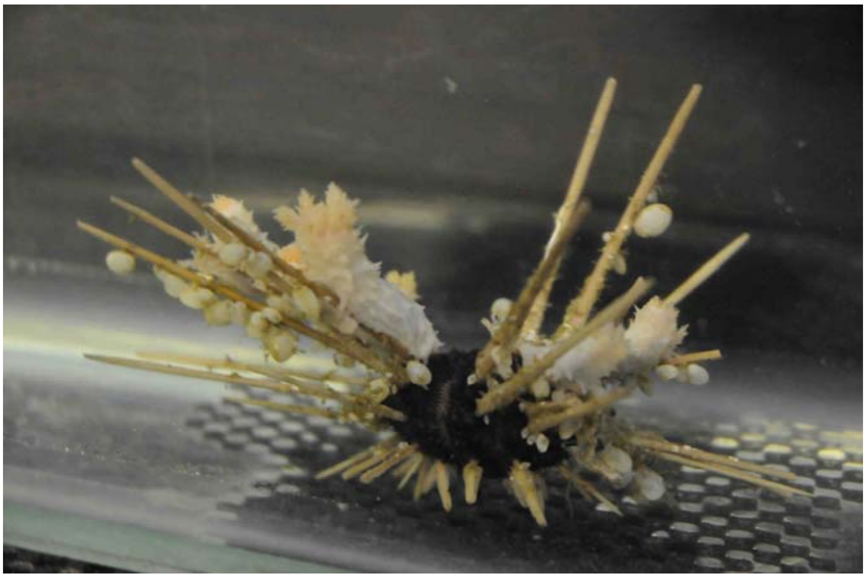


Figure 12: Live *Echinopsolus* attached to a sea urchin along with the Antarctic bivalve *Lissarca.* Photo: Melanie Mackenzie

Figure 13: Various species of *Bathyplotes* such as the *Bathyplotes gourdoni* above were found at sites from ~400 to 1000 m depth. Photo: Camille Moreau

Preliminary Results

A diverse holothuroid assemblage was collected, with over 1,200 holothuroids (~13.5 kgs) from the Agassiz trawls alone, making these echinoderms one of the most abundant groups collected and reinforcing previous records of high holothuroid abundance and diversity in this area. Even in the detritus 'grave yards' of events 91 to 93, smaller holothuroids were found living, along with their relatives the ever-present ophiuroids. Preliminary identifications show that at least 4 of the 6 holothuroid orders are represented in this cruise collection, with the Dendrochirotids, Elasipods and Aspidochirotid orders most species-rich.

Table 3: Relative abundance of 4 orders of Holothuroids collected in AGT nets on JR275

Deployment	Dendrochirotida	Elasipodida	Aspidochirotida	Apodida	Unidentified
8	5	0	3	0	0
20	0	43	0	0	2
21	4	189	0	0	0

22	0	2	0	0	7
25	5	1	5	0	5
26	1	2	6	0	1
27	1	2	3	0	2
29	25	6	1	0	1
30	35	6	0	0	4
31	4	4	1	0	1
33	5	63	0	0	1
34	4	0	0	0	42
35	1	45	0	0	2
38	31	0	0	5	4
39	18	4	2	1	7
43	2	0	0	0	4
44	1	0	0	0	3
47	1	171	0	0	6
48	8	0	1	0	9
49	1	0	0	0	17
52	6	1	1	0	1
53	0	0	0	0	3
54	2	0	0	0	3
55	0	0	0	0	4
58	10	1	6	0	0
59	4	0	1	10	2
60	4	0	1	6	6
63	11	8	0	0	1
64	0	0	0	1	0
68	1	3	1	0	1

69	0	0	6	0	1
70	0	2	0	0	0
75	5	1	1	0	12
77	6	4	2	0	1
81	0	3	1	0	0
82	0	0	0	0	2
85	17	0	0	0	91
86	7	0	0	0	0
88	27	0	0	0	0
91	10	0	0	0	0
92	16	0	0	0	0
93	6	0	0	0	1
96	3	0	7	0	0
97	3	0	5	0	0
98	3	0	4	0	0
101	1	1	0	0	0
102	1	4	2	0	0
103	0	0	0	0	3
106	1	3	3	0	0
107	0	1	0	0	1
108	1	3	1	0	0

*Note: Site 8 is in the South Orkneys, all other sites are Eastern Weddell Sea

Holothuroids were most abundant at Site 21 (~650m depth) and Site 47 (~580m depth) with collections of 192 and 178 respectively, largely due to the abundance of the elasipod *Rhipidothuria racowitzi* Herouard, 1901 at these sites. Preliminary identifications along with site and depth data can be found in Appendix 6.

More accurate identification of AGT and EBS material sent to Museum Victoria will involve sampling the remnant skeletal elements (ossicles) from specimen tentacles and body wall. Samples will be cleared for observation using commercial bleach, viewed under a compound microscope and a selection of voucher and new species subsequently photographed using a Leica microscope and Automontage software.

It is anticipated that additional species – most likely smaller dendrochirotids, psolids and apodids, will be recovered from the EBS samples which are yet to be sorted and examined. It is hoped that further taxonomic and molecular identification will aid investigations into how these species are distributed across sampling sites, and whether diversity and community structure are influenced by depth, habitat, competition or other variables such as proximity to the Antarctic ice sheet or susceptibility to variations in benthic water temperatures, building an even better understanding of relationships and evolution within this intriguing group of animals.

References:

Gutt, J., 1991. Are Weddell Sea holothurians typical representatives of the Antarctic benthos? Meeresforschung 33, 312-329.

O'Loughlin, Mark P., et al., The Antarctic region as a marine biodiversity hotspot for echinoderms: Diversity and diversification of sea cucumbers. Deep-Sea Research II (2010)

Thermal tolerance of Southern Ocean bivalves from the Weddell Sea

Adam Reed

The primary objective of this study was to assess tolerance to acute thermal shock of different bivalve species representing different latitudes, depth ranges, ecology and evolutionary history. Much of the benthic fauna in the Southern Ocean may only be exposed to small changes in temperature annually and this stenothermal environment may be driving a community vulnerable to future environmental changes. By studying respiration rates over a range of temperatures within and outside of their natural ranges, the stenothermal nature of Southern Ocean bivalves can be tested.

Live bivalves were targeted from AGT trawls for respiration experiments on an opportunistic basis, where more then 10 specimens of a species presented themselves in good condition. After 24 hour acclimation in captivity, animals were isolated in 2.8 ml respiration vials and given 6 hours to acclimate to 1˚C temperature increases in a controlled waterbath. Starting temperatures were chosen according to the known bottom temperature at the station where they were captured. At each temperature increase, the vials were filled entirely with water, sealed and left for a known period of time (varying according to species and temperature). Five control vials containing seawater but no animals were also used to measure microbial activity. Oxygen concentration in the respiration vials was measured using a temperature adjusted oxygen meter and microoptode, and oxygen consumed calculated by taking the experimental concentration from the control. After treatment, all animals were fixed in ethanol or 4% formalin and will be weighed back in the UK to calculate true respiration rates.

Four species were used for the experiments, at least 7 specimens from each, and experiments took place for no more than 14 days, dictated by the thermal tolerances of the different species. For the final species used, 7 animals were used in a separate experiment to measure respiration rates over time when kept at a constant temperature. This experiment is to be used to measure the effects of starvation and captive stress. The species used in these experiments represent a range of life histories. Both species of *Lissarca* are likely to be the filter feeding brooder *Lissarca notorcadensis* and represent two populations, one from the Scotia Sea and one from the Weddell Sea. *Yoldiella* and *Propeleda* are both deposit feeding protobranch bivalves more commonly associated with the deep-sea.

Table 4. Species of bivalves used for thermal tolerance respiration experiments with locations, temperature range tested and number of animals used.

Date	Event	Species	Temp. Range	No. Animals
11 Feb 2012	AGT 8	*Lissarca* sp. (Scotia Sea)	-1 – 8˚C	10
20 Feb 2012	AGT 30	*Propeleda* sp.	-1.9- 3˚C	8
21 Feb 2012	AGT 42	*Yoldiella* sp.	-1.9 – 3˚C	7
29 Feb 2012	AGT 85	*Lissarca* sp. (Weddell Sea)	-1 – 5˚C	22

CTD

Celine Heuzé

At every station, a CTD was performed prior to any biology work (for locations see appendix 7). The CTD provided the biologists with details about the temperature, salinity and oxygen concentration at the depth they were studying, but also gave them global information about the whole water column, such as the concentration of phytoplankton at the surface or the mixing and currents on the way down.

About the CTD

The CTD measures the conductivity (hence salinity) and the temperature variations, with depth, of the sea water. Oxygen and chlorophyll sensors are attached next to the CTD so that their concentrations are also measured. Finally, there is also a LADCP on the CTD rosette, which measures currents.

Measures are performed every 0.07s, which gives a good accuracy even at the depths of strong mixing. Plus this accuracy allows the operator to detect the layers of interest – maximum of chlorophyll and minimum of oxygen for example- with a very small error.

Once the CTD was back on deck, dissolved oxygen, chlorophyll and salinity samples were taken from the Niskin bottles to be analysed onboard. Results obtained from the CTD could then be confirmed and sensors which needed to be recalibrated or changed were detected.

Data obtained

The results of main interest that we obtain thanks to the CTD are the temperature, salinity and oxygen profiles.

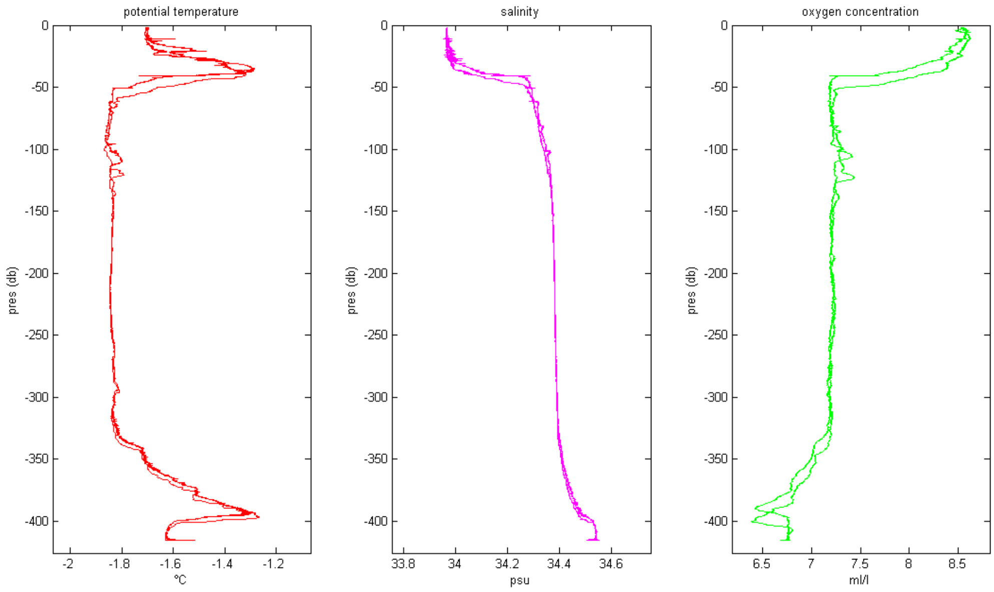


Figure 14: Potential temperature (left), salinity (center) and oxygen (right) profiles for the event 105

Another point of interest for oceanographers and biologists in the Southern Ocean is the density of the sea water they are sampling. The CTD analysis software provided a value for the density, but another way to visualise it is through the use of T/S diagrams which we plotted for every station. We superimposed on them Alejandro Orsi's neutral density lines for the Southern Ocean, so that depth of same density can be easily detected.

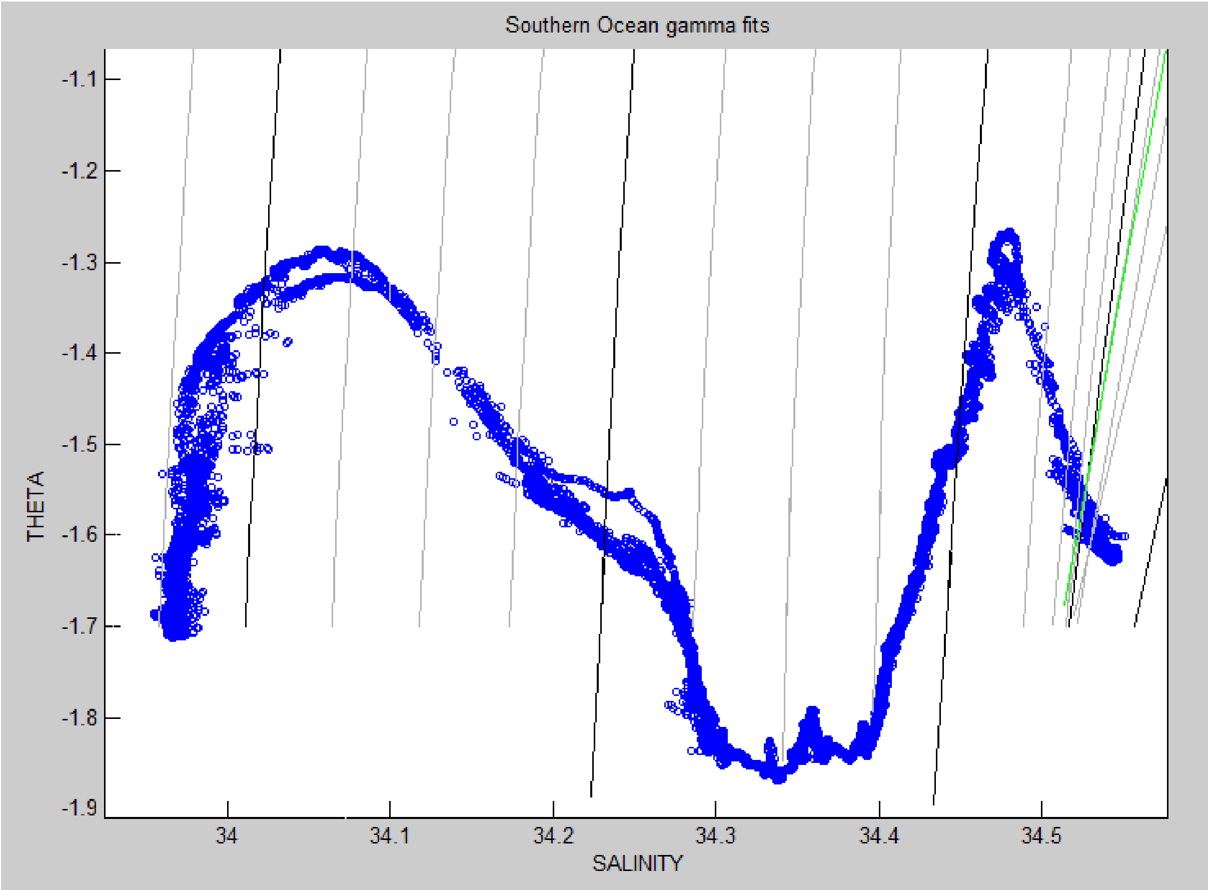


Figure 15: T/S diagram for the event 105: blue dots are the measures of temperature and salinity; black, grey and green lines are lines of same neutral density

Comparison with the small CTD on the EBS

The last deployment of each station was the EBS, on which a small CTD was attached. Even if the ship slightly changed location between this CTD and ours, we considered it interesting to compare our two results.

This small CTD sampled only every 30s and had no oxygen or chlorophyll sensors. Yet, as we can see on the figure below, the results obtained with this one in terms of temperature and salinity are pretty similar to ours, and their bottom values are the same. Only the areas with strong mixing and fast changes in salinity and temperature are far more accurate with the full CTD.

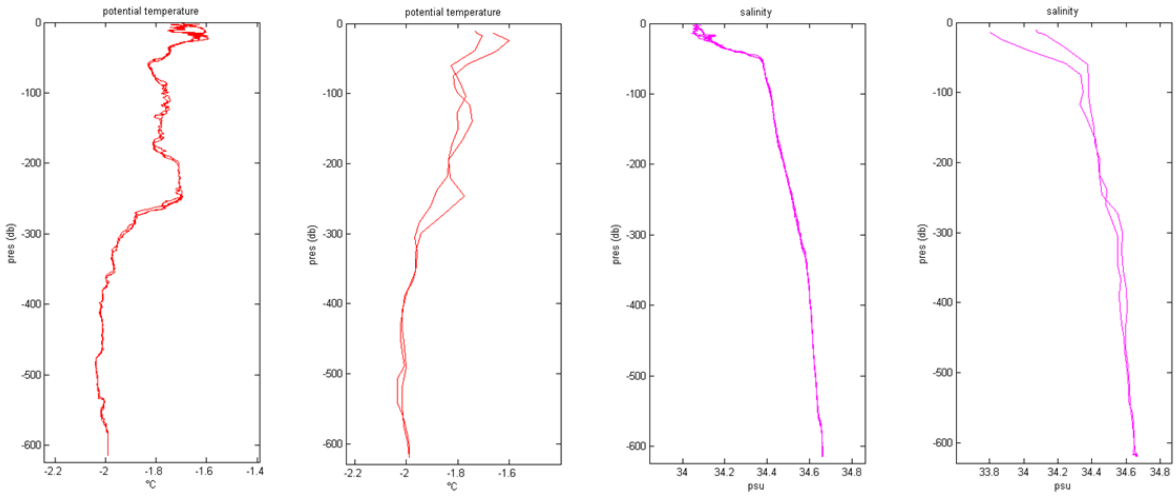


Figure 16: Deployments 62 (left of each parameter) and 66 (right): comparison of the temperature and salinity

Different water masses

As well as providing the biologists with information about the conditions of living of their subjects, performing the CTD was a good way of spotting the repartition of the different water masses in the South-Eastern Weddell Sea. In fact, we were close to the area of formation and spreading of the Weddell Sea Bottom Water (WSBW), the coldest on Earth and very salty, hence the densest. And as we were expecting, we actually spotted some WSBW in the westernmost of the stations we studied (Fig. 17).

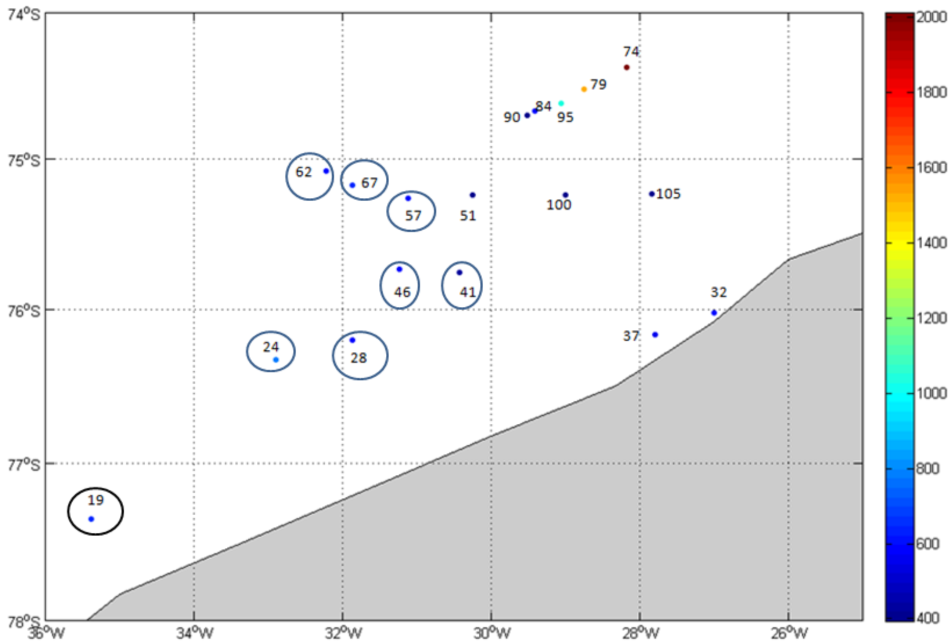


Figure 17: Location and depth of the stations sampled – the circled ones are those with WSBW

CTDs 19, 24, 28, 41, 46, 57, 62 and 67 were those where we found some WSBW. They all had a bottom temperature close to -2°C and a bottom salinity superior to 34.7psu, hence a bottom sigma-100 density of 32.7 kg/m^3.

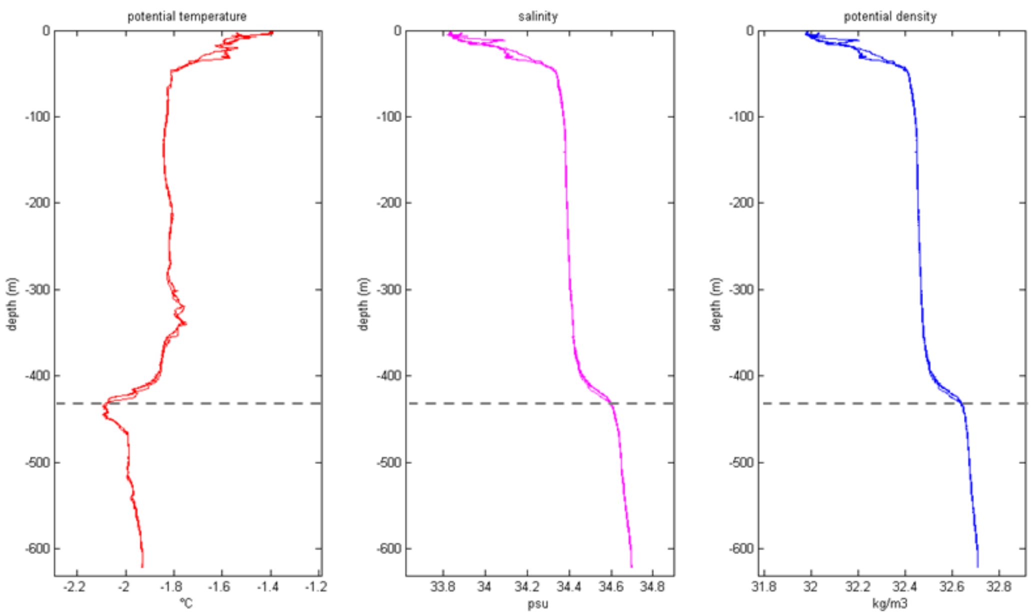


Figure 18: Temperature, salinity and density of CTD 19: Weddell Sea Bottom Water below the line

On the contrary, CTDs 74 and 79 were really warm with a positive temperature on the bottom. As they were also the northernmost ones, what we see is probably some Warm Deep Water coming from the Weddell Gyre.

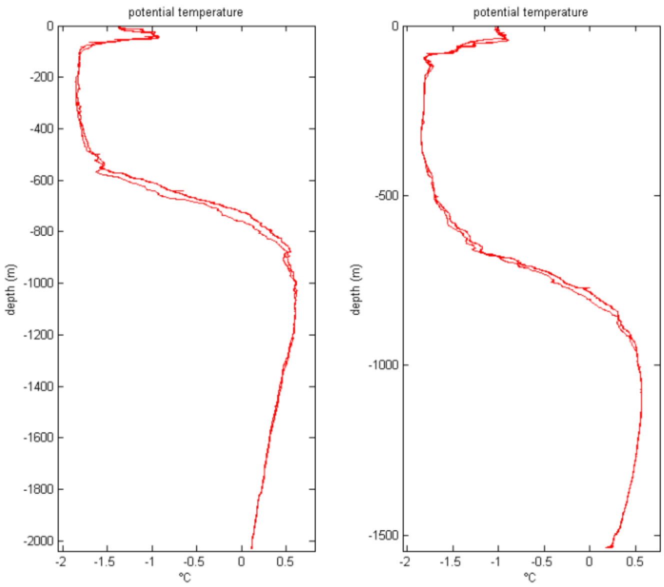


Figure 19: Temperature of deployments 74 (left) and 79 (right)

Regarding other CTD bottom water, even where there was no WSBW we observed some Weddell Sea Deep Water: cold and salty but less than the WSDW, with a bottom sigma-100 density between 32.4 and 32.6 kg/m^3.

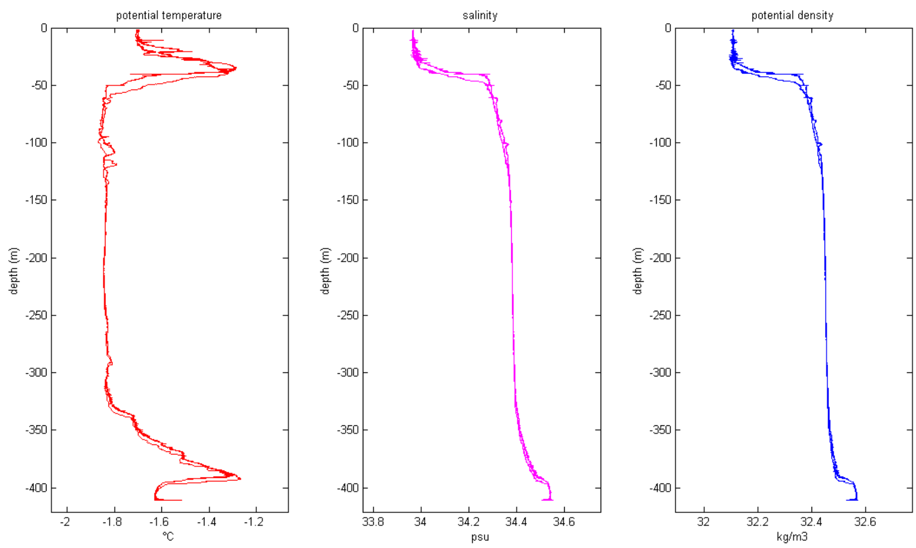


Figure 20: Temperature, salinity and density of the CTD 105

Dissolved Oxygen

Douglas Hamilton

Objective

- Use Winkler titrations to accurately measure the dissolved oxygen (DO) concentration of discrete water samples drawn from Niskin bottles.
- To provide a dataset in order to calibrate and assess the performance of the Seaglider mounted Aanderaa 4330F optode using proximal CTD cast dissolved oxygen data
- To provide a dataset for the bottom water [DO] for use in biology cruise JR275 benthic sampling analysis.

A total of 161 samples from 12 stations were collected over 22 days. Of this the last 15 were for the glider SG539 recovery (cruise JR255b) and previous 146 from the biology (cruise JR275) CTD casts. These samples were analysed by whole-bottle Winkler titration with photometric endpoint detection, using a computerised auto-burette system designed by Carol Robinson (University of East Anglia.) A thiosulphate solution at concentration 0.2 mol dm^{-3} was used, and this was standardized by iodemetry against a 2.225 mmol dm^{-3} KIO_3 solution (prepared gravimetrically at UEA, and shipped as a solution).

Changes in sample volume with differing temperature were corrected using values collected from an electronic thermometer employed during the transfer of water from the Niskin bottles to the titration flasks. During the first DO sampling station (Event 019) it became apparent the current calibrated thermometer (Oakton) was giving temperatures with a difference of up to 2.5°C of that recorded from the CTD sensor. A test of the calibrated thermometer, an uncalibrated thermometer (Testo) and an Hg thermometer show the uncalibrated thermometer giving the most succinct results and was used for the remaining sampling (fig 21). Due to this uncertainty in the temperature for Event 019 results for [DO] are only from Event 024 onwards.

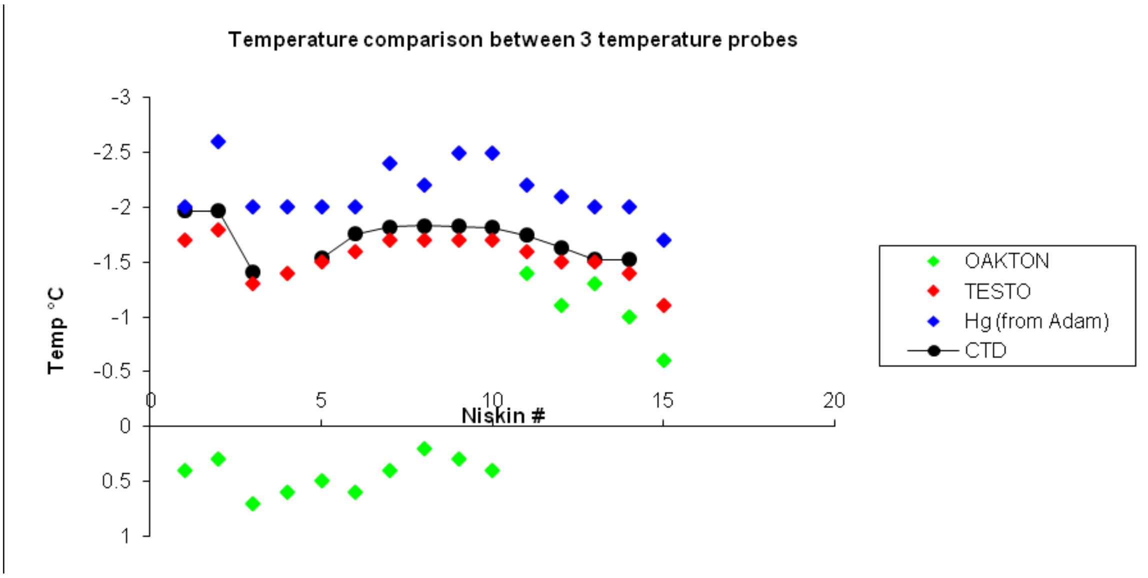


Figure 21: Varying temperatures recorded with 3 different temperature probes.

Calibration was carried out in accordance with WOCE procedures (Dickson, 1996), using a distilled water blank as opposed to a seawater blank. The measured value used was that from the JR255a cruise of 0.0035 ml. Triplicate samples were taken from each sample depth following the procedure laid out in appendix 8 (with the exception of event 110 detailed later). The median standard deviation of [DO] of the titrations for the biology cruise JR275 analysis is 0.0387 ml/l and 0.0032 ml/l for JR255a recovery cruise. Salinity and pressure checks were completed on JR255a.

Upon comparison of the biology cruise Winkler DO samples and corresponding SBE43 DO samples it became apparent that there was a shift in the measurement values from the sensor compared to the last cruise. This shift was approximately 61.5% as seen in figure 22. This change occurred after station 40 of JR275.

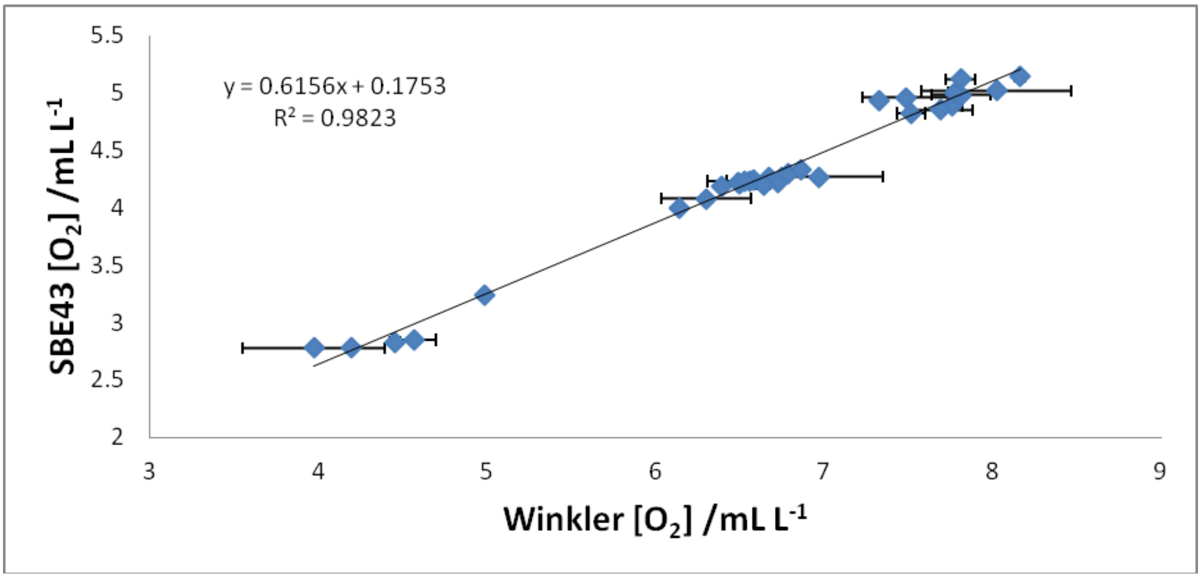


Figure 22: Comparison of SBE43 sensor dissolved oxygen concentrations with that of the Winkler titrations during cruise JR275.

The bottom depth [DO] is of particular interest for the JR275 biology cruise, as the sensor was now less reliable than in past deployments the manual Winkler titration values are given in table 5.

Table 5: Bottom depth dissolved oxygen concentration with respective standard deviations as determined by Winkler titration.

Event	Pressure /db	$[O_2]$ /µM	SD $[O_2]$ /µM	$[O_2]$ /mL L^{-1}	SD $[O_2]$ /mL L^{-1}
024	781	289.94	0.15	6.50	0.00
028	588	289.58	0.26	6.49	0.01
037	572	291.94	0.16	6.75	0.00
046	567	288.28	0.94	6.67	0.02
057	602	283.15	5.71	6.56	0.13
067	658	289.04	0.10	6.73	0.00
074	2060	195.45	5.46	4.57	0.13
079	1558	192.08	1.23	4.46	0.03
090	394	283.28	1.76	6.64	0.04

095	1039	187.34	1.26	4.19	0.03
105	415	273.80	0.29	6.10	0.01

A new SBE43 sensor was employed alongside the malfunctioning one and a full depth profile of Niskins were also fired to compare as seen in figure 2. During sampling 3 Niskins misfired and were recorded with higher than expected temperatures (deviating ~4^{o}C). These are included in figure 23 as anomalies for completeness only.

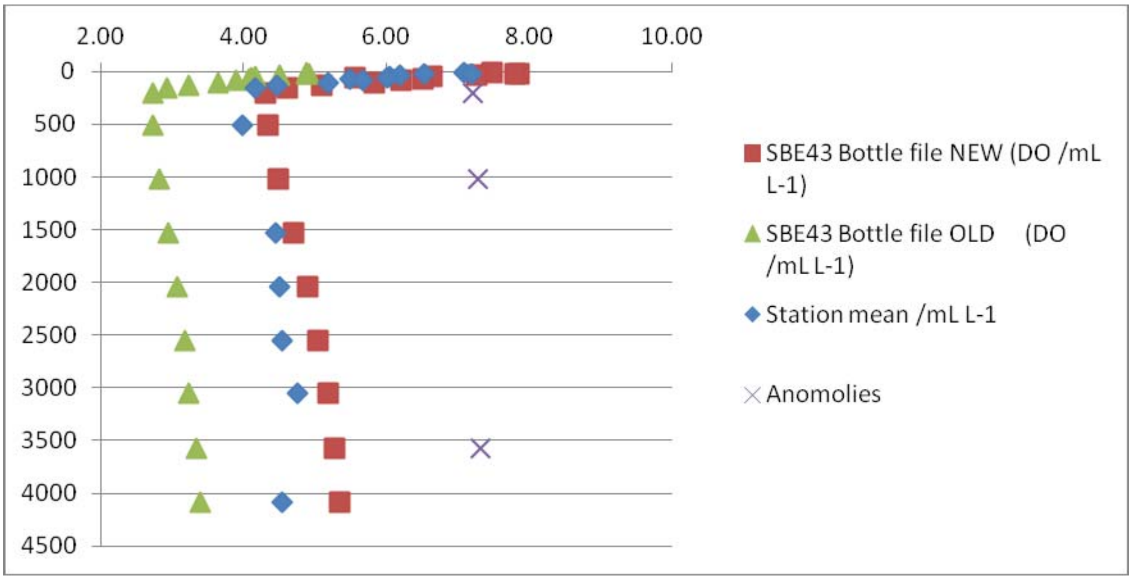


Figure 23: Comparison of old and new sensors with corresponding Winkler titrations.

The new sensor was recording results 11% ± 6% higher than the Winkler titration with the malfunctioning sensor being 30% ± 3% lower than the Winkler titrations and 36 ± 3% lower than the new sensor. To help stop passive degassing of DO it should be noted that due to time constraints on the large amount of samples they were only taken in duplicate and not triplicate, thus lowering the precision.

There appears to be a lot more noise in the signal of the previous sensor compared to the newer one and a larger deviation from the Winkler concentrations, the new sensor values are therefore the ones shown in table 6 for comparison in the recovery of SG539, although both sensors data is available if needed. Due to the lack of available salinity measurements (n=3) for the replacement sensor, there is no check to see if salinity has affected the results in the new sensor.

Table 6: Salinity and dissolved oxygen measurements from the CTD deployed during the recovery of SG539.

Pressure /db	salinity CTD /psu	salinity analysed /psu	[DO] CTD /mL L^{-1}	[DO] analysed /mL L^{-1}	[DO] analysed SD /mL L^{-1}
404	34.56	34.44	5.4369	4.9840	0.002
252			6.0958	5.6086	0.009
202	34.42	34.38	6.3273	5.7570	0.007
176			6.4252	5.9379	0.003
5	34.34	34.38	6.9855	6.3921	0.002

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We are very grateful to Captain Burgan and officers and crew of the RRS *James Clark Ross* for their tremendous help in carrying out this science cruise. We would also like to thank Phil Leat, Alex Tate and Gwen Buys (JR259) for providing support, data and advice with the swath bathymetry.

Appendices:

Appendix 1: Station details for AGT and EBS deployments.

Deployment Number	Gear Type	Start Latitude	End Latatitude	Start Longitude	End Longitude	Minimum Depth (m)	Maximum Depth (m)
8	AGT	-60.6774	-60.67754	-44.01327	-44.01438	279.04	281.57
9	EBS	-60.6778	-60.67864	-44.01644	-44.02198	278.57	287.51
10	EBS	-60.6778	-60.67872	-44.01701	-44.02248	278.75	288.42
20	AGT	-77.359	-77.35763	-35.37029	-35.36416	654.34	654.35
21	AGT	-77.3548	-77.35286	-35.35131	-35.34232	648.18	652.8
22	AGT	-77.3494	-77.34829	-35.32627	-35.32139	650.78	654.2
23	EBS	-77.3569	-77.35788	-35.36059	-35.36497	649.74	655.86
25	AGT	-76.3295	-76.32695	-32.90046	-32.8956	778.81	781.73
26	AGT	-76.321	-76.31971	-32.88435	-32.88189	780.3	789.24
27	AGT	-76.3151	-76.31442	-32.87307	-32.87185	779.51	781.36
29	AGT	-76.1991	-76.19816	-31.86015	-31.85561	575.95	578.97
30	AGT	-76.1956	-76.19471	-31.84258	-31.83826	575.99	578.94
31	AGT	-76.1919	-76.19099	-31.82427	-31.81973	564.11	573
33	AGT	-76.0231	-76.0222	-26.99542	-26.99088	605.21	610
34	AGT	-76.0196	-76.01868	-26.97793	-26.97352	608	613
35	AGT	-76.016	-76.01513	-26.9604	-26.95604	607	613.01
36	EBS	-76.0083	-76.0098	-26.941	-26.94333	490.1	547.86
38	AGT	-76.1697	-76.1685	-27.79567	-27.79901	544.89	561
39	AGT	-76.1694	-76.16887	-27.79659	-27.79802	549.28	555.26
40	EBS	-76.1669	-76.16565	-27.8038	-27.80733	533.05	550.82
42	AGT	-75.7612	-75.76213	-30.43723	-30.44131	429.41	433.85
43	AGT	-75.7645	-75.76497	-30.45297	-30.45472	427.94	430
44	AGT	-75.767	-75.76738	-30.46317	-30.46484	429.39	436.8
45	EBS	-75.762	-75.76412	-30.44267	-30.45131	427.92	439.74
47	AGT	-75.7406	-75.74176	-31.23803	-31.24128	578.94	584.88
48	AGT	-75.7451	-75.74617	-31.25064	-31.2538	584.83	590.75
49	AGT	-75.7496	-75.75076	-31.2636	-31.26678	583.36	584.94
50	EBS	-75.7433	-75.74587	-31.24615	-31.25353	583.34	590.45

52	AGT	-75.2434	-75.24473	-30.24534	-30.24715	418.73	419.21
53	AGT	-75.2478	-75.24906	-30.25152	-30.25333	417.39	417.78
54	AGT	-75.2526	-75.25386	-30.25835	-30.26024	418.7	419.11
55	AGT	-75.2567	-75.25798	-30.26436	-30.26619	418.38	418.61
56	EBS	-75.246	-75.24865	-30.24888	-30.25268	415.9	417.5
58	AGT	-75.2631	-75.26378	-31.12627	-31.131	604.29	607.13
59	AGT	-75.2658	-75.26645	-31.14481	-31.15042	607.1	610.24
60	AGT	-75.2686	-75.26921	-31.16355	-31.168	614.3	616.52
61	EBS	-75.2647	-75.2661	-31.13846	-31.14796	606.52	609.65
63	AGT	-75.0852	-75.08658	-32.21766	-32.21765	609.48	612.28
64	AGT	-75.091	-75.09233	-32.21768	-32.21767	610.62	611.83
65	AGT	-75.0966	-75.09788	-32.21773	-32.21772	615.23	616.16
66	EBS	-75.0886	-75.09142	-32.21791	-32.21797	608.6	610.96
68	AGT	-75.1767	-75.17805	-31.8702	-31.86902	655.78	676.11
69	AGT	-75.1754	-75.1768	-31.87114	-31.86995	654.87	657.46
70	AGT	-75.1743	-75.17568	-31.87206	-31.87083	654.65	691.31
71	EBS	-75.1775	-75.18163	-31.86928	-31.86577	555.62	676.86
75	AGT	-74.37	-74.37177	-28.10797	-28.09996	2052.26	2053.91
76	AGT	-74.3797	-74.38169	-28.06634	-28.05902	2056.14	2058.19
77	AGT	-74.3886	-74.3904	-28.1561	-28.14818	2006.54	2011.16
78	EBS	-74.4047	-74.40649	-28.08486	-28.07692	2019.49	2026.16
80	AGT	-74.5202	-74.51747	-28.75306	-28.75118	1537.72	1545.99
81	AGT	-74.5084	-74.50573	-28.74527	-28.74355	1558.28	1570.08
82	AGT	-74.4962	-74.49309	-28.73726	-28.73518	1580.27	1595.46
83	EBS	-74.4853	-74.4846	-28.77472	-28.78469	1577.88	1588.23
85	AGT	-74.6741	-74.67504	-29.42462	-29.43436	586.74	604.49
86	AGT	-74.6769	-74.67659	-29.45447	-29.45068	573.42	580.99
87	AGT	-74.6767	-74.67648	-29.45172	-29.44921	573.92	583.74
88	AGT	-74.6747	-74.67446	-29.43061	-29.42842	592.71	602.27
89	EBS	-74.6716	-74.6706	-29.39886	-29.38834	639.32	657.44
91	AGT	-74.7067	-74.70542	-29.50822	-29.50656	401.67	410
92	AGT	-74.7013	-74.70085	-29.50091	-29.50021	427.17	428.55
93	AGT	-74.6982	-74.69752	-29.49652	-29.49558	439.76	450.09
94	EBS	-74.6919	-74.68928	-29.48786	-29.4842	476.94	494.03

96	AGT	-74.6252	-74.62676	-29.05155	-29.04293	1018.91	1028.48
97	AGT	-74.6304	-74.63194	-29.0236	-29.01513	985.75	1010.63
98	AGT	-74.6357	-74.63723	-28.99501	-28.98652	941.94	971.14
99	EBS	-74.6341	-74.63571	-29.00812	-28.99958	958.98	986.19
101	AGT	-75.2427	-75.2437	-29.00356	-29.00721	391.66	398.3
102	AGT	-75.246	-75.24708	-29.01541	-29.01895	392.77	396.83
103	AGT	-75.2495	-75.25056	-29.02708	-29.0304	390.17	392.2
104	EBS	-75.2456	-75.24777	-29.01217	-29.01915	393.69	396.84
106	AGT	-75.2389	-75.23971	-27.84859	-27.85297	413.67	415.71
107	AGT	-75.2416	-75.24234	-27.8633	-27.86773	414.23	415.15
108	AGT	-75.244	-75.24481	-27.87707	-27.88155	417.56	424.41
109	EBS	-75.241	-75.24248	-27.86192	-27.87098	413.3	415.71

Appendix 2: Tissue samples taken from holothuroids during JR275

Event #	Final lat-long	Max depth	JR275 IDs
20	77.36S 35.36W	654.4	88,103,106,111,112
21	77.35S 35.34W	652.8	133-135,137-140,143
22	77.35S 35.32W	654.2	150,158
25	76.33S 32.90W	781.7	167-170,184,187,188-190,194
26	76.32S 32.87W	789.2	205,206,210
27	76.32S 32.87W	781.4	228-230
29	76.20S 31.86W	579.0	264,274,281
30	76.19S 31.84W	578.9	300-302,310
31	76.19S 31.82W	573.0	347-349,351
33	76.02S 26.99W	610.0	372,375
34	76.02S 26.97W	613.0	415,419,422
35	76.02S 26.96W	613.0	452-455
38	76.17S 27.80W	561.0	489.491,493,499,502,505,1654
39	76.17S 27.80W	555.3	520,521,523,530,541,566
43	75.76S 30.45W	430.0	611
44	75.77S 30.46W	436.8	629,632
47	75.74S 31.24W	584.9	654,659,660,664,674
48	75.75S 31.25W	590.8	694,702
49	75.75S 31.27W	584.9	744,745,747
52	75.24S 30.25W	419.2	755
53	75.25S 30.25W	417.8	784
54	75.25S 30.26W	419.1	813
55	75.26S 30.27W	418.6	836,837
58	75.26S 31.13W	607.1	870
59	75.27S 31.15W	610.2	892,897-899,902
60	75.27S 31.17W	616.5	906,910,929
63	75.09S 32.22W	612.3	940-946,959,964
64	75.09S 32.22W	611.8	966
68	75.18S 31.87W	676.1	982
69	75.18S 31.87W	657.5	1019,1021

70	75.18S 31.87W	691.3	1027
75	74.37S 28.10W	2053.9	1043,1047,1052
76	74.38S 28.06W	2058.2	1085
77	74.39S 28.15W	2011.2	1125,1127
82	74.49S 28.74W	1595.5	1208,1209
85	74.68S 29.43W	604.5	1236,1623
88	74.67S 29.43W	602.3	1319
91	74.71S 29.51W	410.0	1346
92	74.70S 29.50W	428.6	1364
96	74.63S 29.04W	1028.5	1394,1409
97	74.63S 29.02W	1010.6	1425,1432
98	74.64S 28.99W	971.1	1454,1457
101	75.24S 29.01W	398.3	1492
102	75.25S 29.02W	396.8	1511
103	75.25S 29.03W	392.2	1543
106	75.24S 27.85W	415.7	1564
107	75.24S 27.87W	415.2	1597
108	75.24S 27.88W	424.4	1609

Appendix 3: Ophiuroidea sampled for DNA bar-coding during the JR275 cruise.

Date	Station	Event	Trawl	Depth	Tissue sampled ophiuroids
11/02/2012	South Orkney Shelf	8	AGT	281 m	28 x *Ophiacantha* spp. 24 x *Ophioplinthus* spp. 9 x *Ophiura* spp. 7 x *Ophiochondrus* spp. 5 x *Amphiura*-type spp. 3 x *Astrohamma*-type spp. 2 x *Ophiomitrella* sp. 1 x Unknown ophiuroid sp.
13/02/2012	South Orkney Shelf	11	RD	993 m	2 x *Amphiura*-type spp. 1 x *Ophioleuce* sp.
19/02/2012	Southern Weddell	20	AGT	654 m	2 x *Ophiocten* spp. 1 x *Ophioleuce* sp. 1 x Unknown ophiuroid sp.
19/02/2012	Southern Weddell	21	AGT	654 m	1 x *Ophiacantha* sp.
19/02/2012	Southern Weddell	22	AGT	654 m	6 x *Ophiocten* spp. 1 x *Ophiacantha* sp.
20/02/2012	Mid East Filchner Trough Edge	26	AGT	789 m	17 x *Ophiocten* spp. 11 x Unknown ophiuroid spp. 3 x *Ophiacantha* spp. 2 x *Ophioplinthus* spp. 1 x *Amphiura*-type sp. 1 x *Ophioleuce* sp. 1 x *Ophionotus victoriae*
20/02/2012	Mid East Filchner Trough Edge	27	AGT	781 m	11 x *Ophiocten* spp. 5 x Unknown ophiuroid spp. 2 x *Ophioplinthus* spp. 4 x *Ophioleuce* spp. 3 x *Amphiura*-type spp. 1 x *Ophiacantha* sp.
20/02/2012	Mid East Filchner Trough Edge	29	AGT	579 m	8 x *Ophiacantha* spp. 4 x *Amphiura*-type spp.

					3 x *Ophiocten* spp. 2 x *Ophioleuce* spp. 2 x *Ophiosteira* spp. 1 x *Ophionotus victoriae* 1 x *Ophioperla* sp. 1 x *Ophioplinthus* sp. 1 x Unknown ophiuroid sp.
20/02/2012	Mid East Filchner Trough Edge	30	AGT	579 m	9 x *Ophiacantha* spp. 7 x *Ophiocten* spp. 2 x *Amphiura*-type spp. 1 x *Ophioperla* sp. 1 x *Ophioplinthus* sp.
20/02/2012	Mid East Filchner Trough Edge	31	AGT	573 m	4 x *Amphiura*-type spp. 3 x *Ophiacantha* spp. 2 x *Ophioperla* spp. 1 x *Ophioplinthus* sp.
22/02/2012	Mid-West Brunt Shelf	43	AGT	430 m	1 x *Ophiochondrus* sp.
22/02/2012	Mid-West Brunt Shelf 2	47	AGT	585 m	7 x *Ophiocten* spp. 2 x *Ophiosteira* spp. 1 x *Amphiura*-type sp.
22/02/2012	Mid-West Brunt Shelf 2	49	AGT	585 m	2 x *Ophioperla* spp. 2 x *Ophiacantha* spp. 2 x Unknown ophiuroid spp. 1 x *Ophioleuce* sp. 1 x *Amphiura*-type sp.
23/02/2012	West Brunt Shelf	53	AGT	418 m	8 x *Ophiacantha* spp. 3 x *Ophiocten* spp. 1 x *Amphiura*-type sp. 1 x *Ophioplinthus* sp.
23/02/2012	West Brunt Shelf 2	60	AGT	616 m	18 x *Ophiocten* spp. 14 x *Ophiacantha* spp. 10 x *Amphiura*-type spp. 7 x *Ophioleuce* spp. 3 x *Ophioplinthus* spp.

					3 x Unknown ophiuroid spp. 1 x *Ophioperla* sp.
24/02/2012	North-West Brunt Shelf	63	AGT	612 m	4 x *Ophiacantha* spp. 3 x *Ophioplinthus* spp. 1 x *Ophiocten* sp. 1 x *Ophiosteira* sp. 1 x *Ophioperla* sp.
24/02/2012	North-West Brunt Shelf	64	AGT	612 m	7 x *Ophiacantha* spp. 1 x *Ophioplinthus* sp.
24/02/2012	North-West Brunt Shelf	65	AGT	616 m	1 x *Ophioplinthus* sp. 1 x *Ophiocten* sp.
24/02/2012	Shelf Scour	68	AGT	676 m	2 x Ophiocten spp. 1 x Amphiura-type sp.
24/02/2012	Shelf Scour	69	AGT	657 m	1 x *Ophioperl*a sp. 1 x *Amphiura*-type sp. 1 x *Ophiocten* sp.
24/02/2012	Shelf Scour	70	AGT	691 m	2 x *Ophiocten* spp. 3 x *Ophiacantha* spp. 1 x *Ophioplinthus* sp.
26/02/2012	2000m Station	75	AGT	2053 m	7 x *Ophioplinthus* spp. 4 x *Ophiacantha* spp. 2 x *Amphiura*-type spp. 2 x Unknown ophiuroid spp.
26/02/2012	2000m Station	76	AGT	2058 m	9 x *Ophiacantha* spp. 6 x *Amphiura*-type spp.
26/02/2012	2000m Station	77	AGT	2011 m	3 x *Ophiacantha* spp. 3 x *Amphiura*-type spp. 1 x *Ophioplinthus* sp. 4 x Unknown ophiuroid spp.
28/02/2012	1500m Station	80	AGT	1546 m	1 x *Ophionotus victoriae*
28/02/2012	1500m Station	81	AGT	1570 m	1 x *Ophioplinthus* sp. 1 x *Ophiacantha* sp. 1 x *Ophiocten* sp. 1 x Unknown ophiuroid sp.

29/02/2012	600m Station	89	AGT	657 m	2 x *Ophiacantha* spp. 1 x *Ophiura* sp.
29/02/2012	400m Station	91	AGT	410 m	1 x *Ophioplinthus* sp.

Appendix 4: Holothuroidea sampled for DNA bar-coding during the JR275 cruise.

Date	Station	Event	Trawl	Depth	Tissue sampled holothuroids
11/02/2012	South Orkney Shelf	8	AGT	281 m	8 x holothuroidea spp.
19/02/2012	Southern Weddell	20	AGT	654 m	5 x holothuroidea spp.
19/02/2012	Southern Weddell	21	AGT	654 m	8 x holothuroidea spp.
19/02/2012	Southern Weddell	22	AGT	654 m	2 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	25	AGT	782 m	10 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	26	AGT	789 m	3 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	27	AGT	781 m	3 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	29	AGT	579 m	3 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	30	AGT	579 m	5 x holothuroidea spp.
20/02/2012	Mid East Filchner Trough Edge	31	AGT	573 m	4 x holothuroidea spp.
21/02/2012	Shelf Canyon	33	AGT	610 m	2 x holothuroidea spp.
21/02/2012	Shelf Canyon	34	AGT	613 m	3 x holothuroidea spp.
21/02/2012	Shelf Canyon	35	AGT	613 m	4 x holothuroidea spp.
21/02/2012	Shelf Canyon 2	38	AGT	561 m	25 x holothuroidea spp.
21/02/2012	Shelf Canyon 2	39	AGT	555 m	11 x holothuroidea spp.
22/02/2012	Mid-West Brunt Shelf	42	AGT	434 m	6 x holothuroidea spp.
22/02/2012	Mid-West Brunt Shelf	43	AGT	430 m	1 x holothuroidea sp.
22/02/2012	Mid-West Brunt Shelf	44	AGT	437 m	2 x holothuroidea spp.
22/02/2012	Mid-West Brunt Shelf 2	47	AGT	585 m	5 x holothuroidea spp.
22/02/2012	Mid-West Brunt Shelf 2	48	AGT	591 m	2 x holothuroidea spp.
22/02/2012	Mid-West Brunt Shelf 2	49	AGT	585 m	4 x holothuroidea spp.
23/02/2012	West Brunt Shelf	52	AGT	419 m	1 x holothuroidea sp.
23/02/2012	West Brunt Shelf	53	AGT	418 m	1 x holothuroidea sp.
23/02/2012	West Brunt Shelf	54	AGT	419 m	1 x holothuroidea spp.

23/02/2012	West Brunt Shelf	55	AGT	419 m	2 x holothuroidea spp.
23/02/2012	West Brunt Shelf 2	58	AGT	607 m	1 x holothuroidea sp.
23/02/2012	West Brunt Shelf 2	59	AGT	610 m	5 x holothuroidea spp.
23/02/2012	West Brunt Shelf 2	60	AGT	616 m	3 x holothuroidea spp.
24/02/2012	North-West Brunt Shelf	63	AGT	612 m	9 x holothuroidea spp.
24/02/2012	North-West Brunt Shelf	64	AGT	612 m	1 x holothuroidea sp.
24/02/2012	Shelf Scour	68	AGT	676 m	1 x holothuroidea sp.
24/02/2012	Shelf Scour	69	AGT	657 m	2 x holothuroidea spp.
24/02/2012	Shelf Scour	70	AGT	691 m	1 x holothuroidea sp.
26/02/2012	2000m Station	75	AGT	2053 m	3 x holothuroidea spp.
26/02/2012	2000m Station	76	AGT	2058 m	1 x holothuroidea sp.
26/02/2012	2000m Station	77	AGT	2011 m	2 x holothuroidea spp.
28/02/2012	1500m Station	82	AGT	1595 m	2 x holothuroidea spp.
29/02/2012	600m Station	85	AGT	604 m	2 x holothuroidea spp.
29/02/2012	600m Station	88	AGT	602 m	1 x holothuroidea sp.
29/02/2012	400m Station	91	AGT	410 m	6 x holothuroidea spp.
29/02/2012	400m Station	92	AGT	428 m	5 x holothuroidea spp.
01/03/2012	1000m Station	96	AGT	1028 m	2 x holothuroidea spp.
01/03/2012	1000m Station	97	AGT	1011 m	3 x holothuroidea spp.
01/03/2012	1000m Station	98	AGT	971 m	3 x holothuroidea spp.
04/03/2012	Halley Transect 1	101	AGT	398 m	2 x holothuroidea spp.
04/03/2012	Halley Transect 1	102	AGT	397 m	3 x holothuroidea spp.
04/03/2012	Halley Transect 1	103	AGT	392 m	2 x holothuroidea spp.
04/03/2012	Halley Transect 2	106	AGT	416 m	4 x holothuroidea spp.
04/03/2012	Halley Transect 2	107	AGT	415 m	2 x holothuroidea spp.
04/03/2012	Halley Transect 2	108	AGT	424 m	3 x holothuroidea spp.

Appendix 5: Asteroidea sampled for DNA bar-coding during the JR275 cruise.

Date	Station	Event	Trawl	Depth	Tissue sampled asteroids

11/02/2012	South Orkney Shelf	8	AGT	281 m	7 x asteroidea spp.
19/02/2012	Southern Weddell	20	AGT	654 m	1 x asteroidea sp.
19/02/2012	Southern Weddell	21	AGT	654 m	4 x asteroidea spp.
20/02/2012	Mid East Filchner Trough Edge	25	AGT	782 m	9 x asteroidea spp.
20/02/2012	Mid East Filchner Trough Edge	26	AGT	789 m	2 x asteroidea spp.
20/02/2012	Mid East Filchner Trough Edge	27	AGT	781 m	5 x asteroidea spp.
20/02/2012	Mid East Filchner Trough Edge	29	AGT	579 m	6 x asteroidea spp.
20/02/2012	Mid East Filchner Trough Edge	30	AGT	579 m	2 x asteroidea spp.
21/02/2012	Shelf Canyon	33	AGT	610 m	4 x asteroidea spp.
21/02/2012	Shelf Canyon	34	AGT	613 m	2 x asteroidea spp.
21/02/2012	Shelf Canyon	35	AGT	613 m	1 x asteroidea sp.
21/02/2012	Shelf Canyon 2	39	AGT	555 m	15 x asteroidea spp.
22/02/2012	Mid-West Brunt Shelf	43	AGT	430 m	4 x asteroidea spp.
22/02/2012	Mid-West Brunt Shelf	44	AGT	437 m	2 x asteroidea spp.
22/02/2012	Mid-West Brunt Shelf 2	47	AGT	585 m	1 x asteroidea sp.
22/02/2012	Mid-West Brunt Shelf 2	48	AGT	591 m	6 x asteroidea spp.
22/02/2012	Mid-West Brunt Shelf 2	49	AGT	585 m	2 x asteroidea spp.
23/02/2012	West Brunt Shelf	52	AGT	419 m	5 x asteroidea spp.
23/02/2012	West Brunt Shelf	54	AGT	419 m	2 x asteroidea spp.
23/02/2012	West Brunt Shelf	55	AGT	419 m	4 x asteroidea spp.
23/02/2012	West Brunt Shelf 2	59	AGT	610 m	4 x asteroidea spp.
23/02/2012	West Brunt Shelf 2	60	AGT	616 m	6 x asteroidea spp.
24/02/2012	North-West Brunt Shelf	63	AGT	612 m	1 x asteroidea sp.
24/02/2012	Shelf Scour	69	AGT	657 m	3 x asteroidea spp.
24/02/2012	Shelf Scour	70	AGT	691 m	1 x asteroidea sp.
26/02/2012	2000m Station	75	AGT	2053 m	14 x asteroidea spp.
26/02/2012	2000m Station	77	AGT	2011 m	1 x asteroidea sp.
28/02/2012	1500m Station	82	AGT	1595 m	2 x asteroidea spp.
29/02/2012	600m Station	85	AGT	604 m	22 x asteroidea spp.
29/02/2012	600m Station	86	AGT	581 m	3 x asteroidea spp.
29/02/2012	600m Station	88	AGT	602 m	2 x asteroidea spp.
29/02/2012	400m Station	91	AGT	410 m	6 x asteroidea spp.
29/02/2012	400m Station	92	AGT	428 m	7 x asteroidea spp.
29/02/2012	400m Station	93	AGT	450 m	7 x asteroidea spp.

01/03/2012	1000m Station	96	AGT	1028 m	6 x asteroidea spp.
01/03/2012	1000m Station	98	AGT	971 m	1 x asteroidea sp.
04/03/2012	Halley Transect 1	101	AGT	398 m	2 x asteroidea spp.
04/03/2012	Halley Transect 1	103	AGT	392 m	3 x asteroidea spp.
04/03/2012	Halley Transect 2	106	AGT	416 m	9 x asteroidea spp.
04/03/2012	Halley Transect 2	108	AGT	424 m	6 x asteroidea spp.

Appendix 6: Preliminary identifications of Holothuroids from JR275

lot	Count	Order	Family	Genus	Species	Author	Trawl
27	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	8
28	1	Dendrochirotida	Cucumariidae				8
29	1	Dendrochirotida	Cucumariidae				8
30	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	8
31	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	8
32	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	8
33	1	Dendrochirotida	Cucumariidae	*Parathyonidium*	*incertum*	Heding, 1954	8
34	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	8
88	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	20
103	41	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	20
106	1	Elasipodida	Elpidiidae				20
111	1	unidentified					20
112	1	unidentified					20
133	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	21
134	1	Elasipodida	Elpidiidae				21
135	1	Dendrochirotida	Psolidae				21
136	1	Dendrochirotida	Psolidae	*Psolidium*			21
137	1	Dendrochirotida	Cucumariidae	*Trachythyone*	*bouvetensis*	(Ludwig & Heding, 1935)	21
138	1	Dendrochirotida	Cucumariidae				21
139	1	Elasipodida	Elpidiidae				21
140	1	Elasipodida	Elpidiidae				21
143	180	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	21
143.1	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	21
144.1	3	Elasipodida	Elpidiidae				21
144.2	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	21
150	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	22
152	1	Elasipodida	Elpidiidae				22
158	7	unidentified					22
167	1	unidentified					25
168	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	25
169	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		25
170.1	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		25
184	2	Dendrochirotida	Cucumariidae	*Trachythyone*	*bouvetensis*	(Ludwig & Heding, 1935)	25
184.1	1	Dendrochirotida	Cucumariidae	*Trachythyone*	*bouvetensis*	(Ludwig & Heding, 1935)	25
187	1	unidentified					25

188	1	unidentified					25
189	1	unidentified					25
190.1	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		25
193	2	Dendrochirotida					25
194.1	1	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	25
195	1	unidentified					25
198	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	25
204	4	Aspidochirotida					26
205	1	Aspidochirotida	Synallactidae	*Bathyplotes*			26
206	1	Elasipodida	Elpididae				26
207	1	Elasipodida	Laetmogonidae	Laetmogone	wyvillethomsoni	Theel, 1879	26
208	1	unidentified					26
209	1	Dendrochirotida	Cucumariidae	*Trachythyone*	*bouvetensis*	(Ludwig & Heding, 1935)	26
210	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		26
228	2	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	27
229	2	unidentified					27
230	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	27
231	1	Dendrochirotida					27
232	1	Elasipodida	Elpidiidae				27
234	1	Elasipodida	Elpidiidae				27
258	21	Dendrochirotida	Psolidae				29
264	1	unidentified					29
274.1	1	Elasipodida	Elpidiidae				29
277	2	Dendrochirotida	Cucumariidae				29
281	2	Elasipodida	Elpidiidae				29
281.1	1	Elasipodida	Elpidiidae				29
282.1	1	Aspidochirotida					29
282.2	1	Elasipodida	Elpidiidae				29
282.3	1	Elasipodida	Elpidiidae				29
285	1	Dendrochirotida	Cucumariidae				29
286	1	Dendrochirotida	Cucumariidae				29
299.1	2	Dendrochirotida	Psolidae				30
299.2	29	Dendrochirotida	Psolidae				30
299.3	1	Dendrochirotida	Psolidae				30
300	1	Elasipodida	Elpididae				30
301	3	Elasipodida	Elpidiidae				30
302.1	1	Elasipodida	Elpidiidae				30
302.2	1	Elasipodida	Elpididae				30
303	1	Dendrochirotida	Cucumariidae				30
310	1	unidentified					30
310.1	1	unidentified					30
311	2	unidentified					30
315	1	Dendrochirotida	Cucumariidae				30
316	1	Dendrochirotida	Cucumariidae				30
347	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	31
348	1	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	31
349	1	unidentified					31
350	1	Aspidochirotida	Synallactidae	*Pseudostichopus*			31
351	1	Elasipodida	Elpidiidae				31
352	1	Dendrochirotida	Cucumariidae				31
357	3	Dendrochirotida	Psolidae				31
364	1	Elasipodida	Elpidiidae				31
372	3	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	33

372.1	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	33
373	1	Dendrochirotida	Cucumariidae				33
374	1	unidentified					33
375	62	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	33
375.1	1	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	33
414	2	unidentified					34
415	39	unidentified mixed lot					34
419	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	34
419.1	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	34
420	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	34
421	1	unidentified					34
422	1	Dendrochirotida	Cucumariidae	*Trachythyone*			34
452	45	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	35
453	1	unidentified					35
454	1	unidentified					35
455	1	Dendrochirotida	Cucumariidae				35
484	3	Apodida	Chirdotidae	*Sigmodota*	*magnibacula*	(Massin & Heterier, 2004)	38
489	1	Apodida					38
489.1	1	Apodida					38
490	1	unidentified					38
491	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	38
492	1	Dendrochirotida					38
493	1	Dendrochirotida	Cucumariidae				38
499	2	unidentified					38
502.1	1	Dendrochirotida					38
502.2	1	Dendrochirotida					38
502.3	1	Dendrochirotida					38
502.4	1	Dendrochirotida					38
502.5	1	Dendrochirotida					38
502.6	1	Dendrochirotida					38
502.7	1	Dendrochirotida					38
502.8	1	Dendrochirotida					38
502.9	1	Dendrochirotida					38
502.10	1	Dendrochirotida					38
502.11	1	Dendrochirotida					38
502.12	1	Dendrochirotida					38
502.13	1	Dendrochirotida					38
502.14	1	Dendrochirotida					38
502.15	1	Dendrochirotida					38
502.16	1	Dendrochirotida					38
502.17	1	Dendrochirotida					38
502.18	1	Dendrochirotida					38
502.19	1	Dendrochirotida					38
505	4	Dendrochirotida	Psolidae				38
506	1	Dendrochirotida					38
507	1	Dendrochirotida					38
508	1	unidentified					38
520	1	unidentified					39
521	3	unidentified					39
522	3	Dendrochirotida	Cucumariidae				39
523	1	unidentified					39
524	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	39
525	2	Dendrochirotida	Psolidae				39

530	2	unidentified					39
541.1	1	Dendrochirotida	Cucumariidae				39
541.1a	1	Dendrochirotida	Cucumariidae				39
541.2	1	Dendrochirotida	Cucumariidae				39
541.3	1	Apodida					39
541.4	1	Dendrochirotida	Cucumariidae				39
541.5	1	Dendrochirotida	Cucumariidae	*Trachythyone*			39
541.6	2	Dendrochirotida	Cucumariidae				39
541.7	1	Dendrochirotida	Cucumariidae				39
566.1	1	Aspidochirotida	Synallactidae	*Pseudostichopus*			39
566.2	3	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	39
566.2a	1	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	39
566.3	1	Aspidochirotida					39
566.4	1	Dendrochirotida	Psolidae				39
566.4a	1	Dendrochirotida	Psolidae				39
566.5	1	Dendrochirotida					39
566.6	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	39
611	4	unidentified					43
612	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	43
613	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	43
629	1	unidentified					44
632	2	unidentified					44
633	1	Dendrochirotida					44
653	1	Dendrochirotida	Cucumariidae	*Trachythyone*			47
654	3	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	47
659	1	unidentified					47
660	1	unidentified					47
664	168	Elasipodida	Elpidiidae	*Rhipidothuria*	*racowitzi*	Herouard, 1901	47
674	4	unidentified					47
693	5	Dendrochirotida	Psolidae				48
694	8	unidentified					48
702	1	unidentified					48
707	1	Dendrochirotida	Cucumariidae				48
709	1	Dendrochirotida	Cucumariidae	*Trachythyone*			48
710	1	Aspidochirotida	Synallactidae	*Bathyplotes*			48
711	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	48
744	15	unidentified mixed					49
745	1	unidentified					49
746	1	Dendrochirotida	Psolidae				49
747	1	unidentified					49
755	1	unidentified					52
758	1	Aspidochirotida	Synallactidae	*Pseudostichopus*			52
762.1	1	Elasipodida	Elpidiidae				52
762.2	3	Dendrochirotida					52
762.3	1	Dendrochirotida	Cucumariidae				52
762.4	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	52
762.5	1	Dendrochirotida					52
784	3	unidentified					53
811	2	Dendrochirotida	Psolidae				54
813	3	unidentified					54
836	1	unidentified					55
837	2	unidentified					55
846	1	unidentified					55

853	3	Dendrochirotida	Psolidae				58
857	4	Aspidochirotida					58
859	4	Dendrochirotida	Cucumariidae				58
863	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	58
864	2	Aspidochirotida	Synallactidae	*Bathyplotes*			58
868	1	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	58
869	1	Dendrochirotida	Cucumariidae	*Trachythyone*			58
870	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	58
892	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	59
897	9	Apodida					59
897.1	1	Apodida					59
898	1	Dendrochirotida	Cucumariidae				59
898.1	1	Dendrochirotida	Cucumariidae				59
899	1	unidentified					59
901	1	Dendrochirotida	Psolidae				59
902	1	unidentified					59
903	1	Dendrochirotida	Psolidae				59
906	2	unidentified					60
910	2	unidentified					60
912	1	Dendrochirotida	Cucumariidae	*Trachythyone*			60
915	1	unidentified					60
920	1	Dendrochirotida	Cucumariidae				60
921	1	Dendrochirotida					60
927	1	unidentified					60
928	1	Aspidochirotida	Synallactidae	*Bathyplotes*			60
929	5	Apodida					60
929.1	1	Apodida					60
940	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
941	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
942	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
943	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
944	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
945	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
946	1	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	63
959	1	unidentified					63
962	10	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	63
964	1	Elasipodida	Elpidiidae				63
965	1	Dendrochirotida	Psolidae				63
966	1	Apodida					64
982	1	unidentified					68
983	1	Dendrochirotida	Psolidae				68
985	3	Elasipodida	Elpidiidae				68
986	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	68
1019	6	Aspidochirotida					69
1021	1	unidentified					69
1027	2	Elasipodida	Elpidiidae	*Protelpidia*	*murrayi*	(Theel, 1879)	70
1040	1	Dendrochirotida	Psoliidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield, 2010)	75
1041	2	Dendrochirotida	Psoliidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield, 2010)	75
1043	1	Dendrochirotida	Psolidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield, 2010)	75
1044	4	unidentified					75
1047	4	unidentified					75
1048	1	Dendrochirotida	Psoliidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield,	75

						2010)	
1049	1	unidentified					75
1050	1	Elasipodida	Elpidiidae				75
1051	1	unidentified					75
1052	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	75
1057	1	unidentified					75
1058	1	unidentified					75
1084	3	Dendrochirotida	Psoliidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield, 2010)	76
1085	1	Elasipodida	Elpidiidae				76
1122	6	Dendrochirotida	Psoliidae	*Psolus*	*lockhartae*	(O'Loughlin & Whitfield, 2010)	77
1123	2	Elasipodida					77
1125	2	Aspidochirotida	Synallactidae	*Pseudostichopus*	*spiculiferous*	(O'Loughlin, 2005)	77
1127	1	Elasipodida					77
1127.1	1	Elasipodida					77
1131	1	unidentified					77
1180	3	Elasipodida	Elpidiidae				81
1187	1	Aspidochirotida	Synallactidae	*Bathyplotes*			81
1208	1	unidentified					82
1209	1	unidentified					82
1236	6	Dendrochirotida	Psolidae	*Echinopsolus*			85
1237	10	Dendrochirotida	Psolidae	*Echinopsolus*			85
1253	91	unidentified mixed lot					85
1292.1	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	86
1292.2	1	Dendrochirotida	Cucumariidae				86
1292.3	1	Dendrochirotida	Cucumariidae				86
1292.4	1	Dendrochirotida	Cucumariidae				86
1292.5	1	Dendrochirotida	Cucumariidae				86
1292.6	1	Dendrochirotida	Psolidae				86
1298	1	Dendrochirotida					86
1319	12	Dendrochirotida	Psolidae	*Echinopsolus*			88
1319.1	1	Dendrochirotida	Psolidae	*Echinopsolus*			88
1320.1	4	Dendrochirotida					88
1320.2	1	Dendrochirotida	Cucumariidae	*Heterocucumis*	*steineni*	(Ludwig, 1898)	88
1320.3	4	Dendrochirotida	Psolidae				88
1320.4	1	Dendrochirotida					88
1320.5	3	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	88
1320.6	1	Dendrochirotida					88
1346.1	1	Dendrochirotida	Cucumariidae				91
1346.2	1	Dendrochirotida					91
1346.3	2	Dendrochirotida	Cucumariidae				91
1346.3a	1	Dendrochirotida	Cucumariidae				91
1346.4	1	Dendrochirotida	Psolidae				91
1346.4a	1	Dendrochirotida	Psolidae				91
1346.5	1	Dendrochirotida	Cucumariidae	*Psolicrux*			91
1346.5a	1	Dendrochirotida	Cucumariidae	*Psolicrux*			91
1346.6	1	Dendrochirotida					91
1364.1	2	Dendrochirotida	Psolidae				92
1364.2	1	Dendrochirotida	Cucumariidae				92
1364.3	1	Dendrochirotida	Psolidae	*Psolidium*			92
1364.4	2	Dendrochirotida					92
1364.4a	1	Dendrochirotida					92

1364.5	8	Dendrochirotida					92
1364.5a	1	Dendrochirotida					92
1374.1	5	Dendrochirotida					93
1374.2	1	Dendrochirotida					93
1374.3	1	unknown					93
1394	6	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	96
1394.1	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	96
1397	5	Dendrochirotida	Psolidae				96
1400	1	Dendrochirotida	Psolidae				96
1401	1	Dendrochirotida	Psolidae				96
1409	1	Dendrochirotida	Cucumariidae				96
1425	2	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	97
1425.1	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	97
1425.2	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		97
1429	1	Dendrochirotida	Cucumariidae				97
1430	1	Aspidochirotida					97
1431	1	Dendrochirotida	Psolidae				97
1432	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	97
1454.1	2	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	98
1454.1a	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*gourdoni*	(Vaney, 1914)	98
1454.2	1	Aspidochirotida	Synallactidae	*Bathyplotes*	sp.		98
1457	1	Dendrochirotida	Cucumariidae				98
1458	1	Dendrochirotida	Psolidae				98
1459	1	Dendrochirotida	Psolidae				98
1492.1	1	Elasipodida	Elpididae				101
1492.2	1	Dendrochirotida					101
1510	1	Aspidochirotida	Synallactidae	*Bathyplotes*	*cf. bongraini*	(Vaney, 1914)	102
1511.1	1	Aspidochirotida					102
1511.2	4	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	102
1511.3	1	Dendrochirotida	Psolidae	*Psolus*	sp.		102
1543	3	unidentified					103
1564.1	2	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	106
1564.1a	1	Elasipodida	Elpididae	*Peniagone*	*vignoni*	Herouard, 1901	106
1564.2	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	sp.	(O'Loughlin, 2005)	106
1564.3	1	Aspidochirotida	Synallactidae	*Pseudostichopus*	sp.	(O'Loughlin, 2005)	106
1564.4	1	Aspidochirotida					106
1568	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	106
1597.1	1	unknown					107
1597.2	1	Elasipodida	Elpidiidae				107
1609.1	2	Elasipodida	Elpidiidae				108
1609.1a	1	Elasipodida	Elpidiidae				108
1609.2	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	108
1609.3	1	Aspidochirotida					108
1623	1	Dendrochirotida	Psolidae				85
1638	1	Dendrochirotida	Cucumariidae	*Staurocucumis*	*liouvillei*	(Vaney, 1914)	60
1654	1	Dendrochirotida	Cucumariidae	*Psolidiella*	*c.f. mollis*		38

Appendix 7: CTD locations and details.

Deployment Number	Gear Type	Date	Start Time	Start Latitude	Start Longitude	Start Depth
7	CTD	11/02/2012	11:06:00	-60.6766	-44.00929	300
19	CTD	19/02/2012	15:41:00	-77.3608	-35.37846	650
24	CTD	20/02/2012	11:26:00	-76.3317	-32.9046	780
28	CTD	20/02/2012	17:37:00	-76.2003	-31.86614	587
32	CTD	21/02/2012	11:15:00	-76.0245	-27.00203	602
37	CTD	21/02/2012	19:23:00	-76.1713	-27.79085	562
41	CTD	22/02/2012	11:05:00	-75.76	-30.43226	429
46	CTD	22/02/2012	16:39:00	-75.7389	-31.23664	580
51	CTD	23/02/2012	11:03:00	-75.2417	-30.24278	419
57	CTD	23/02/2012	17:30:00	-75.262	-31.11879	601
62	CTD	24/02/2012	11:06:00	-75.0831	-32.21813	612
67	CTD	24/02/2012	17:24:00	-75.1747	-31.87152	654
74	CTD	26/02/2012	10:01:00	-74.38	-28.17627	2014
79	CTD	28/02/2012	11:16:00	-74.5251	-28.75628	1531
84	CTD	29/02/2012	11:08:00	-74.6733	-29.4173	612
90	CTD	29/02/2012	17:54:00	-74.7084	-29.51068	394
95	CTD	01/03/2012	11:02:00	-74.6233	-29.06073	1028
100	CTD	04/03/2012	10:56:00	-75.2417	-28.99967	398

Appendix 8: Oxygen Protocol.

Following the procedure laid out in “Determination of dissolved oxygen in sea water by Winkler titration” version 1.01 section 6.2.4-6.2.9.

Step 1 (Prep):

Check Tygon tube and bottles for defects before sampling. Ensure bottle stopper number matches correct bottle. Write in the sampling log the relevant bottle information for each depth (bottom, middle and top).

Step 2 (CTD):

Once CTD is ready check the Niskins; if one has incorrectly fired use the backup Niskin for sampling. The Tygon tube is to be pinched to allow a steady and slow outflow of water, with no air bubbles.

Step 3 (Sampling):

Fill the sample bottle. This is done by placing the tubing to the side and base of the sample bottle. Counting the time it takes to fill the bottle. Slowly rotate the bottle/tube to allow water to flow upwards over the entire inside breadth of the bottle for 3x the time it took to fill the bottle. This ensures any traces of air attached to the sides are removed. Visibly check for air bubbles and repeat. Finally with the tube placed in the centre of the bottle at the base hold steady and allow the water to overflow for a count of 3x time taken to fill bottle and remove tubing very slowly. Wet the stopper. Record the water temperature.

Step 4 (Fixing):

The reagents manganese chloride ($MnCl_2$) and Sodium Iodide (NaI) are added immediately after the sample is drawn to fix the oxygen. 1 mL of $MnCl_2$ is added by submerging the dispenser tip under the surface to ensure no additional air is added to the sample. 1 mL of NaI is added in the same method.

Step 5 (Mixing):

The wet stopper is then placed securely on the sample and it is vigorously shaken and inverted to ensure thorough mixing of reagents and substrate. A seal of seawater is placed around the collar of the bottle and stopper to provide a further airtight barrier. Repeat after 20 minutes.

Step 6 (Storage):

Store flasks in cool, dark location for 24 hours before titrating.