





Characterising pelagic biodiversity at South Georgia through novel sampling methods

6 Month Field and Laboratory Report

January 2024

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Quick Summary

Fieldwork has now been underway for six months with regular patrols taking place on the *MV Pharos SG* on a near monthly basis, from which a suite of equipment is deployed collecting samples and data. The Underwater Vision Profiler (UVP6) has been collecting numerous images and classification through EcoTaxa is ongoing. Alterations were made to the UVP6 settings between the October and November patrol which resulted in a dramatic increase in the number of images being captured. Zooplankton nets have been operating well and samples have been collected and preserved in ethanol for transportation via the RRS Sir David Attenborough in spring 2024. Water has been collected from 20 m and 75 m using Niskin bottles before being filtered for eDNA analysis. Some technical issues arose with the filtration process resulting in smaller volumes of water being filtered than anticipated. Therefore, work is ongoing to improve filtration techniques and allow greater volumes to be filtered, and a series of modifications have been made to date. Historical zooplankton samples have been scanned using the ZooScan with six of these samples being sent to the Marine Biological Association for taxonomic identification. Further taxonomy, scanning and eDNA extraction are planned for summer 2024 when samples are back in the UK.

Project Introduction

The Characterising Pelagic Biodiversity at South Georgia through Novel Sampling Methods project (referred to as the SG Pelagic Biodiversity project) aims to assess the zooplankton and ichthyoplankton communities around South Georgia (SG) through a variety of novel methods. The zooplankton community of SG is a critical component of the Scotia Sea ecosystem, connecting primary producers, supporting globally important populations of higher predators, and sustaining valuable commercial fisheries. Zooplankton and ichthyoplankton are also key bioindicators of environmental change since they are small, have short life-cycles and respond rapidly to environmental changes. Establishing a pelagic biodiversity baseline at SG is crucial to assess the stability of this system and its response to climate-related changes and species invasions. Whilst routine sampling of the plankton is regularly carried out, analysis is currently limited to a small range of taxa such as euphausiids, fish and *Themisto* spp. Detailed monitoring of a wider range of taxa, particularly the extremely abundant copepods, is vital if we are to understand and monitor changes to the ecosystem. However, achieving this through net sampling alone is costly and time-consuming, and it can miss important fractions of the plankton community, particularly gelatinous taxa that can be easily damaged in net retrievals.

This project uses three techniques simultaneously to characterise the plankton community: i) image analysis of historical and newly collected samples; ii) water sampling for eDNA analysis; and iii) traditional net based sampling to validate the previous techniques and maintain consistency with previous sample collection.

Fieldwork

Overview

To build a year-round baseline for the plankton around SG, field campaigns are taking place approximately every 4-6 weeks from July 2023 to June 2024, coinciding with patrols carried out by the MV Pharos SG (Table 1). During these campaigns a suite of equipment is deployed. This is made up of zooplankton nets (a 1 m rectangular mid-water trawl (RMT1) and miniBongo), an underwater visual profiler (UVP6), Niskin bottles to collect water for molecular analysis, and a conductivity-temperature-depth profiler (CTD) to collect contextual environmental data. Data generated by all approaches will be used to develop indices of plankton biodiversity, enabling comparisons of the methods and their applications in understanding and monitoring zooplankton communities.

To initiate the SG Pelagic Biodiversity project fieldwork Cecilia Liszka and Vicky Fowler joined King Edward Point (KEP) scientists, Carrie Gunn and George Perry, on the *MV Pharos SG* to test and deploy the UVP6, miniBongo net and Niskin bottles. In addition to this, and as part of another Darwin+ project and ongoing long-term monitoring RMT1 nets and CTDs were deployed. Water filtration was carried out for eDNA analysis and protocols were written and adapted during this initial research cruise. Further details of this trip can be found in the cruise report (https://www.bas.ac.uk/project/south-georgia-pelagic-biodiversity/#data). Since this initial research cruise, KEP scientists have undertaken monthly trips to Cumberland Bay (CBE) and bi-monthly cruises to the Eastern Core Box (ECB) on the *MV Pharos SG*. Where possible deployments have also been made within the Western Core Box (WCB) to the northeast of SG. Although a few issues have arisen (detailed below) fieldwork has, thus far, been highly successful with project equipment being successfully deployed at all sampled stations throughout the six months (see Appendix 1 for event logs detailing deployments made between July and December 2023).

There have been a few alterations made to protocols in response to issues that have arisen during the project. These are detailed for each piece of equipment or process in the relevant sections below. However, in October 2023 Avian Influenza was reported in the SG area. It was unclear whether this would impact the October patrol or future fieldwork due to the *MV Pharos SG* and KEP scientists being involved in the response to the outbreak. Despite this, the KEP scientists and *MV Pharos SG* crew were able to continue sampling for the SG Pelagic Biodiversity project after a slight delay resulting in no impact upon the project fieldwork.

Table 1: SG Pelagic Biodiversity sampling dates and locations for July 2023- December 202	Table 1: SG Pelaaic Biodive	sitv samplina dates a	nd locations for Jul	v 2023- December 2023
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	Sampling										
Pharos Patrol	Start Date	End Date	Locations								
July 23	15/07/2023	22/07/2023	CBE, ECB, WCB								
August 23	11/08/2023		CBE								
September 23	08/09/2023	20/09/2023	CBE, ECB, WCB								
October 23	12/10/2023		CBE								
November 23	28/11/2023	03/12/2023	CBE, ECB								
December 23	02/01/2024		CBE								

Underwater Vision Profiler

The UVP6 has been deployed 22 times, 8 of these being at CBE, 12 in the inshore ends of the ECB and 2 in the WCB. Images were captured on all deployments and have resulted in over 300,000 vignettes being uploaded onto EcoTaxa (https://ecotaxa.obs-vlfr.fr/gui/prj/). Following further training on the use of the UVP6, it was decided to adjust the acquisition setting. Prior to the November fieldwork the acquisition setting was changed from 0.5Hz to 25Hz. This alteration led to the UVP6 taking images at its maximum speed and has resulted in close to a ten-fold increase from 200-300 images to 2,000 images being obtained on a 100 m deployment. During the processing stages, the UVP software assesses pixel colour and uses this parameter to pick out objects within the images to create vignettes (Error! Reference source not found.). In general, each vignette displays one object, these could be zooplankton, detritus, or other particles the UVP comes across. Prior to changing the settings, the CBE deployments were resulting in ~ 100-200 vignettes being uploaded to EcoTaxa for further classification (Error! Reference source not found.). After changing the acquisition frequency, the November patrol resulted in 17,730 vignettes being uploaded for CBE. For

the ECB the number of vignettes uploaded for the November patrol were even greater with 95,392 at ECB1 and 122,894 at ECB3.

In January 2024, a connector sleeve that is used to securely fasten the cable connecting the UVP6 camera to the power supply during deployment cracked. With the assistance of AME engineers at BAS, KEP scientists were able to swap this sleeve with another sleeve from a cable that is not used during deployments. Spare connector sleeves have been ordered and will be sent to KEP to replace the broken one and ensure there are spares should any more break.

Table 2: Number of vignettes uploaded to EcoTaxa for each sampling station following a UVP6 deployment on MV Pharos SG patrols from July 2023- November 2023. * denotes UVP6 operating with acquisition frequency set to 25Hz.

Pharos Patrol		Num	nber of vigne	ettes Uploade	d to EcoTa	ха	
	CBE	ECB1	ECB2	ECB3	ECB4	ECB5	ECB6
July	105	15		19		8	13
August	95						
September	154	50	155	99	47	47	
October	263						
November*	17,730	95,392		122,894			

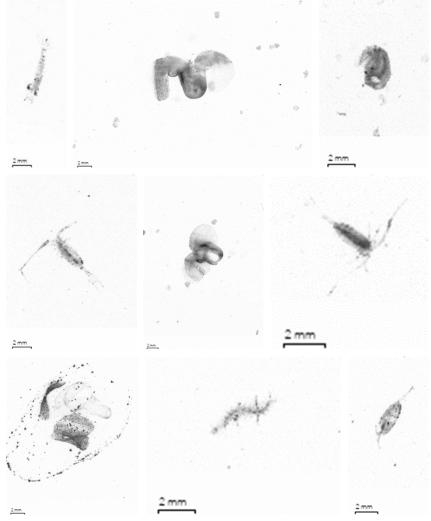


Figure 1: Examples of vignettes uploaded onto EcoTaxa following processing of images obtained by the UVP6.

Zooplankton Nets

Since July 2023, the miniBongo net has been deployed 20 times, 8 of these being at CBE, 11 in the inshore ends of the ECB and 1 in the WCB. The miniBongo is deployed to a depth of 100 m where possible or 10 m above the sea floor in shallower areas. In the same time period, the RMT1 has been deployed 46 times, 15 of these being at CBE, 16 in the inshore ends of the ECB, 7 at the offshore ends of ECB transects and 8 in the WCB. Further details of how these nets are deployed from the MV Pharos SG can be found in https://www.bas.ac.uk/project/south-georgia-pelagic-biodiversity/#data.

Zooplankton samples collected in the cod ends of both the RMT1 and miniBongo are preserved in ethanol ready for transportation to the UK. During the July patrol the RMT1 twisted during a deployment resulting in no catch from that deployment. Alterations were made with details being recorded in https://www.bas.ac.uk/project/south-georgia-pelagic-biodiversity/#data. Following this, both zooplankton nets have worked well on all patrols and no changes to the deployment and sample preservation protocols have been required during the project. Net catches have changed throughout the season with greater volumes being caught in the summer months. There were reports of large numbers of naked pteropods in the December samples, while the November samples had large quantities of phytoplankton. Further analysis of zooplankton samples will be conducted when samples return to the UK.



Figure 2: miniBongo net sample collected on the November patrol (left) and the December patrol (right) during the preservation process. Photographs from Carrie Gunn

eDNA water collection and Filtration

Two five litre Niskin bottles have been deployed on every patrol to collect water from 20 m and 75 m. There have been a total of 35 Niskin bottle deployments, with 17 deployments to 20 m and 18 to 75 m, between July and December 2023. Sixteen (8 to each depth) of these were at CBE, 16 (8 to each depth) at the inshore ends of the ECB transects and 3 (one to 20 m and two to 75 m) were collected from stations in the WCB. Water from these bottles is filtered onto a $0.2 \mu m$ filter paper which is then frozen for preservation and transportation as per the protocol laid out in KEP eDNA Sampling Protocol.docx.

During the October patrol the plastic handle came away from the body of the 20 m Niskin bottle during its recovery onto the MV *Pharos SG*, leaving a hole in the side of the bottle (Figure 3). The team on board the vessel were able to retrieve the collected water quickly allowing filtration to take place. As the handle broke away once the bottle was out of the water, we are confident that the water had not been contaminated by any water from shallower depths during its recovery. The KEP station team were able to drill out the remaining plastic and reattach the handle filling any holes and ensuring the Niskin was watertight ready for the next patrol.



Figure 3: Photograph of the detached handle and 20 m Niskin bottle following deployment on the October patrol. Photograph from Carrie Gunn

Originally, it was hoped that three replicates of 1 L would be filtered from each depth at every sample location using a peristaltic pump. This methodology was problematic as pressure commonly built up in the tubing running through the peristaltic pump, forcing the filter housing off the end of the tubing. Attempts to prevent this by using cable ties to secure the tubing to the filter housing, and reducing the pressure, were not successful. In addition, the filter rate was very slow, often taking longer than 30 minutes to filter less than 250 ml. As a result, the person filtering had to hold the filter housing onto the tubing throughout the filtration process making this a time-consuming and inefficient process. As an interim solution, Luer-lok hand syringes were trialled on the September patrol where 300 ml or more was filtered per replicate. These syringes securely attach to the filter housing containing the filter paper and water is manually pushed through using the plunger. Syringes have continued to be used on patrols since September. This has been working reasonably well with KEP scientists filtering three replicates totalling 1500 ml or more for each depth at all sampling stations on the October, November and December patrols (see Appendix 2 for filtration volumes).

A new system using a vacuum pump has been sent to KEP and will hopefully arrive before the February 2024 patrol. This system has been used by other BAS researchers on ships in the Arctic and has been found to be very efficient. It is hoped that this will allow greater volumes of water to be filtered and small experiments will be carried out comparing the eDNA extractions from the different methods and differing volumes of sample water.

Laboratory Work

Historical Zooplankton Samples

Zooplankton samples have been collected from SG for many years, however many samples are not fully analysed and are hence underutilised. As part of this project, we are planning to use these samples to build a historic baseline of the zooplankton community around SG. Samples from 2002 to 2008 have been transported to the Cambridge stores and catalogued. An attempt has been made to match these samples to a KEP event log to provide further details for each sample. Unfortunately, this has not always been possible; 174 out of 304 samples have been matched to a corresponding event.

Fifty-two samples have been scanned using the BAS ZooScan. Where possible one sample from each calendar month has been scanned for the years 2003, 2004, 2005, 2006 and 2007. This has resulted in a complete set of 12 months of scans for 2003 and 2006. For the other years there are either months missing or the samples from some months are too degraded to be scanned. Scanned images have been uploaded onto EcoTaxa for image classification. Image classification has begun and will be continued alongside a verification process to ensure images are correctly identified.

Samples from more recent campaigns are currently being transported to Cambridge and will be catalogued and ZooScanned when available.

Six samples from 2006 were selected and sent to the MBA for taxonomic analysis this financial year. Samples from 2006 were chosen due to this year being one of the most complete in terms of samples and was representative in environmental characteristics e.g. an average ice year. Voucher specimens will be picked out of these samples to aid with the image classification of the ZooScan images.

Appendix 1 Pelagic Biodiversity Project Event Log, July -December 2023

Table 3: MV Pharos SG Event log covering July 2023 to December 2023. Each individual event was assigned an event number. Gear is assigned as 'CTD' (Conductivity Temperature Depth profiler), 'UVP' (Underwater Visual Profiler 6), 'EDNA' (Niskin bottle deployment), 'MBN' (miniBongo net) or 'RMT1 (Rectangular Midwater Trawl, 1m2 mouth opening plankton net). Times presented are local (GMT/UTC -2). 'Lat' and 'long' refer to latitude and longitude respectively. Further details of plankton trawls and CTD data can be found in the KEP plankton trawl database and CTD database respectively.

NB Bridge event numbers were started again at the start of the WCB for the July patrol so to avoid confusion these have been renumbered to continue from the ECB numbers and the original bridge numbers are provided in brackets.

Pharos Patrol	Event #.	Gear	Location	Туре	Start date	Start time (local)	Start Lat	Start Long	End date	End time (local)	End Lat	End Long
July 23	1	EDNA	CBE	Practice Run	15/07/2023	10:08	-54.2825	-36.2017	15/07/2023	10:11	-54.2825	-36.435
July 23	2	EDNA	CBE	Practice Run	15/07/2023	10:16	-54.2825	-36.435	15/07/2023	10:24	-54.2767	-36.435
July 23	3	UVP	CBE	Practice Run	15/07/2023	10:46	-54.285	-36.4433	15/07/2023	10:55	-54.2833	-36.4267
July 23	4	MBN	CBE	Practice Run	15/07/2023	11:09	-54.2817	-36.4417	15/07/2023	11:19	-54.2817	-36.4283
July 23	5	CTD	CBE	Practice Run	15/07/2023	11:24	-54.28	-36.4433	15/07/2023	11:44	-54.2783	-36.4317
July 23	6	RMT1	CBE	Practice Run	15/07/2023	12:39	-54.2783	-36.43	15/07/2023	13:09	-54.2917	-36.4133
July 23	7	CTD	ECB1 Start	Night Inshore	15/07/2023	16:44	-54.0917	-36.265	15/07/2023	17:03	-54.09	-36.2717
July 23	8	RMT1	ECB1 Start	Night Inshore	15/07/2023	17:12	-54.0917	-36.2733	15/07/2023	17:42	-54.1067	-36.2667
July 23	12	EDNA	ECB1 Start	Day Inshore	16/07/2023	05:38	-54.095	-36.2617	16/07/2023	05:42	-54.095	-36.2617
July 23	13	EDNA	ECB1 Start	Day Inshore	16/07/2023	05:45	-54.0967	-36.2617	16/07/2023	05:53	-54.0967	-36.2633
July 23	14	UVP	ECB1 Start	Day Inshore	16/07/2023	05:59	-54.0967	-36.2633	16/07/2023	06:06	-54.0967	-36.265
July 23	15	MBN	ECB1 Start	Day Inshore	16/07/2023	06:09	-54.0967	-36.2667	16/07/2023	06:17	-54.0983	-36.2667
July 23	16	CTD	ECB1 Start	Day Inshore	16/07/2023	06:22	-54.0983	-36.265	16/07/2023	06:39	-54.0983	-36.265
July 23	17	RMT1	ECB1 Start	Day Inshore	16/07/2023	07:17	-54.0933	-36.2683	16/07/2023	07:47	-54.1067	-36.2467
July 23	19	CTD	ECB1/2	Day Offshore	16/07/2023	11:55	-53.9117	-35.6583	16/07/2023	12:13	-53.9117	-35.66
July 23	21	CTD	ECB2 End	Night Inshore	16/07/2023	16:55	-54.17	-36.1733	16/07/2023	17:09	-54.1683	-36.175
July 23	22	RMT1	ECB2 End	Night Inshore	16/07/2023	17:19	-54.1683	-36.1783	16/07/2023	17:49	-54.1783	-36.1567
July 23	25	EDNA	ECB3 Start	Day Inshore	17/07/2023	05:37	-54.266	-36.065	17/07/2023	05:39	-54.2663	-36.066

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July 23	26	EDNA	ECB3 Start	Day Inshore	17/07/2023	05:43	-54.2662	-36.0153	17/07/2023	05:49	-54.2662	-36.0662
July 23	27	UVP	ECB3 Start	Day Inshore	17/07/2023	05:52	-54.2667	-36.0668	17/07/2023	05:59	-54.2672	-36.0683
July 23	28	MBN	ECB3 Start	Day Inshore	17/07/2023	06:03	-54.2672	-36.069	17/07/2023	06:10	-54.268	-36.0698
July 23	29	CTD	ECB3 Start	Day Inshore	17/07/2023	06:14	-54.2682	-36.0697	17/07/2023	06:23	-54.2687	-36.0072
July 23	30	RMT1	ECB3 Start	Day Inshore	17/07/2023	07:15	-54.265	-36.0733	17/07/2023	07:45	-54.2683	-36.0433
July 23	32	CTD	ECB3/4	Day Offshore	17/07/2023	12:14	-54.9783	-36.2033	17/07/2023	12:32	-53.9767	-35.21
July 23	34	CTD	ECB4 End	Night Inshore	17/07/2023	16:42	-54.3485	-35.9618	17/07/2023	16:54	-54.3482	-35.962
July 23	35	UVP	ECB4 End	Night Inshore	17/07/2023	16:56	-54.3482	-35.962	17/07/2023	17:03	-54.3477	-35.9623
July 23	36	RMT1	ECB4 End	Night Inshore	17/07/2023	17:15	-54.3483	-35.9533	17/07/2023	17:45	-54.3517	-35.925
July 23	39	EDNA	ECB5 Start	Day Inshore	18/07/2023	05:33	-54.4008	-35.9118	18/03/2023	05:36	-54.4003	-35.9118
July 23	40	EDNA	ECB5 Start	Day Inshore	18/07/2023	05:40	-54.3998	-35.912	18/03/2023	05:47	-54.399	-35.9125
July 23	41	UVP	ECB5 Start	Day Inshore	18/07/2023	05:49	-54.3985	-35.9127	18/03/2023	05:56	-54.3977	-35.912
July 23	42	MBN	ECB5 Start	Day Inshore	18/07/2023	05:59	-54.3973	-35.9115	18/03/2023	06:05	-54.3967	-35.9118
July 23	43	CTD	ECB5 Start	Day Inshore	18/07/2023	06:10	-54.398	-35.9135	18/03/2023	06:16	-54.3985	-35.9135
July 23	44	RMT1	ECB5 Start	Day Inshore	18/07/2023	07:12	-54.3967	-35.9133	18/03/2023	07:42	-54.425	-35.905
July 23	46	CTD	ECB5/6	Day Offshore	18/07/2023	12:13	-54.1105	-35.3497	18/03/2023	12:31	-54.1077	-35.0543
July 23	48	RMT1	ECB6 End	Night Inshore	18/07/2023	17:03	-54.4917	-35.8117	18/03/2023	17:33	-54.4867	-35.7817
July 23	49	UVP	ECB6 End	Night Inshore	18/07/2023	17:44	-54.485	-35.7767	18/03/2023	17:51	-54.485	-35.7767
July 23	50	MBN	ECB6 End	Night Inshore	18/07/2023	17:54	-54.485	-35.7767	18/03/2023	18:01	-54.485	-35.7783
July 23	51	CTD	ECB6 End	Night Inshore	18/07/2023	18:05	-54.485	-35.7767	18/03/2023	18:13	-54.485	-35.7767
July 23	52	EDNA	CBE	Routine	19/07/2023	05:25	-54.3083	-36.3983	19/07/2023	05:28	-54.3083	-36.3983
July 23	53	EDNA	CBE	Routine	19/07/2023	05:30	-54.3067	-36.3967	19/07/2023	05:37	-54.3083	-36.3967
July 23	54	UVP	CBE	Routine	19/07/2023	05:43	-54.3067	-36.3967	19/07/2023	05:50	-54.3067	-36.3967
July 23	55	MBN	CBE	Routine	19/07/2023	05:53	-54.3067	-36.3967	19/07/2023	06:00	-54.3067	-36.395
July 23	56	CTD	CBE	Routine	19/07/2023	06:03	-54.3067	-36.395	19/07/2023	06:18	-54.3067	-36.3933
July 23	57	RMT1	CBE	Routine	19/07/2023	06:36	-54.3067	-36.4233	19/07/2023	07:06	-54.2917	-40.1667
July 23	58	RMT1	CBE	Routine	19/07/2023	07:14	-54.29	-36.4233	19/07/2023	07:44	-54.275	-36.4383
July 23	61 (3)	EDNA	WCB1 Start	Day Inshore	20/07/2023	05:32	-53.8733	-37.7233	20/07/2023	05:33	-53.8733	-37.7233
July 23	62 (4)	EDNA	WCB1 Start	Day Inshore	20/07/2023	05:35	-53.874	-37.7218	20/07/2023	05:42	-53.8747	-37.7215
July 23	63 (5)	UVP	WCB1 Start	Day Inshore	20/07/2023	05:45	-53.8748	-37.7207	20/07/2023	05:51	-53.875	-37.7212

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July 23	64 (6)	MBN	WCB1 Start	Day Inshore	20/07/2023	05:53	-53.875	-37.7212	20/07/2023	06:00	-53.8752	-37.7208
July 23	65 (7)	CTD	WCB1 Start	Day Inshore	20/07/2023	06:03	-53.8757	-37.7207	20/07/2023	06:09	-53.8758	-37.7203
July 23	66 (8)	RMT1	WCB1 Start	Day Inshore	20/07/2023	07:06	-53.8717	-37.7167	20/07/2023	07:36	-53.8717	-37.7433
July 23	68 (10)	CTD	WCB1/2	Day Offshore	20/07/2023	12:11	-53.2903	-37.8623	20/07/2023	12:29	-53.292	-37.8693
July 23	70 (12)	CTD	WCB2 End	Night Inshore	20/07/2023	16:36	-53.8558	-37.5925	20/07/2023	16:43	-53.8567	-37.5962
July 23	71 (13)	RMT1	WCB2 End	Night Inshore TARGET	20/07/2023	19:05	-53.5185	-37.8363	20/07/2023	19:35	-53.6045	-37.8175
July 23	72 (14)	UVP	WCB2 End	Night Inshore	20/07/2023	20:08	-53.612	-37.8253	20/07/2023	20:15	-53.6125	-37.8293
July 23	73 (15)	EDNA	WCB2 End	Night Inshore	20/07/2023	20:18	-53.6122	-37.8305	20/07/2023	20:25	-53.613	-37.8328
July 23	75 (17)	CTD	STROMNESS BAY	Calibration	21/07/2023	08:37	-54.1817	-36.6817	21/07/2023	08:44	-54.1817	-36.6817
July 23	77 (19)	EDNA	CBE	Routine	22/07/2023	05:46	-54.2757	-36.433	22/07/2023	05:48	-54.2758	-36.4332
July 23	78 (20)	EDNA	CBE	Routine	22/07/2023	05:51	-54.276	-36.433	22/07/2023	05:57	-54.2758	-36.4335
July 23	79 (21)	UVP	CBE	Routine	22/07/2023	06:00	-54.276	-36.4337	22/07/2023	06:06	-54.2757	-36.4348
July 23	80 (22)	MBN	CBE	Routine	22/07/2023	06:08	-54.2755	-36.4352	22/07/2023	06:16	-54.2748	-36.436
July 23	81 (23)	CTD	CBE	Routine	22/07/2023	06:18	-54.2752	-36.4362	22/07/2023	06:35	-54.274	-36.4387
July 23	82 (24)	RMT1	CBE	Routine	22/07/2023	06:44	-54.2767	-36.435	22/07/2023	07:14	-54.2917	-36.4233
July 23	83 (25)	RMT1	CBE	Routine	22/07/2023	07:22	-54.285	-36.42	22/07/2023	07:52	-54.3	-36.4
August 23	1	EDNA	CBE	Routine	11/08/2023	16:05	-54.32	-36.3967	11/08/2023	16:07	n/a	n/a
August 23	2	EDNA	CBE	Routine	11/08/2023	16:11	-54.32	-36.3967	11/08/2023	16:17	n/a	n/a
August 23	3	MBN	CBE	Routine	11/08/2023	16:20	-54.32	-36.3967	11/08/2023	16:28	n/a	n/a
August 23	4	UVP	CBE	Routine	11/08/2023	16:31	-54.3183	-36.395	11/08/2023	16:38	n/a	n/a
August 23	5	CTD	CBE	Routine	11/08/2023	16:40	-54.3183	-36.395	11/08/2023	16:53	n/a	n/a
August 23	6	RMT1	CBE	Routine	11/08/2023	17:16	-54.3183	-36.3933	11/08/2023	17:46	-54.3033	-36.405
August 23	7	RMT1	CBE	Routine	11/08/2023	17:54	-54.2983	-36.4083	11/08/2023	18:24	-54.285	-36.42
September 23	1	RMT1	CBE	ROUTINE	08/09/2023	18:03	-54.3159	-36.3948	08/09/2023	18:33	-54.302	-36.415
September 23	2	RMT1	CBE	ROUTINE	08/09/2023	18:40	-54.2996	-36.4169	08/09/2023	19:10	-54.282	-36.425
September 23	3	EDNA	CBE	ROUTINE	08/09/2023	19:28	-54.2745	-36.427	08/09/2023	19:31	N/A	N/A
September 23	4	EDNA	CBE	ROUTINE	08/09/2023	19:34	-54.2747	-36.4267	08/09/2023	19:42	N/A	N/A

September 23	5	UVP	CBE	ROUTINE	08/09/2023	19:44	-54.2749	-36.4265	08/09/2023	19:52	N/A	N/A
September 23	6	MBN	CBE	ROUTINE	08/09/2023	19:54	-54.2751	-36.4265	08/09/2023	20:02	N/A	N/A
September 23	7	CTD	CBE	ROUTINE	08/09/2023	20:05	-54.2752	-36.4261	08/09/2023	20:22	N/A	N/A
September 23	9	CTD	HUSVIK	CALIBRATION	10/09/2023	08:28	-54.1808	-36.688	10/09/2023	08:31	N/A	N/A
September 23	11	RMT1	ECB1	DAY INSHORE	11/09/2023	05:20	-54.1147	-36.2283	11/09/2023	05:50	-54.099	-36.243
September 23	12	MBN	ECB1	DAY INSHORE	11/09/2023	06:02	-54.0948	-36.2474	11/09/2023	06:09	N/A	N/A
September 23	13	UVP	ECB1	DAY INSHORE	11/09/2023	06:12	-54.0945	-36.2459	11/09/2023	06:19	N/A	N/A
September 23	14	EDNA	ECB1	DAY INSHORE	11/09/2023	06:22	-54.0942	-36.2447	11/09/2023	06:24	N/A	N/A
September 23	15	EDNA	ECB1	DAY INSHORE	11/09/2023	06:27	-54.094	-36.2443	11/09/2023	06:33	N/A	N/A
September 23	16	CTD	ECB1	DAY INSHORE	11/09/2023	06:36	-54.094	-36.2433	11/09/2023	06:54	N/A	N/A
September 23	19	CTD	ECB1/2	DAY OFFSHORE	11/09/2023	12:00	-53.8061	-35.3883	11/09/2023	12:18	N/A	N/A
September 23	22	CTD	ECB2	NIGHT INSHORE	11/09/2023	17:26	-54.178	-36.2184	11/09/2023	17:40	N/A	N/A
September 23	23	UVP	ECB2	NIGHT INSHORE	11/09/2023	17:43	-54.1781	-36.2169	11/09/2023	17:50	N/A	N/A
September 23	24	MBN	ECB2	NIGHT INSHORE	11/09/2023	17:52	-54.178	-36.2151	11/09/2023	17:59	N/A	N/A
September 23	25	RMT1	ECB2	NIGHT INSHORE	11/09/2023	18:43	-54.1103	-36.2417	11/09/2023	19:13	-54.107	-36.271
September 23	28	RMT1	ECB3	DAY INSHORE	12/09/2023	05:23	-54.273	-36.0506	12/09/2023	05:53	-54.26	-36.08
September 23	29	MBN	ECB3	DAY INSHORE	12/09/2023	06:07	-54.2575	-36.0845	12/09/2023	06:14	N/A	N/A
September 23	30	UVP	ECB3	DAY INSHORE	12/09/2023	06:16	-54.2574	-36.0831	12/09/2023	06:23	N/A	N/A
September 23	31	EDNA	ECB3	DAY INSHORE	12/09/2023	06:26	-54.2571	-36.0821	12/09/2023	06:28	N/A	N/A
September 23	32	EDNA	ECB3	DAY INSHORE	12/09/2023	06:31	-54.2573	-36.0826	12/09/2023	06:37	N/A	N/A
September 23	33	CTD	ECB3	DAY INSHORE	12/09/2023	06:39	-54.2574	-36.0822	12/09/2023	06:49	N/A	N/A
September 23	36	CTD	ECB3/4	DAY OFFSHORE	12/09/2023	12:01	-53.9822	-35.1967	12/09/2023	12:18	N/A	N/A
September 23	38	CTD	ECB4	NIGHT INSHORE	12/09/2023	17:54	-54.3504	-35.9846	12/09/2023	18:05	N/A	N/A
September 23	39	UVP	ECB4	NIGHT INSHORE	12/09/2023	18:07	-54.352	-35.9867	12/09/2023	18:14	N/A	N/A

September 23	40	MBN	ECB4	NIGHT INSHORE	12/09/2023	18:18	-54.3531	-35.9893	12/09/2023	18:25	N/A	N/A
September 23	41	RMT1	ECB4	NIGHT INSHORE	12/09/2023	18:41	-54.345	-35.9894	12/09/2023	19:12	-54.324	-35.988
September 23	44	RMT1	ECB5	DAY INSHORE TARGET	13/09/2023	07:19	-54.3967	-35.9217	13/09/2023	07:23	-54.4	-35.919
September 23	45	CTD	ECB5	DAY INSHORE	13/09/2023	08:08	-54.3917	-35.92	13/09/2023	08:15	N/A	N/A
September 23	48	CTD	ECB5/6	DAY OFFSHORE	13/09/2023	13:01	-54.116	-35.0331	13/09/2023	13:19	N/A	N/A
September 23	51	CTD	ECB6	NIGHT INSHORE	13/09/2023	18:05	-54.4998	-35.8058	13/09/2023	18:12	N/A	N/A
September 23	52	RMT1	ECB6	NIGHT INSHORE	13/09/2023	18:44	-54.4735	-35.8322	13/09/2023	19:14	-54.459	-35.851
September 23	55	MBN	ECB5	DAY INSHORE	14/09/2023	06:18	-54.4021	-35.9141	14/09/2023	06:25	N/A	N/A
September 23	56	UVP	ECB5	DAY INSHORE	14/09/2023	06:27	-54.4027	-35.9159	14/09/2023	06:33	N/A	N/A
September 23	57	EDNA	ECB5	DAY INSHORE	14/09/2023	06:36	-54.4035	-35.9177	14/09/2023	06:38	N/A	N/A
September 23	58	EDNA	ECB5	DAY INSHORE	14/09/2023	06:41	-54.4037	-35.9184	14/09/2023	06:48	N/A	N/A
September 23	59	CTD	ECB5	DAY INSHORE	14/09/2023	06:50	-54.4041	-35.9202	14/09/2023	06:57	N/A	N/A
September 23	60	RMT1	ECB5	DAY INSHORE TARGET	14/09/2023	08:41	-54.4193	-35.9373	14/09/2023	09:01	-54.411	-36.922
September 23	61	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	13:58	-54.0568	-35.5726	14/09/2023	14:12	-54.049	-35.573
September 23	63	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	19:14	-53.8836	-35.9969	14/09/2023	19:27	-53.881	-36.008
September 23	64	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	19:42	-53.877	-36.029	14/09/2023	19:48	-53.876	-36.035
September 23	65	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	19:57	-53.8749	-36.0416	14/09/2023	20:06	-53.873	-36.049
September 23	66	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	20:15	-53.8717	-36.0571	14/09/2023	20:28	-53.873	-36.049
September 23	67	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	20:35	-53.8711	-36.0763	14/09/2023	20:55	-53.871	-36.094

September 23	68	RMT1	ECB	DAY OFFSHORE TARGET	14/09/2023	21:37	-53.8712	-36.0557	14/09/2023	21:58	-53.871	-36.076
September 23	69	CTD	WCB1	NIGHT INSHORE	19/09/2023	18:29	-53.8707	-37.7306	19/09/2023	18:36	-53.87	-37.731
September 23	70	RMT1	WCB1	NIGHT INSHORE	19/09/2023	19:02	-53.8704	-37.7344	19/09/2023	19:33	-53.884	-37.77
September 23	73	RMT1	WCB2	DAY INSHORE TARGET	20/09/2023	05:22	-53.8581	-37.5983	20/09/2023	05:23	-53.858	-37.601
September 23	74	RMT1	WCB2	DAY INSHORE TARGET	20/09/2023	05:48	-53.8624	-37.5833	20/09/2023	05:57	-53.857	-37.592
September 23	75	CTD	WCB2	DAY INSHORE	20/09/2023	06:07	-53.8527	-37.598	20/09/2023	06:14	-53.853	-37.596
September 23	78	CTD	WCB1/2	DAY OFFSHORE	20/09/2023	11:58	-53.2905	-37.8501	20/09/2023	12:13	N/A	N/A
September 23	80	RMT1	WCB2	NIGHT OFFSHORE TARGET	20/09/2023	19:34	-53.6233	-37.6528	20/09/2023	19:49	-53.629	-37.668
September 23	81	RMT1	WCB2	NIGHT OFFSHORE TARGET	20/09/2023	20:02	-53.6287	-37.6812	20/09/2023	20:19	-53.624	-37.697
September 23	82	RMT1	WCB2	NIGHT OFFSHORE TARGET	20/09/2023	20:44	-53.6111	-37.7118	20/09/2023	20:54	-53.608	-37.721
October 23	1	RMT1	CBE	Routine	12/10/2023	19:12	-54.3121	-36.4009	12/10/2023	19:42	-54.2979	-36.4178
October 23	2	RMT1	CBE	Routine	12/10/2023	19:50	-54.2942	-36.422	12/10/2023	20:20	-54.2793	-36.4381
October 23	3	EDNA	CBE	Routine	12/10/2023	20:35	-54.28	-36.4435	12/10/2023	20:37	-54.275	-36.4433
October 23	4	EDNA	CBE	Routine	12/10/2023	20:39	-54.275	-36.443	12/10/2023	20:45	-54.2749	-36.4429
October 23	5	UVP	CBE	Routine	12/10/2023	20:48	-54.2748	-36.4429	12/10/2023	20:55	-54.2747	-36.4431
October 23	6	MBN	CBE	Routine	12/10/2023	20:57	-54.2747	-36.4431	12/10/2023	21:04	-54.2746	-36.4479
October 23	7	CTD	CBE	Routine	12/10/2023	21:07	-54.2745	-36.4428	12/10/2023	21:24	-54.2745	-36.4418
November 23	1	RMT1	CBE	ROUTINE	28/11/2023	20:54	-54.2767	-36.4267	28/11/2023	21:24	-54.262	-36.415
November 23	2	RMT1	CBE	ROUTINE	28/11/2023	21:36	-54.26	-36.4417	28/11/2023	22:06	-54.273	-36.425
November 23	3	EDNA	CBE	ROUTINE	28/11/2023	22:20	-54.2708	-36.4279	28/11/2023	22:22	-54.27	-36.427

November 23	4	EDNA	CBE	ROUTINE	28/11/2023	22:25	-54.2705	-36.4277	28/11/2023	22:32	-54.27	-36.427
November 23	5	UVP	CBE	ROUTINE	28/11/2023	22:35	-54.2705	-36.4265	28/11/2023	22:42	-54.27	-36.427
November 23	6	MBN	CBE	ROUTINE	28/11/2023	22:44	-54.2703	-36.4273	28/11/2023	22:52	-54.27	-36.427
November 23	7	CTD	CBE	ROUTINE	28/11/2023	22:57	-54.27	-36.4297	28/11/2023	23:14	-54.27	-36.427
November 23	8	EDNA	ECB1	NIGHT INSHORE	01/12/2023	20:39	-54.0954	-36.2593	01/12/2023	20:42	-54.095	-36.258
November 23	9	EDNA	ECB1	NIGHT INSHORE	01/12/2023	20:44	-54.0957	-36.2577	01/12/2023	20:51	-54.096	-36.256
November 23	10	UVP	ECB1	NIGHT INSHORE	01/12/2023	20:53	-54.0955	-36.2541	01/12/2023	21:00	-54.095	-36.253
November 23	11	MBN	ECB1	NIGHT INSHORE	01/12/2023	21:04	-54.0951	-36.2543	01/12/2023	21:11	-54.095	-36.254
November 23	12	RMT1	ECB1	NIGHT INSHORE	01/12/2023	21:29	-54.0978	-36.2536	01/12/2023	21:59	-54.082	-36.267
November 23	15	CTD	ECB1	DAY INSHORE	02/12/2023	06:13	-54.0933	-36.2783	02/12/2023	06:41	-54.095	-36.262
November 23	17	CTD	ECB1/2	DAY OFFSHORE	02/12/2023	11:42	-53.7976	-35.4048	02/12/2023	12:00	-53.797	-35.407
November 23	19	CTD	ECB2	DAY INSHORE	02/12/2023	17:32	-54.1713	-36.1765	02/12/2023	17:46	-54.173	-36.177
November 23	20	CTD	ECB3	DAY INSHORE	02/12/2023	19:11	-54.2655	-36.0698	02/12/2023	19:20	-54.267	-36.068
November 23	21	EDNA	ECB3	NIGHT INSHORE	02/12/2023	20:31	-54.2677	-36.0724	02/12/2023	20:34	-54.268	-36.072
November 23	22	EDNA	ECB3	NIGHT INSHORE	02/12/2023	20:37	-54.2678	-36.0718	02/12/2023	20:43	-54.268	-36.07
November 23	23	UVP	ECB3	NIGHT INSHORE	02/12/2023	20:45	-54.2673	-36.0704	02/12/2023	20:52	-54.268	-36.07
November 23	24	MBN	ECB3	NIGHT INSHORE	02/12/2023	20:56	-54.2678	-36.0697	02/12/2023	21:02	-54.268	-36.069
November 23	25	RMT1	ECB3	NIGHT INSHORE	02/12/2023	21:26	-54.2671	-36.0478	02/12/2023	21:56	-54.256	-36.063
November 23	29	CTD	ECB3/4	DAY OFFSHORE	03/12/2023	11:38	-53.9767	-35.1995	03/12/2023	11:56	-53.972	-35.199
November 23	31	CTD	ECB4	DAY INSHORE	03/12/2023	16:32	-54.3497	-35.9591	03/12/2023	16:43	-54.349	-35.963
December 23	1	RMT1	CBE	Routine	02/01/2024	21:26	-54.3067	-36.41	02/01/2024	21;56	-54.29	-36.42

December 23	2	RMT1	CBE	Routine	02/01/2024	22:03	-54.2867	-36.4233	02/01/2024	22:33	-54.2717	-36.4317
December 23	3	EDNA	CBE	Routine	02/01/2024	22:47	-54.2733	-36.435	02/01/2024	22:50	-54.2733	-36.435
December 23	4	EDNA	CBE	Routine	02/01/2024	22:52	-54.2733	-36.435	02/01/2024	22:59	-54.2733	-36.435
December 23	5	MBN	CBE	Routine	02/01/2024	23:02	-54.2733	-36.435	02/01/2024	23:09	-54.2733	-36.435
December 23	6	UVP	CBE	Routine	02/01/2024	23:12	-54.2717	-36.435	02/01/2024	23:19	-54.2717	-36.435
December 23	7	CTD	CBE	Routine	02/01/2024	23:23	-54.2717	-36.435	02/01/2024	23:40	-54.2717	-36.435

Appendix 2 eDNA water filtration volumes

Table 4: eDNA filtration volumes for all sampling stations from July 2023 to December 2023. * denotes filtration being carried out using a luer-lok syringe.

Patrol	Date	Station	Water	Volume	Filter#
			collection	filtered	
			Depth	(ml)	
			(m)	()	
July 2023	17/07/2023	ECB3	20	615	1
July 2023	17/07/2023	ECB3	20	225	2
July 2023	18/07/2023	ECB5	20	200	1
July 2023	18/07/2023	ECB5	20	200	2
July 2023	18/07/2023	ECB5	20	200	3
July 2023	18/07/2023	ECB5	75	200	1
July 2023	18/07/2023	ECB5	75	220	2
July 2023	18/07/2023	ECB5			3
July 2023	19/07/2023	CBE	20	250	1
July 2023	19/07/2023	CBE	20	290	2
July 2023	19/07/2023	CBE	20	460	3
July 2023	19/07/2023	CBE	75	275	1
July 2023	19/07/2023	CBE	75	270	2
July 2023	19/07/2023	CBE	75	495	3
July 2023	20/07/2023	WCB1	20	165	1
July 2023	20/07/2023	WCB1	20	175	2
July 2023	20/07/2023	WCB1	20	225	3
July 2023	20/07/2023	WCB1	75	335	1
July 2023	20/07/2023	WCB1	75	510	2
July 2023	20/07/2023	WCB1	75	290	3
July 2023	20/07/2023	WCB2	75	100	1
	20/27/2022	(Target)		10-	
July 2023	20/07/2023	WCB2	75	125	2
July 2023	20/07/2023	(Target) WCB2	75	160	3
July 2023	20/07/2023	(Target)	75	100	3
July 2023	22/07/2023	CBE(2)	20	225	1
July 2023	22/07/2023	CBE(2)	20	210	2
July 2023	22/07/2023	CBE(2)	20	200	3
July 2023	22/07/2023	CBE(2)	75	125	1
July 2023	22/07/2023	CBE(2)	75	130	2
July 2023	22/07/2023	CBE(2)	75	110	3
August 2023	11/08/2023	CBE	N/A	255	1
August 2023	11/08/2023	CBE	N/A	220	2
August 2023	11/08/2023	CBE	N/A	500	3
August 2023	11/08/2023	CBE	20	230	1
August 2023	11/08/2023	CBE	20	300	2

August 2023	11/08/2023	CBE	20	260	3
August 2023	11/08/2023	CBE	75	280	1
August 2023	11/08/2023	CBE	75	270	2
August 2023	11/08/2023	CBE	75	220	3
September	08/09/2023	CBE	20	300	1
2023*	00/00/2020	052	20	000	·
September 2023*	08/09/2023	CBE	20	310	2
September 2023*	08/09/2023	CBE	20	315	3
September 2023*	08/09/2023	CBE	75	310	1
September 2023*	08/09/2023	CBE	75	300	2
September 2023*	08/09/2023	CBE	75	320	3
September 2023*	09/09/2023	BLANK	N/A	300	1
September 2023*	09/09/2023	BLANK	N/A	350	2
September 2023*	09/09/2023	BLANK	N/A	285	3
September 2023*	11/09/2023	ECB1	20	315	1
September 2023*	11/09/2023	ECB1	20	350	2
September 2023*	11/09/2023	ECB1	20	320	3
September 2023*	11/09/2023	ECB1	75	320	1
September 2023*	11/09/2023	ECB1	75	325	2
September 2023*	11/09/2023	ECB1	75	310	3
September 2023*	12/09/2023	ECB3	20	320	1
September 2023*	12/09/2023	ECB3	20	310	2
September 2023*	12/09/2023	ECB3	20	320	3
September 2023*	12/09/2023	ECB3	75	310	1
September 2023*	12/09/2023	ECB3	75	330	2
September 2023*	12/09/2023	ECB3	75	325	3
September 2023*	14/09/2023	ECB5	20	310	1
September 2023*	14/09/2023	ECB5	20	300	2

September 2023*	14/09/2023	ECB5	20	350	3
September 2023*	14/09/2023	ECB5	75	330	1
September 2023*	14/09/2023	ECB5	75	300	2
September 2023*	14/09/2023	ECB5	75	345	3
September 2023*	15/09/2023	BLANK	N/A	300	1
September 2023*	15/09/2023	BLANK	N/A	300	2
September 2023*	15/09/2023	BLANK	N/A	300	3
October 2023*	12/10/2023	CBE	20	520	1
October 2023*	12/10/2023	CBE	20	500	2
October 2023*	12/10/2023	CBE	20	500	3
October 2023*	12/10/2023	CBE	75	500	1
October 2023*	13/10/2023	CBE	75	500	2
October 2023*	13/10/2023	CBE	75	520	3
October 2023*	13/10/2023	BLANK	N/A	500	1
October 2023*	13/10/2023	BLANK	N/A	520	2
October 2023*	13/10/2023	BLANK	N/A	500	3
November 2023*	28/11/2023	CBE	20	500	1
November 2023*	28/11/2023	CBE	20	500	2
November 2023*	28/11/2023	CBE	20	500	3
November 2023*	28/11/2023	CBE	75	500	1
November 2023*	28/11/2023	CBE	75	500	2
November 2023*	28/11/2023	CBE	75	500	3
November 2023*	28/11/2023	BLANK	N/A	500	1
November 2023*	28/11/2023	BLANK	N/A	500	2
November 2023*	28/11/2023	BLANK	N/A	500	3

November 2023*	01/12/2023	ECB1	20	500	1
November 2023*	01/12/2023	ECB1	20	525	2
November 2023*	01/12/2023	ECB1	20	500	3
November 2023*	01/12/2023	ECB1	75	500	1
November 2023*	01/12/2023	ECB1	75	510	2
November 2023*	02/12/2023	ECB1	75	510	3
November 2023*	02/12/2023	BLANK	N/A	500	1
November 2023*	02/12/2023	BLANK	N/A	550	2
November 2023*	02/12/2023	BLANK	N/A	500	3
November 2023*	02/12/2023	ECB3	20	600	1
November 2023*	02/12/2023	ECB3	20	600	2
November 2023*	02/12/2023	ECB3	20	450	3
November 2023*	02/12/2023	ECB3	75	500	1
November 2023*	02/12/2023	ECB3	75	600	2
November 2023*	02/12/2023	ECB3	75	520	3
December 2023*	03/01/2024	CBE	20	500	1
December 2023*	03/01/2024	CBE	20	500	2
December 2023*	03/01/2024	CBE	20	500	3
December 2023*	03/01/2024	CBE	75	500	1
December 2023*	03/01/2024	CBE	75	500	2
December 2023*	03/01/2024	CBE	75	500	3
December 2023*	03/01/2024	BLANK	N/A	500	1
December 2023*	03/01/2024	BLANK	N/A	500	2
December 2023*	03/01/2024	BLANK	N/A	500	3