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ENRICH Voyage Report, IN2019_V01: The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean

Double, M.C., Bell, E., Miller, B., Kelly, N., Kawaguchi, S., Lawrence, J., Leaper, R., Olson, P., Westwood, K. et al.

Australian Government
Department of the Environment and Energy
Australian Antarctic Division

ENRICH Voyage Report

ENRICH Voyage Report, IN2019_V01: The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean

Prepared by:

Chief and Alternate Chief Scientists:

Brian Miller, Elanor Bell, Mike Double

Science Voyage Planner:

Natalie Kelly

Science team leaders:

So Kawaguchi

Joshua Lawrence

Russell Leaper

Paula Olson

Karen Westwood

Science Team members:

Charlotte Boyd

Susannah Calderan

David Donnelly

Thomas Holmes

Lyn Irvine

Olivia Johnson

Ailbhe Kavanagh

Rob King

Jessica Melvin

Elanor Miller

James O'Brien

Lavenia Ratnarajah

Vanesa Reyes Reyes

Clara Rodriguez Vives

Ana Širović

Abigail Smith

Kathleen Stafford

Haiting Zhan

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For any information regarding this report please contact:

Voyage Chief Scientist: Michael Double
Telephone: +61 3 6232 3407
E-mail: mike.double@aad.gov.au

Cover image: Scientist and crew aboard the RV *Investigator* taken using a DJI Inspire 2 UAV (© Alex Vail and James Cox).

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Executive Summary

In March, 2017 the project entitled, '*The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean*', was awarded ship time on Australia's Marine National Facility's research vessel, *RV Investigator*. The resulting 49-day *ENRICH* Voyage (**E**uphausiids and **N**utrient **R**ecycling **I**n **C**etacean **H**otspots) departed from Hobart, Tasmania on the 19 January and returned to the same port on the 5 March, 2019.

Multi-disciplinary marine science was conducted off Antarctica from 64°S to 67°S and between 138°E and 154°W. Active acoustic data were collected continuously throughout the voyage totalling over 9,000 km of effort south of 60°S. The survey of the study area included six formal broad-scale transects (approx. orientated N-S). During the 1,670km of effort on these transects 975 distinct krill swarms were detected and the acoustic density and 3D structure was recorded for each swarm using the calibrated echosounder. The open-ocean cold-water calibration of the EK60 echsounder was conducted south of 64°S on the 26th January. Krill were distributed throughout the survey region with the highest densities in the western region close to but offshore of the shelf-break whereas in the east the highest densities were further north. A total of 41 target trawls were conducted using the RMT1+8 scientific trawl net in order to determine the krill size, maturity stage composition of various swarms. Morphometric data were collected from 4,385 krill and the growth rates of 5,472 were measured in 20 Integrated Growth Rate experiments.

In total 295 sonobuoys were deployed during the voyage, which provided 574 hours of passive acoustic monitoring. Antarctic blue whales were detected most commonly with 33,435 calls on 238 sonobuoys. The geographic location of calling whales could be determined over a period of 205 hours when two or three sonobuoys provided data simultaneously.

Visual sightings effort totalled 317 hours over 4,471km. In total there were 569 sightings of 1,380 cetaceans. Sightings of humpbacks whales were most common (201) followed by fin (124), blue (26) and minke whales (23). Nineteen groups of blue whales were approached for photo-identification from which suitable imagery was collected from 25 whales. To obtained data on the surfacing and movement behaviour of whales relative to their local prey field video-tracking of blue and fin whales was conducted ion 24 occasions for a total of 18 hours.

During the voyage 134 Unmanned Aerial Vehicle (UAV or drone) flights were conducted to undertake photogrammetry, photo-identification, whale 'blow' sampling, surface water sampling or to collected general whale and scenic imagery. Of these flights 113 were conducted used a DJI Inspire 2 and 21 using a Phantom 4. Photogrammetry video data (8 individuals) and a single blow sample were collected from blue whales.

A total of 110 biogeochemistry deployments were conducted during the voyage including 28 CTDs (Conductivity Temperature Depth; at 22 survey and 5 process stations), 35 Trace Metal Rosettes (TMRs; at 21 stations), 37 eXpendable BathyThermographs (XBTs) and 10 drifters. Not all CTD and TMR provided water samples for experimental work but in total these deployments together with UAV water sampling produced over 3,500 samples for a diverse range of analyses examining primary production, bacterial production, dissolved organic carbon, viral abundance, eDNA/RNA, dissolved chlorophyll-a, DMSP, trace metals and organic ligands.

This voyage provided the first opportunity to conduct a detailed survey of the distribution and characteristics of krill swarms while recording physical and biological oceanographic data together

with the distribution and behaviour of the largest krill predators, primarily blue, fin and humpback whales. While this was an ambitious and complex multidisciplinary voyage it stands as example of how many scientific data streams can be collected simultaneously to provide a more complete description of the dynamic physical and ecosystem processes occurring off Antarctica albeit over a relatively short period of time.

Many of the datasets generated by this voyage are large and complex and integrated analyses will take many years but they will generate novel insights into Antarctic krill, their environment and the predators that depend upon them.

We estimate the data collected during the ENRICH voyage will contribute to at least 50 peer reviewed publications and will be included in at least five PhD theses. The voyage provided training opportunities for 6 graduate students, and 6 early career researchers. Outreach from the ENRICH voyage included 5 remote classroom presentations during the voyage, and generated nearly 40 articles and stories on mainstream media.

1 Introduction

In March, 2017 the project entitled, '*The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean*', was awarded 49 days of ship time on the Marine National Facility's research vessel, *RV Investigator*. The Australian Antarctic Division (AAD) signed an Agreement as Sponsoring Agency for this project in July, 2017.

The multi-disciplinary marine science voyage, dubbed *ENRICH* (**E**uphausiids and **N**utrient **R**ecycling **I**n **C**etacean **H**otspots), was conducted 19 January – 5 March. The area of operation spanned 64°S to 67°S and between 138°E and 154°W.

The overarching aim of the research voyage was to describe the characteristics of Antarctic krill swarms and determine whether these characteristics predict the distribution and behaviour of Antarctic predators, particularly Antarctic blue whales, and how these predators interact with krill in time and space.

The density, distribution, and fine-scale 3D structure of krill swarms was described with active acoustics and target trawls. Krill observations occurred alongside estimates of cetacean density and distribution passive acoustics and visual surveys. In addition, the voyage aimed to test the theory of iron-fertilisation by whales and determine whether iron concentrations were higher within aggregations of feeding whales than within krill only aggregations, or than in adjacent areas.

The data collected on this voyage will inform the development of management tools for both baleen whales and krill, and will contribute to the International Whaling Commission's Southern Ocean Research Partnership's (IWC-SORP) and to CCAMLR's management of Antarctic krill.

This document reports on the voyage, the sampling methods and experiments undertaken, and the principal data streams collected throughout the survey that will contribute to meeting the voyage's scientific objectives.

2 Research objectives and survey design

2.1 Research objectives

The research objectives of the *ENRICH* voyage were to:

1) Characterise the density, distribution and fine-scale 3D structure of Antarctic krill swarms using the latest active acoustic multibeam technology.
2) Compare the krill prey field in the vicinity and absence of a large predator by remotely detecting and tracking the location of Antarctic blue whale aggregations using novel passive acoustic methods.
3) Describe the behaviour of Antarctic blue whales on foraging grounds by investigating the relationships among vocalisations, density, movements and surface behaviour, and compare the local prey field around whales exhibiting different behaviours.
4) Conduct the first field study of the theory of iron fertilisation by whales and krill.

Every team on this multi-disciplinary voyage conducted coordinated research to meet these objectives.

2.2 Summary of survey design

The survey area for the *ENRICH* voyage was identified before departure as a region that would minimise transit time, maximise the likelihood of surveying the putative Antarctic krill niche (i.e., between the shelf-break/1000 isobath), and likely have an higher density of Antarctic blue whales (CPIII Branch 2007) when compared to the wider region.

The survey design was a hybrid between a 'traditional' systematic line-transect survey and a targeted, adaptive survey.

The systematic line-transect design component aimed to describe the variability in dynamics of krill, whale distribution and biogeochemistry at the mesoscale (i.e., ~100-1000 km) and covered the north-south extent of Antarctic krill distribution (including the shelf-break, but outside sea ice covered areas). This north-south transition from open-ocean to the sea ice is considered a dominant gradient for krill habitat in East Antarctica (Nicol *et al.* 2012).

The adaptive design component targeted aggregations of Antarctic blue whales, detected using passive acoustics, in order to:

1) describe the characteristics of the prey-field in the vicinity of foraging Antarctic blue whales;
2) describe the surface behaviours of individual Antarctic blue whales, with potential to match this with vocal behaviour;
3) to collect photo-identification data from Antarctic blue whales;
4) to assess the iron levels in the presence of baleen whales (at least correlated with presence of baleen whales in the absence of directly collecting whale faecal matter); and
5) assess local Antarctic blue whale densities with call detection rates.

When modelling the key variables (krill swarm characteristics, biogeochemistry and oceanography) in relation to the local 'presence' of Antarctic blue whales and their 'absence', it was assumed that sampling along the broader-scale systematic transects away from Antarctic blue whale detections (sightings or acoustic) would deliver sufficient 'absence' data.

The surveyed area spanned several tens of degrees of longitude in an attempt to both 'replicate' sampling along the north-south gradient, as many times as possible, and to characterise some environmental variability in the east-west dimension. The data collected about Antarctic krill, whale distribution and density, biogeochemistry, and oceanography will inform models of the macroecology of the respective species in East Antarctica and the broader Southern Ocean region.

The survey design is described in full in the ENRICH Voyage Science Plan (Kelly *et al.* 2019).

2.3 Synoptic outputs

The multi-disciplinary data collected during the ENRICH voyage offers a unique and valuable opportunity to address the strategically and ecologically important research questions posed within the voyage objectives (Section 2.1). The strength of this multi-disciplinary research voyage was that every scientist on board conducted research that was coordinated within and between teams to meet these same objectives.

The four overarching research questions will be addressed in a suite of key outputs which synthesize the data and analyses described in Sections 4.1 - 4.6 for publication in high impact, peer-reviewed journals. These outputs will also be highly influential in international forums such as the IWC and CCAMLR, informing both management and conservation decisions, and future, systematic survey designs.

Table 2.3.1 summarises the likely synoptic outputs from the ENRICH voyage. It also presents the resources required to finalise post-voyage data analysis and facilitate timely production.

Table 2.3.1 - Summary of key ENRICH voyage outputs.

Key output	Likely lead
Manuscript: Characterisation of the density, distribution and fine-scale 3D structure of Antarctic krill swarms (*Euphausia superba*) using active acoustic multi-beam technology (objective 1)	Martin Cox/ So Kawaguchi
Manuscript: Towards optimal survey designs to study Antarctic krill (objective 1)	Natalie Kelly
Manuscript: Comparison of the krill prey field in the vicinity of baleen whale aggregations (objective 2)	Brian Miller/ Natalie Kelly
Manuscript: The behaviour of Antarctic blue whales (*Balaenoptera musculus intermedia*) on foraging grounds (objective 3)	Brian Miller
Manuscript: The ecological niche of endangered Antarctic blue whales whales (*Balaenoptera musculus intermedia*) in the Southern Ocean (objective 3)	Michael Double
Manuscript: Review of the spatial and temporal distribution of Antarctic blue and fin whales in the Southern Ocean (objective 3)	Natalie Kelly
Manuscript: An overview of the effects of iron fertilisation by whales and krill on the Southern Ocean microbial loop (objective 4)	Elanor Bell/ Karen Westwood
Manuscript: Designing a multidisciplinary/interdisciplinary voyage	Natalie Kelly
Policy advice: Scientific advice to IWC and CCAMLR	Michael Double/ So Kawaguchi

3 Voyage overview

3.1 Voyage personnel

60 people were on board: 28 science staff, 2 filmmakers, 1 AAD medical doctor, 9 MNF staff (including the designated ship's medical doctor) and 20 vessel crew. A personnel list can be found in Appendix 9.1.

An important element of the ENRICH voyage was the involvement, training and support of early-career scientists. Data collected on the voyage will form chapters (and subsequently be published as peer-reviewed scientific papers) of five PhD theses. James O'Brien, Jessica Melvin, Abigail Smith, Clara Rodriguez Vives and Haiting Zhang were PhD students conducting scientific research on the voyage. A number of other scientific personnel were early-career post-doctoral researchers leading important components of the multi-disciplinary work, i.e., Madeleine Brasier, Joshua Lawrence, Lavenia Ratnarajah and Thomas Holmes.

3.2 Vessel track

The voyage departed from and returned to Hobart Tasmania. The area of operation spanned 64°S to 67°S and between 138°E and 154°W. Figure 3.2.1 illustrates vessel position by date and time.

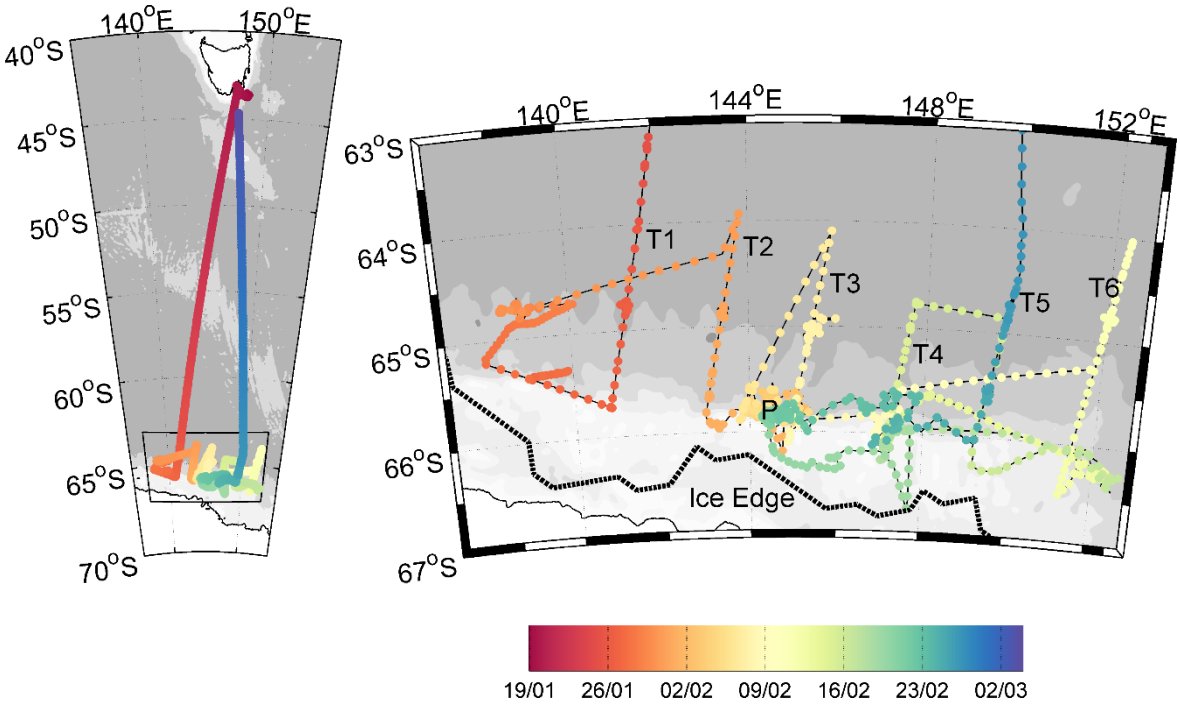


Figure 3.2.1: Map of voyage track including transit (left) and close-up of study site (right). Colour scale indicates the date and time. Labels T1-T6 indicate the broad-scale systematic transect lines. Label P indicates the process station. Black dashed line indicates the ice edge (10% sea ice concentration from AMSR2 satellite imagery) for 2019-02-14.

3.3 Weather and ice conditions

2019 was a “low ice” year and the survey area was already open by the time the ENRICH voyage departed Hobart. Fragmented sea ice was encountered on the shelf between Transects T5 and T2. No significant sea ice was encountered in other areas. Large icebergs were observed at the southern end of transects T2 and T5.

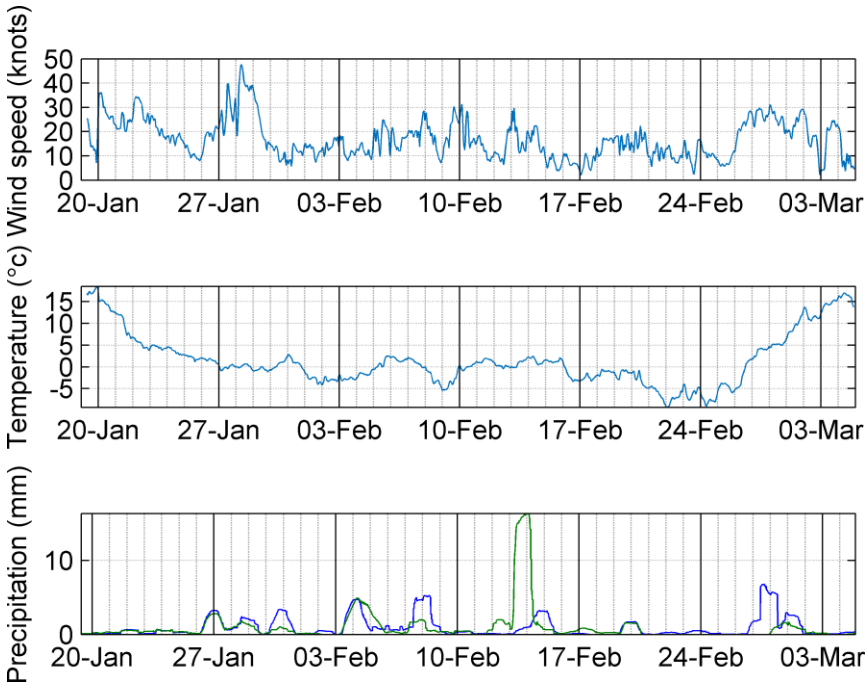


Figure 3.3.1: Summary of weather conditions during the ENRICH Voyage. The top two panels show hourly means for true wind speed and air temperature. The bottom panel summarises precipitation: the blue line represents ‘starboard cumulative day rain’ and the green line, ‘port cumulative day rain’. This figure was created using underway data during the voyage without quality control. The published underway weather data are available on the MNF data portal: *http://www.marlin.csiro.au/geonetwork/srv/eng/search#!2c516bf2-2420-44c1-8412-a0f70bb077aa*

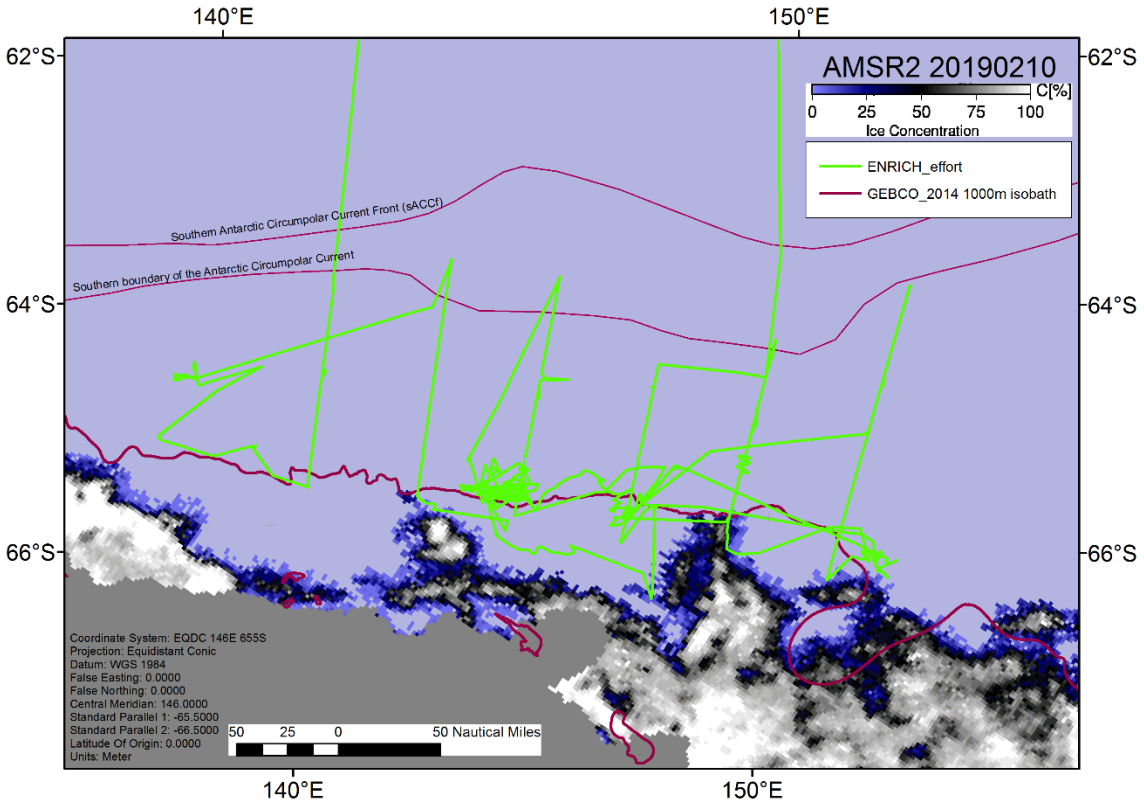


Figure 3.3.2: Ice imagery for 10 February 2019 showing AMSR2 sea-ice concentration data acquired 11/02/2019 and provided by ICDC, Universität. Hamburg.

3.4 Brief voyage narrative

RV *Investigator* departed from Hobart on 19 January 2019. Gear testing in sheltered Tasmanian waters, and transfer of remaining science personnel was conducted on the 19th and 20th of January. The ship headed nearly due south in transit to the Antarctic from 20-24 January undertaking passive acoustic and visual surveys for marine mammals. Antarctic science operations began on January 24th as the ship crossed the 60°S parallel.

Cold water echosounder calibration was conducted on the 25 January. On the 26 and 27 January the ship entered into "Transition phase" and began to conduct an initial site-survey (i.e. broad-scale systematic transects and passive acoustic tracking) to locate Antarctic blue whales. Poor weather was encountered on the 28 and 29 January and all science operations were stopped until the storm had passed. From the 30 January to the 2 February broad-scale systematic surveys resumed. The Biogeochemistry Process Station was conducted over the continental slope from 2-7 February and the Acoustic mooring was deployed on the 3 February. Krill surveys and blue whale studies were conducted during daylight hours around the Process Station. Broad scale systematic transects, small scale active acoustic surveys, and passive acoustic tracking were conducted from the 8 through 26 of February with intermittent periods of poor weather during this time used largely for transit between broad-scale transects. CTD and/or TMR stations were typically conducted most, but not all nights.

Return transit commenced on the 27 February, and the ship returned to Hobart on the 5 March.

The phases and modes of operation throughout the voyage are summarised in Figure 4.1.1. A detailed log of daily activities can be found in Appendix **Error! Reference source not found.**.

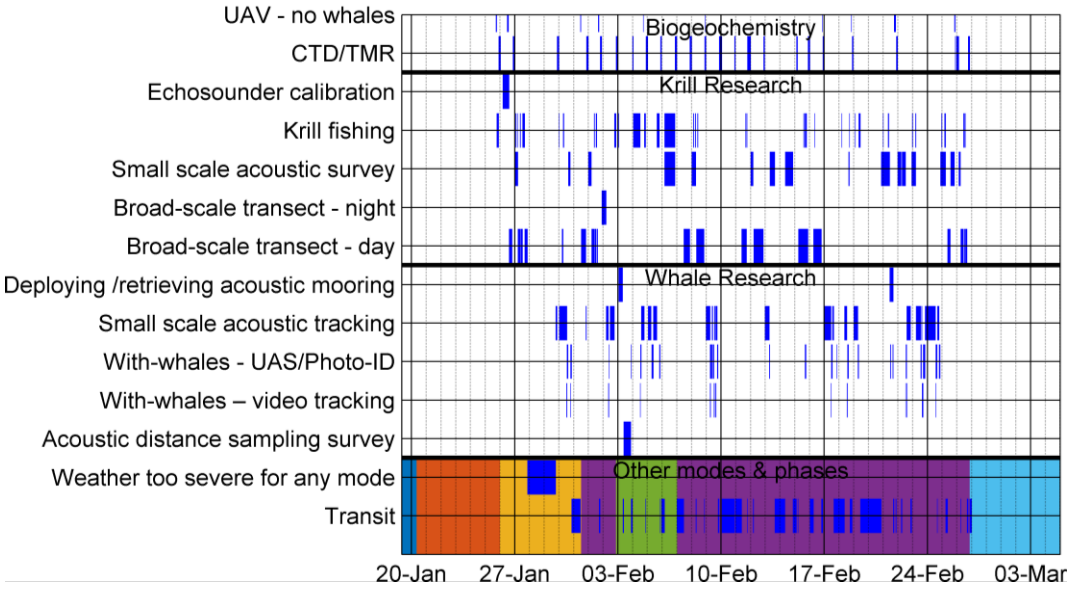
4 Scientific research and data streams

4.1 Active acoustics

4.1.1 Brief background

Numerous previous surveys have set out to describe and characterise the abundance and distribution of Antarctic krill (*Euphausia superba*) in the various regions of Antarctica. These have included, among others, the BIOMASS surveys of the 1980s (El-Sayed 1994), BROKE in (Nicol *et al.* 2000), the CCAMLR 2000 synoptic survey (Watkins 2004), and BROKE-WEST (Nicol *et al.* 2010b) in 2006. Together, these surveys have provided a wealth of knowledge and understanding regarding the biomass, distribution and habitat preferences of krill around the Southern Ocean, relying on well-established techniques (multifrequency scientific echosounders, and routine and target trawls, deployed on structured transects) to quantify and sample the krill populations in each area.

The ENRICH survey set out to build on this prior work, adding to the body of information on krill distribution and abundance in East Antarctica using the accepted methodologies, but also employing more advanced technologies to provide additional information on the 3-dimensional structural characteristics of the krill swarms detected. The survey also used a 'structured-adaptive' survey design to combine a standard krill acoustic survey with a targeted survey of the behaviour and distribution of one of their major predators, blue whales (*Balenoptera musculus*), using both passive acoustic and visual survey techniques.



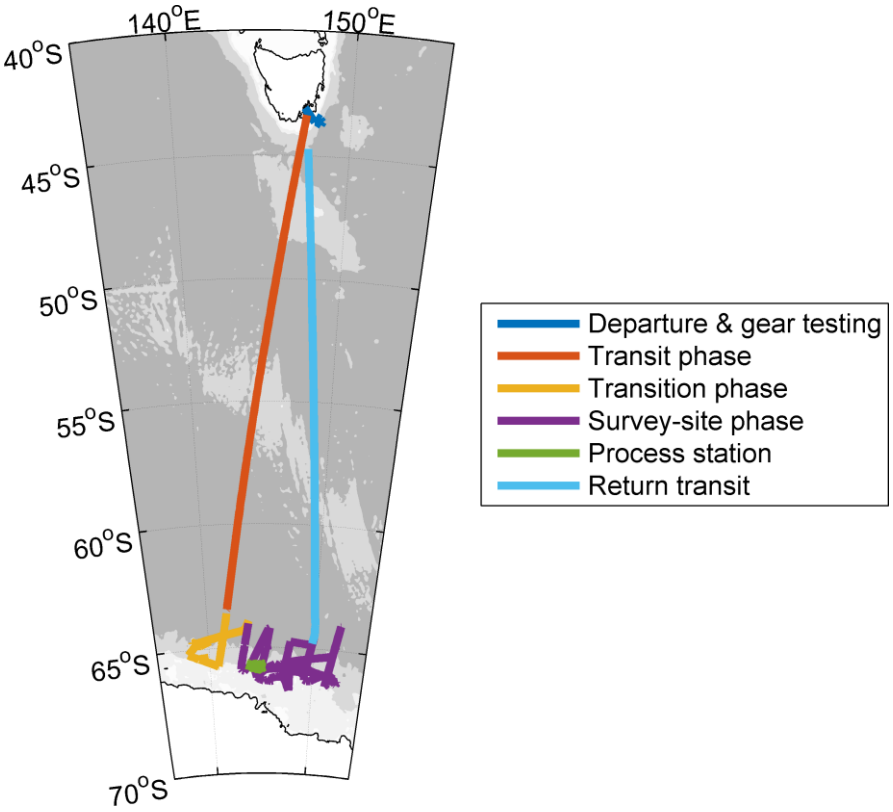
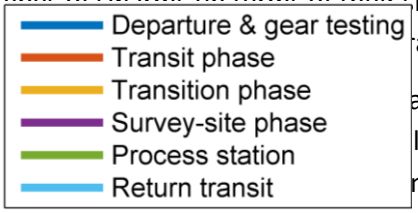


Figure 4.1.1: Timeline and map showing phases and modes of operation during the ENRICH voyage (local time AEDT)

The use of a scientific multibeam echosounder (Simrad ME70) allows measurements of the 3D geometry (volume, surface area, etc.) of krill swarms to be made, which would otherwise be impossible utilising only the standard downward-facing split-beam echosounder (Simrad EK60). Collecting concurrent data with these two technologies, therefore, allows the precise measurement of the density of a swarm (using the calibrated downward-facing sounder), as well accurately characterising its 3D structure (with the multibeam sounder). In combination, along with data from biological samples on the demography and morphometry of the krill within the swarms, these datasets will allow more detailed investigation of the spatial and behavioural ecology of this keystone species than has previously been possible. It will also allow the most detailed examination of the prey field of large baleen whales in the Southern Ocean to date, providing insight into the types of swarms (in terms of their size, shape and density) with which these predators associate, the characteristics found in areas containing and devoid of whales.



active acoustic data collected on krill (as well as utilising an array lows several questions to be addressed which may not otherwise nning survey effort over areas through which the RMT8 trawl net

has been passed, examining in more detail the apparent DVM behaviour of krill in this area through repeated day/night survey effort in the same area, as well as looking specifically at the 3D form of krill swarms on/with which large baleen whales are found to be feeding/associating.

As such, the survey intentions/questions for which the active acoustics team hoped to provide data were:

1) Quantify the biomass and abundance, and describe the broad-scale distribution of Antarctic krill throughout the entire survey region
2) Examine the environmental and ecological drivers of the observed krill distribution, including any spatial/temporal/environmental variation in the 3D swarm characteristics
3) Characterise the prey field in areas of high whale density, or in areas where whales are observed feeding (and contrast that characterisation with that found in areas of low whale density) in terms of krill density and swarm structure
4) Examine the effect of trawling on krill swarming behaviour by resampling the trawled-swarms with the multibeam echosounder and the ADCP (to provide information on the current field through which the krill area moving)
5) Examine the DVM behaviour of krill by re-running survey effort at night through areas previously surveyed during daylight hours

During data collection, two primary acoustic 'modes' were used, depending upon the activities of the vessel. During broad-scale transects, the EK60 and the ME70 were run continuously (synchronously pinging once every 1200ms), alongside the EM122 (the bathymetry multibeam echosounder) which was set to free-run based on the detected bottom depth. When significant echotraces were detected and net sampling or detailed acoustic mapping was to be undertaken, the SH90 (omni-directional sonar designed to assist fishing) was used, pinging alternately with the ME70 (i.e. every 2400ms, and in sync. with the EK60), the EM122 was switched off, and the high frequency ADCP (OS150) was turned on.

As such, in 'broad-scale transect mode', quantitative backscatter data was collected by the calibrated EK60, 3D swarm characteristics were collected by the ME70, and detailed bathymetry data were collected by the EM122. In 'krill mode', no further bathymetry data was required (since this effort was usually re-tracing previously surveyed ground) so the EM122 was unnecessary, and the ADCP provided data on the local current field, while the EK60 and ME70 continued to collect high resolution (lower ship speeds mean higher spatial resolution) backscatter data, and the SH90 provided information about targets ahead of the vessel so that sampling was more efficient and effective.

4.1.2 Preliminary results

An open-ocean cold-water EK60 calibration was carried out, beginning on the morning of 26th January 2019, at 141" 39.3' E, 64" 0.82' S. The calibration was carried out using standard protocols (Demer *et al.* 2015), using a 38.1mm tungsten-carbine sphere with 6% cobalt, calculating values for on-axis gain (g_0), the filter attenuation correction factor ($s_{a\ corr}$) and along- and athwartship 3-dB beam angles ($\alpha_{3\text{-}dB}$ and $\beta_{3\text{-}dB}$). All 6 frequencies (18, 38, 70, 120, 200 & 333 kHz) were calibrated, although the 333 kHz transducer calibration was of marginal quality (all others were very high quality, in terms of RMS error on beam model fit).

Acoustic data were collected continuously throughout the entire voyage, covering over 9,000 km acoustic transect south of 60°S. The broad-scale survey transects consisted of 1,670 km strict 'on-effort' survey tracks (Figure 4.1.2), comprising 6 roughly north-south transects (one of which, *T5*, was surveyed twice). Over the course of this effort, 975 krill swarms were detected by the EK60, and so had their acoustic density and their 3D structure recorded by the echosounders (Figure 4.1.3).

Krill were distributed throughout the survey region, with the highest densities in the western part of the region detected in areas near, but offshore from, the shelf-break (around the 1000m isobath), in contrast to the easternmost transects where the highest densities were found further north, in deeper water (Figure 4.1.2).

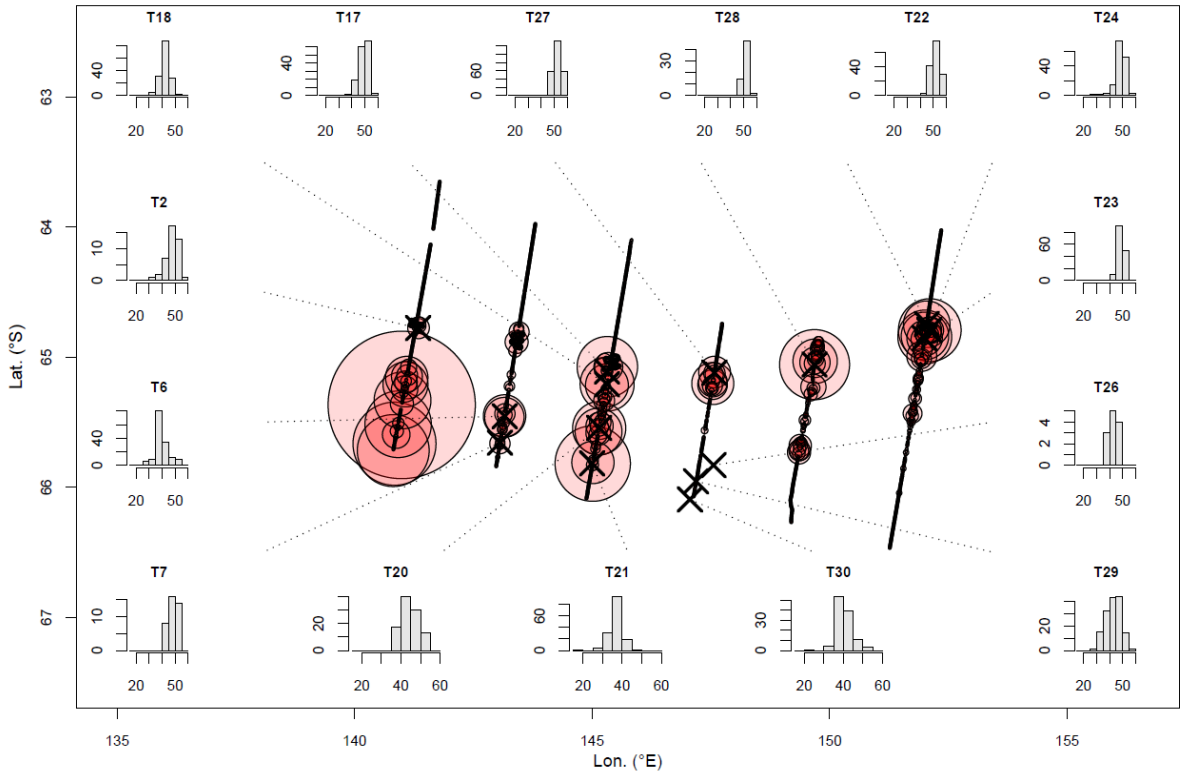


Figure 4.1.2: Broad-scale transect krill acoustic backscatter (NASC, $m^2n.mi^{-2}$) integrated across 1km segments of transect; diameter of the red circles is proportional to krill NASC. Length frequency distributions of krill caught in target trawls on (or very close to) the broad-scale transects, and their locations (black crosses) are also shown.

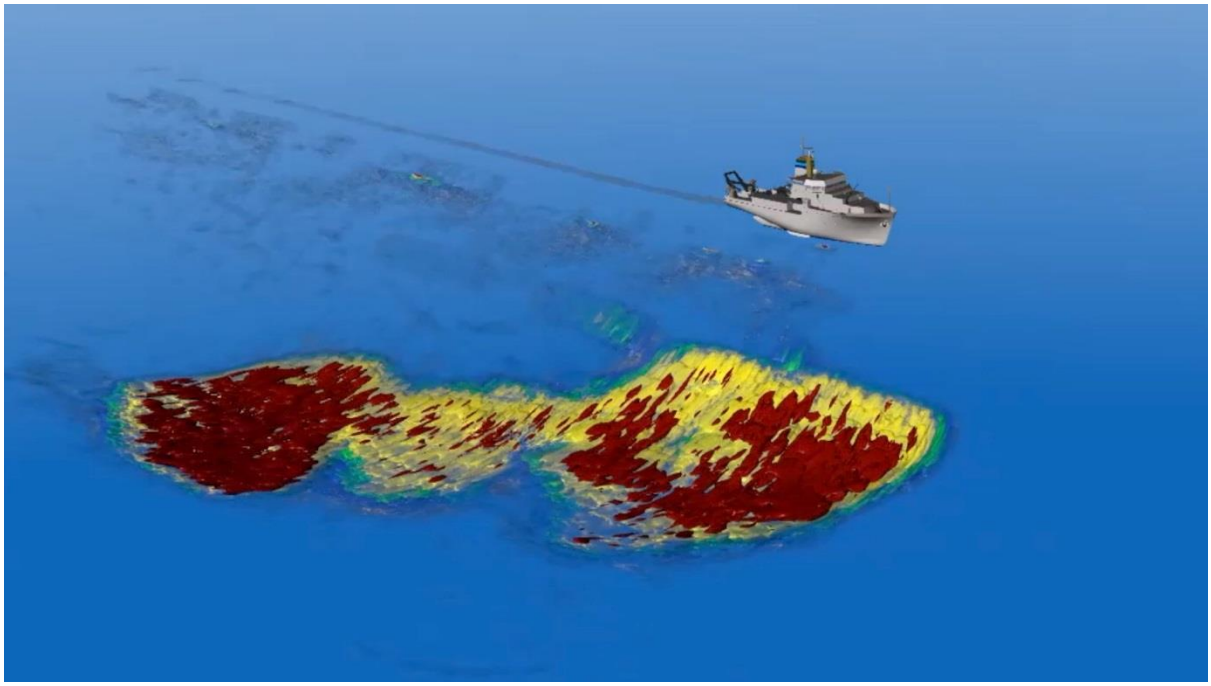


Figure 4.1.3: Example of a 3D model of an Antarctic krill swarm, 400 m long x 200 m wide x 100 m deep. Data collected using the ME70 echosounder © Australian Antarctic Division.

A series of 17 small- and meso-scale structured surveys were also completed, including several (9) at night, re-covering ground surveyed during daylight hours, some in the immediate vicinity of large baleen whales (e.g. after the completion of the video-tracking and photo-ID of a blue whale), and also re-sampling a swarm immediately following a successful trawl to examine the effects of trawling on swarm structure and dynamics (this occurred after 29 of the 41 trawls).

Table 4.1.1*: Summary of the active acoustic data obtained during ENRICH voyage*

Survey type	Effort
Broad-scale transect	1,670 km
Swarm 3D characterisation	975 swarms
Trawl effects	29 trawls (of 41 overall)
Night-time surveys	9
Small/meso-scale daytime surveys	8

4.1.3 Comments and scientific highlights

Overall, the voyage was a great success, with a very large quantity of high quality active acoustic data being collected. One of the most notable achievements was the open ocean cold water calibration which was completed efficiently and effectively by the MNF and active acoustic teams – this was critical to the success of the quantitative aspect of any future data analyses, and so to complete it at the very start of the voyage was a huge success.

Beyond collecting high quality, calibrated EK60 data along the broad-scale transects and throughout the rest of the survey region, there were several other scientific highlights. They included, on several occasions, collecting both EK60 and ME70 data on krill swarms in the immediate vicinity of feeding whales (in particular Antarctic blue whales). Additionally, several very significant swarms were encountered during the course of the survey (including one which was discovered, off track, in the

water below some feeding humpback whales), and efforts were made to measure their extent by encircling them using the ME70. Biological samples were also obtained (on one occasion, twice from one swarm) meaning data on the demography, density, and movement (because concurrent ADCP data were collected, so observed changes in the swarm can be described relative to the current field) of krill within these very large aggregations were collected.

Active acoustic data for this voyage are held and made available by CSIRO's Data Trawler and are available via the following link: https://www.cmar.csiro.au/data/trawler/survey_details.cfm?survey=IN2019%5FV01. Backups of the active acoustic data are also held at the AAD on the local intranet, and offline on the network attached storage (NAS) device that was used to backup data during the voyage. The files are stored in time and date stamped *.raw files, within the 'sounders' folder, and within a folder with the name of the device that collected the data (e.g. sounders/ek60/).

4.1.4 Potential papers

Paper title or scientific question	Likely lead
Krill biomass estimate	Cox
Krill distribution modelling (integrating underway/biogeochemistry data)	TBD
3D characteristics of krill swarms in the immediate vicinity of a feeding Antarctic blue whale (potentially investigating proportion of krill available to blue whales, based on observed characteristics, and include energy content data from demography/morphometrics to estimate 'value' to whales)	TBD
Investigating the effects of trawling on the swarming behaviour of Antarctic krill	Cox
Day/night krill density/swarm characteristics comparison – biomass data only	Cox
Day/night krill density/swarm characteristics comparison – detailed analysis of all data	TBD
Krill distribution/swarm characteristics as an explanatory variable for baleen whale distribution/behaviour (potentially multiple papers in this idea; e.g. krill density included in SDM for whales, then swarm 3D characteristics as a separate exercise)	Miller/Kelly/Cox
A comparison EK60 and ME70 distribution/maps	TBD

4.2 Krill biology

4.2.1 Brief background

Antarctic krill is a key component of the Southern Ocean food web, providing the link between the lower levels and higher-order predators. One of the key objectives of the ENRICH voyage is to test the hypothesis that the density, distribution, and fine-scale 3D structure of krill swarms influences the availability of krill as prey for krill predators and thus the behaviour of predators on their foraging grounds. Maturity stage composition and growth rates determined by the IGR technique from various swarms will provide fundamental information for krill population structure and their condition.

The main objectives of the krill biology team was to undertake biological sampling using RMT-8 net and to

1) Ground truth the object detected acoustically through active acoustic instruments, and collect information on krill demography that are required for deriving conversion factors for acoustic backscatter to biomass.
2) Collect information on maturity stage composition and growth rates from various swarms that will provide fundamental information for krill population structure and their condition.

A total of 41 target trawls were conducted using RMT 1+8 net during 6 broad-scale transects, passive acoustic transects, one mesoscale, 12 small scale active acoustic transects and 1 small scale mapping of a swarm by following the edge of swarm using multibeam and sonar.

4.2.2 Target trawling procedure:

When acoustic targets of interest that needed to be characterised were detected on the echosounder, or when large amount of live krill was required for growth experiment purpose, target trawls were performed using RMT 1+8. Once the position of the target was marked, the ship navigated to run over the target from the direction required within navigation capacity. The ship speed was reduced to 2.0 knots before hitting the target, so that the net could be lowered down to the desired depth when the net reached the target. Fine adjustments were made throughout the trawl by monitoring the echo-sounder EK 60 and Sonar SH 90 in the Operations room.

4.2.2.1 RMT-8 samples

If the catch included enough number of live krill in good condition suitable for growth experiment 288 krill were randomly sampled and used for Instantaneous Growth Rate (IGR) experiments. Gravid females in good condition close to spawning were also counted out of the catch, kept in spawning jars until they spawned. Larvae hatched out of eggs were kept in Kreisel tanks in the constant temperature lab until arrival in Hobart. Whenever possible, up to further 100 krill in good condition were snap frozen in liquid nitrogen and stored in -86C freezer for general biochemical analyses (Table 4.2.1). The remaining catch was visually sorted into taxa to the extent possible, and numbers counted. Up to 150 Antarctic krill were then measured for their size, sex, and maturity stages, and preserved in 10% formalin. Fish samples were preserved in 70% alcohol.

4.2.2.2 RMT-1 Samples

All RMT-1 samples were preserved in 10% formalin for later analysis back ashore.

Table 4.2.1: Summary of krill samples collected other than formalin preservation.

Trawl Number	Trawl type	Date (UTC)	Lat (decimal)	Long (decimal)	Total krill caught (Ind. Per haul)	Frozen Biochemical	Growth Rate Experiment	Genetics sample
T01	Target	NA	NA	NA				
T02	Target	26/01/2019	-64.78	141.33	60			
T03	Target	26/01/2019	-65.36	140.99	0			
T04	Target	27/01/2019	-65.72	140.82	0			
T05	Target	30/01/2019	-64.73	138.53	406	y		
T06	Target	31/01/2019	-65.46	143.15	1800	y	IGR-1	Y
T07	Target	1/02/2019	-65.66	143.06	42			
T08	Target	2/02/2019	-65.82	144.43	268			
T09	Target	3/02/2019	-65.66	144.46	5092	y	IGR-2	Y
T10	Target	3/02/2019	-65.82	144.80	1186	y	IGR-3	Y
T11	Target	3/02/2019	-65.83	144.79	232	y		
T12	Target	4/02/2019	-65.81	144.80	159			
T13	Target	5/02/2019	-65.70	144.63	2147	y	IGR-4	Y
T14	Target	6/02/2019	-65.57	144.01	775	y		
T15	Target	6/02/20019	-65.72	143.91	252	y		
T16	Target	7/02/2019	-65.06	145.51	0			
T17	Target	7/02/2019	-65.11	145.32	759	y		
T18	Target	7/02/2019	-65.21	145.32	4996	y	IGR-5	Y
T19	Target	8/02/2019	-65.55	145.15	65			
T20	Target	8/02/2019	-65.55	145.15	1007	y	IGR-6	Y
T21	Target	8/02/2019	-65.87	145.01	1503	y	IGR-7	Y
T22	Target	11/02/2019	-64.75	152.04	239	y		

T23	Target	11/02/2019	-64.86	151.99	543	y	IGR-8	Y
T24	Target	11/02/2019	-64.79	152.10	2550	y	IGR-9	Y
T25	Target	13/02/2019	-65.62	148.14	0			
T26	Target	14/02/2019	-65.83	147.55	12			
T27	Target	15/02/2019	-65.12	147.58	1799	y	IGR-10	Y
T28	Target	15/02/2019	-65.06	149.68	485	y	IGR-11	Y
T29	Target	18/02/2019	-65.96	147.19	466	y	IGR-12	Y
T30	Target	18/02/2019	-66.09	147.05	113			
T31	Target	18/02/2019	-65.95	147.70	7614	y	IGR-13	Y
T32	Target	20/02/2019	-65.88	144.56	21244	y	IGR-14	Y
T33	Target	20/02/2019	-65.88	144.52	21490	y		
T34	Target	20/02/2019	-65.86	144.52	1227	y	IGR-15	Y
T35	Target	21/02/2019	-65.87	144.61	4267	y	IGR-16	Y
T36	Target	22/02/2019	-65.81	144.85	5392	y	IGR-17	Y
T37	Target	22/02/2019	-65.78	144.62	12095	y		
T38	Target	24/02/2019	-65.90	147.32	6574	y	IGR-18	Y
T39	Target	24/02/2019	-65.83	148.32	17216	y	IGR-19	Y
T40	Target	25/02/2019	-64.94	149.73	652	y		Y
T41	Target	26/02/2019	-64.82	149.78	1390	y	IGR-20	Y

4.2.2.3 Instantaneous Growth Rate (IGR) experiments

Instantaneous Growth Rate (IGR) experiments were run on-board for 4 days following a successful krill trawl (successful: healthy and numerous individuals). Once a successful catch had been released into the cod-end tank in the Dirty Wet Lab, 288 krill were jarred up individually in 200mL jars, with holes to allow for continuous seawater flow during the experiment. 12 krill per trawl were snap frozen in liquid nitrogen, using a sterile technique between each krill (wiping utensils with ethanol and rinsing krill with Milli-Q water) for genetic microbiome analysis. A 2L water sample from the underway scientific seawater line was pumped through a Sterivex filter using a peristaltic pump, as soon as possible after each successful trawl.

Jars were checked every 12 hours for moulted animals, if the animal had moulted, the krill and its moult were measured as soon as possible on board using the Leica DF700 microscope. The first 12 krill and their moults from every experiment were preserved for krill microbiome analysis, using sterile genetic techniques between each krill. After these moults had been collected genetic sterile techniques no longer needed to be used which increased the speed at which krill could be measured at, checks were extended to every 24 hours rather than 12.

4.2.3 Preliminary results

4.2.3.1 Krill demography

Total of 41 target trawls were conducted to collect information on krill size, maturity stage composition and growth rates from various swarms in order to characterise krill population structure and their condition in Blue Whale feeding ground. Morphometric data from 4385 krill were measured during the voyage, with average size of 44.9mm (Table 4.2.2).

Table 4.2.2 - Statistics of krill morphometrics throughout the voyage.

Sex	N	Average (mm)	SD	Min	Max
Juvenile	2926	45.6	5.6	28.5	59.2
Female	301	35.2	4.2	19.5	49.5
Male	1150	45.6	4.3	28.0	59.7
Unidentified	8	40.1	6.2	48.7	34.4
All	4385	44.9	5.8	19.5	59.7

Length frequency distribution from all RMT trawls that caught krill are displayed in Figure 4.2.1. Maturity stage distribution in the broad-scale transect study area is shown in Figure 4.2.2. Our preliminary plot shows that krill population observed in the north east of the area along transects 4,5, and 6 largely consists of mature adult krill with spent females, indicating this part of the area was at the height of its reproduction. Krill population found in south-west of the area along transects 1,2, and 3, and southern end of transect 4 mainly consists of juveniles and sub-adults. This distribution (large mature krill in off-shore waters and smaller sub-adult and juvenile krill closer to the content in shallower waters) is a typical segregation pattern known for Antarctic krill during summer period, for reproductive female krill to lay their sinking eggs in deeper waters. We aim to undertake a more detailed and comprehensive analysis on their population parameters in relation to environment and bathymetry to describe status of krill population in the survey area.

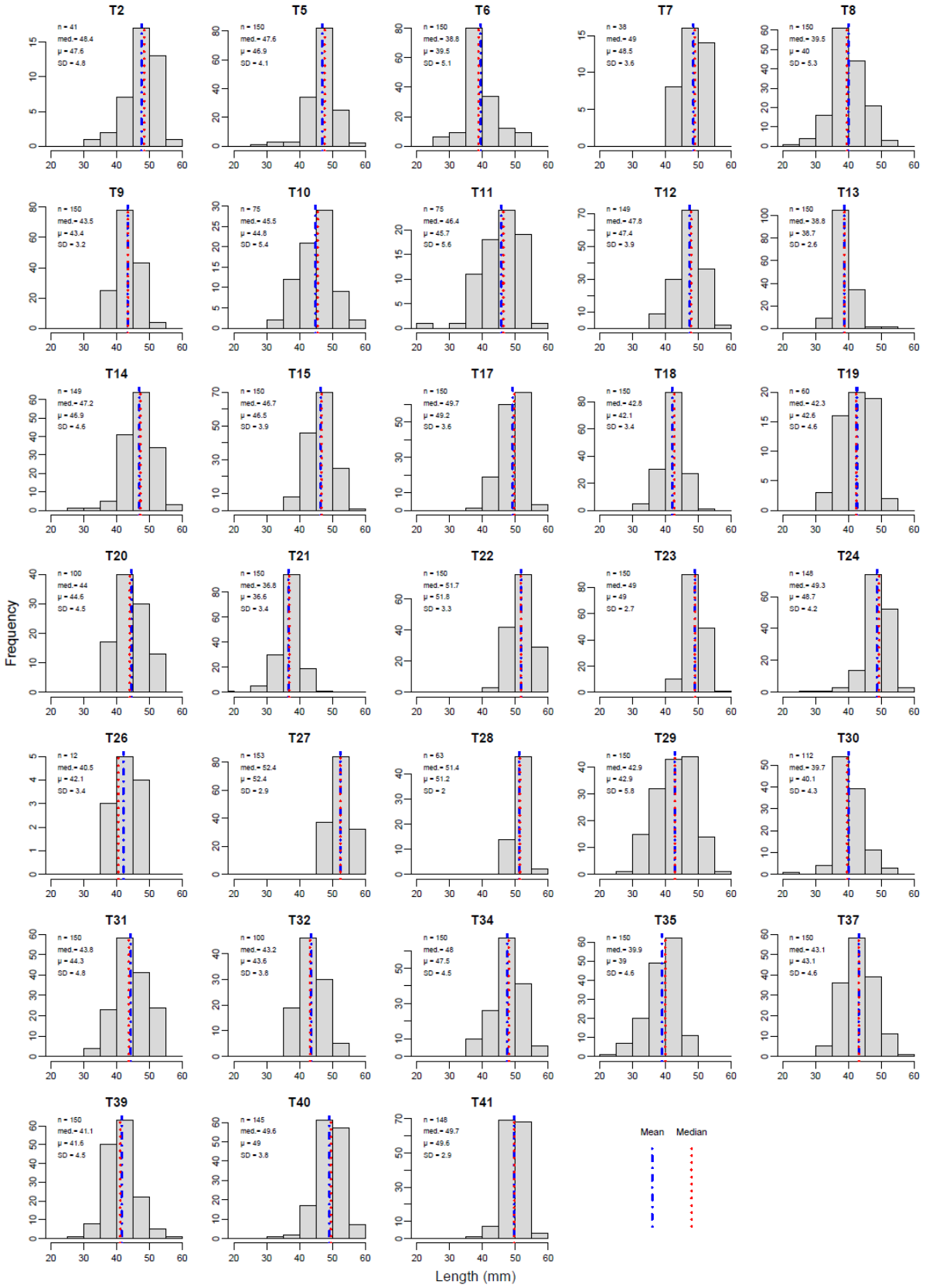


Figure 4.2.1: Length frequency distribution of krill from all RMT-8 trawls that caught krill.

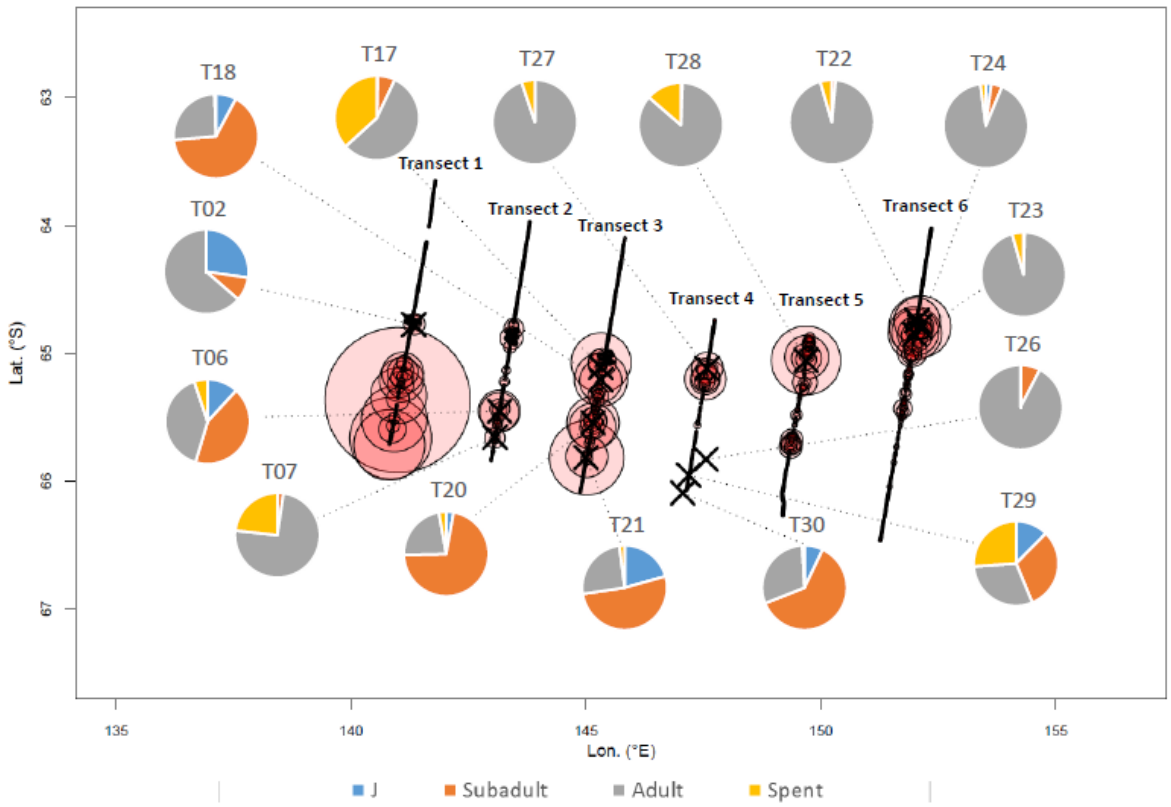


Figure 4.2.2:Maturity composition of krill caught during broad-scale line transect. Pink circle along transects: Acoustic krill density every kilometre. Pie graphs describe maturity composition of krill.

4.2.3.2 Instantaneous Growth Rate (IGR)

In total there were 20 IGR experiments conducted with:

- 5472 krill jarred over all the experiments
- 1002 krill moulted (18% moult success)
- 474 krill to microbiome project
- 20 water samples filtered for microbiome project

Using a new sex-dependent IGR method (Melvin *et al.* 2018), preliminary analysis suggested males and females exhibit different growth rates, with females growing slower than males (Figure 4.2.3). In particular, spent females showed negative growth. Further analysis will be undertaken by analysing inter-moult periods to derive daily growth rates of Antarctic krill in the survey area.

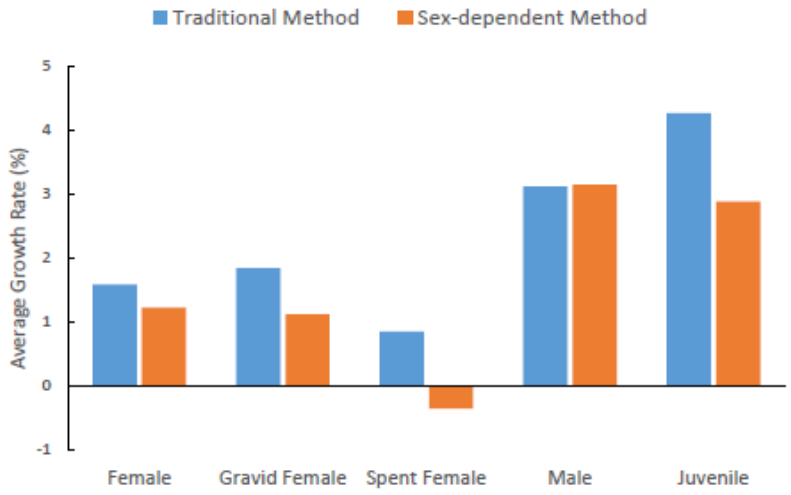


Figure 4.2.3: Sex and stage specific growth rates of krill using traditional and sex-dependent IGR method.

4.2.3.3 Live krill larvae

A total of 105 egg batches were spawned, and approximately 300,000 eggs were collected from gravid females caught throughout the survey. More than 10,000 early stage larvae were successfully maintained in 10 Kreisel systems in the constant temperature lab. These larval krill were brought back to AAD krill aquarium in Kingston to be used for various experiments including effects of climate change on krill early life stage and as starter of known age krill for refining and development of krill aging technique. Although it is possible to spawn and reproduce krill at AAD krill aquarium, this process is a bottle neck as it requires enormous effort to maintain early larval stage in the aquarium. Collecting large number eggs at sea and bring back is now proven to be an excellent way to supply larval krill to the aquarium for experimental purpose.

4.2.4 Comments and scientific highlights

4.2.4.1 Krill demography

This survey was the first time to systematically survey krill in relation to whale distribution. We were able to sample from a range of krill swarms for their sex, maturity composition and conditions. Some of these swarms were those fed actively by whales, and characterised acoustically for its structure in detail using ME-70. Although we will still need to wait for the results of the post-voyage analysis, the range of data collected will certainly give us a new insight into ABWs feeding strategy on krill.

4.2.4.2 Instantaneous Growth Rate (IGR) experiments

The completion of 20 IGR experiments was a huge success on this voyage and the measurement of all krill and their moults on board was an additional success. This voyage was an opportunity to implement revised IGR methods

4.2.5 Potential papers

Paper title or scientific question	Likely lead
Demography, distribution, and quality of krill as the ABW food source during ENRICH voyage.	So Kawaguchi
Can krill growth trends be described by environmental variables of the region?	Jessica Melvin

4.3 Passive acoustics

4.3.1 Brief background

Passive acoustic research during ENRICH expanded upon methods developed during previous Antarctic whale surveys (Miller *et al.* 2017; Miller *et al.* 2015; Miller *et al.* 2016). Methods employed during ENRICH involved structured passive acoustic monitoring of marine mammals using sonobuoys (Gales 2010; Gedamke and Robinson 2010), focusing on acoustic tracking of critically endangered Antarctic blue whales (Miller *et al.* 2015; Miller *et al.* 2016). This included a novel experimental design which aimed to robustly compare the number of calls detected with the number of animals seen in a given area.

The passive acoustic research conducted was fundamental in addressing two key science objectives of the voyage. These were:

1) Comparison of the krill prey field in the presence and absence of a large predator, blue whales, by remotely detecting and tracking the location of Antarctic blue whale aggregations and using active acoustics to map krill swarms.
2) Description of the distribution and behaviour of Antarctic blue whales on foraging grounds by investigating the relationships among vocalisations, density, movements and surface behaviour, and comparing the local prey field around whales exhibiting different behaviours.

4.3.1.1 Methods

We carried out passive acoustic surveys for blue whales and other marine mammals throughout the ENRICH voyage using sonobuoys. Sonobuoy deployments occurred around the clock with listening stations conducted by pairs of acousticians. Passive acoustic research took the form of both broad-scale structured surveys and fine-scale adaptive surveys depending on the operational mode of the ship. Regardless of the mode of operation, listening stations were conducted by deploying SSQ955 HIDAR sonobuoys in DIFAR (standard) mode to monitor for and measure bearings to vocalising whales while the ship was underway (Miller *et al.* 2015).

4.3.1.2 Visual and acoustic distance sampling survey

On 3 February 2019, a novel type of survey was conducted to estimate the total number of Antarctic blue whale visual and acoustic detections in a predefined area. The chosen area was evaluated to have a moderate density of Antarctic blue whales based on sightings and acoustic detections from the previous day. This Acoustic Distance Sampling Survey (ADSS), involved deploying triplets of sonobuoys to precisely triangulate the locations of calls while simultaneously driving a modified sawtooth line-transect visual survey through the area to estimate the number of whales within the vicinity. The purpose of this survey was to test whether there is a relationship between estimates of animal density and call density.

4.3.1.3 Active and passive acoustic mooring

Participation by Kate Stafford and Ana Širović in the voyage was supported by the US National Science Foundation and IWC-SORP funding. In addition to participating in sonobuoy data collection, they also contributed an acoustic mooring consisting of a SIMRAD Wide-Band Autonomous Transceiver (WBAT) with two upward looking transducers (70 and 200 kHz) that emitted continuous-wave and frequency-modulated signals for approximated 4 minutes out of every 10 minutes and a High-frequency Acoustic Recording Package (HARP) that was set to record continuously at 200 k Hz sample rate. The data from the mooring will provide high temporal resolution information on co-occurrence of baleen whales and their krill prey.

The acoustic mooring was deployed on 3 February at approximately 2300 m depth (65°50.210' S, 144°26.047' E) such that the HARP and WBAT would be approximately 350-375 m from the sea surface (Figure 4.3.1). The mooring was deployed in the process station area, where intensive acoustic and oceanographic sampling proceeded to occur for five days after the deployment, which will provide larger spatial-coverage data to complement the moored data. The mooring collected high quality data for the first week after deployment, through February 10, and more limited data from 11 February until its recovery on 21 February when the top floats were observed at the surface. This could have been due to line stretch post-deployment or incorrect line-length.

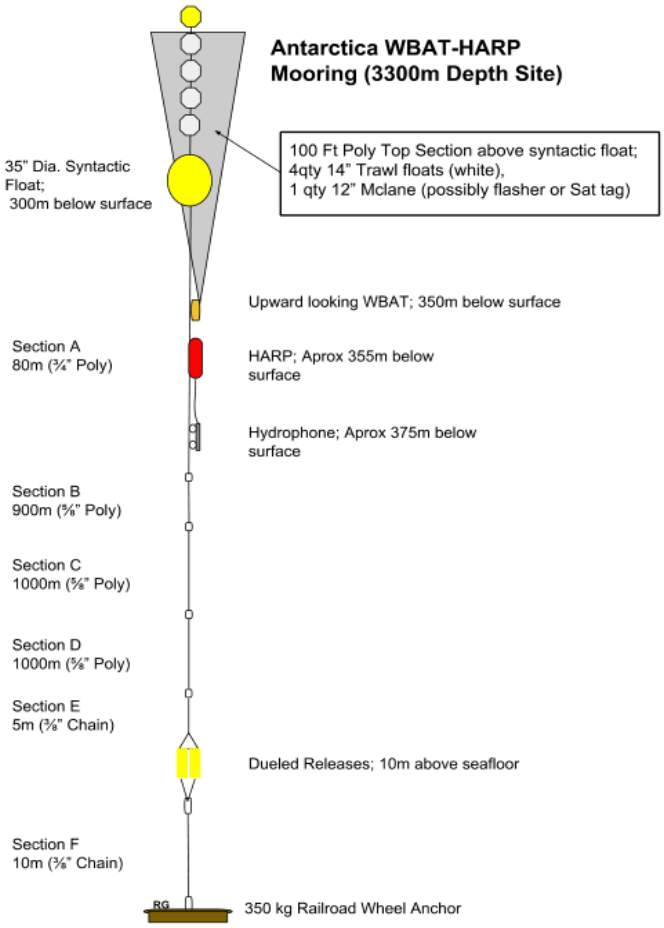


Figure 4.3.1: Schematic detailing the intended configuration of mooring.

4.3.2 Preliminary results

Passive acoustic monitoring via sonobuoys was successfully conducted throughout the voyage. Over the duration of the voyage, 10 different species of marine mammal were acoustically detected in the study area (Table 4.3.1); some were detected much more frequently than others. Antarctic blue whales were detected most commonly, both during transit and in our survey area, with a total of 33,435 calls detected across 238 sonobuoys. During transit, the calls from southeast Indian Ocean blue whales and southwest Pacific blue whales were also detected (Figure 4.3.2).

Whilst blue whale calls were heard on most sonobuoys throughout the area (Figure 4.3.3), the detection rate, bearings, and received level of blue whale calls suggested that blue whales were mainly distributed in the southern part of the study area. Blue whales were seen both in deep water near the continental slope and on the shelf.

Table 4.3.1: *Summary of sonobuoy deployments*

Sonobuoys deployed	295
Sonobuoys failed	15
Sonobuoy-hours total	828.8
Hours with only 1 sonobuoy	368.2
Hours with sonobuoy pairs	150.6
Hours with sonobuoy triplets	55.2
Total time recorded	574.0

Species/type of sound	**Number of buoys with detections**
Antarctic blue whale unit 'a'	238
Antarctic blue whale unit 'b'	154
Antarctic blue whale Z-call	123
Blue whale FM (D-call)	198
Southeast Indian blue	15
Southwest Pacific blue	9
Fin whale FM (downsweep)	150
Fin whale 20 Hz pulse	111
Humpback whale	105
Minke whale	4
Sei whale	0
Southern right	1
Sperm whale	65
Odontocete Whistle	82
Pinniped	38
Crabeater seal	4
Ross seal	1
Weddell seal	0
Leopard seal	37
Seismic airguns	0
Ice	242

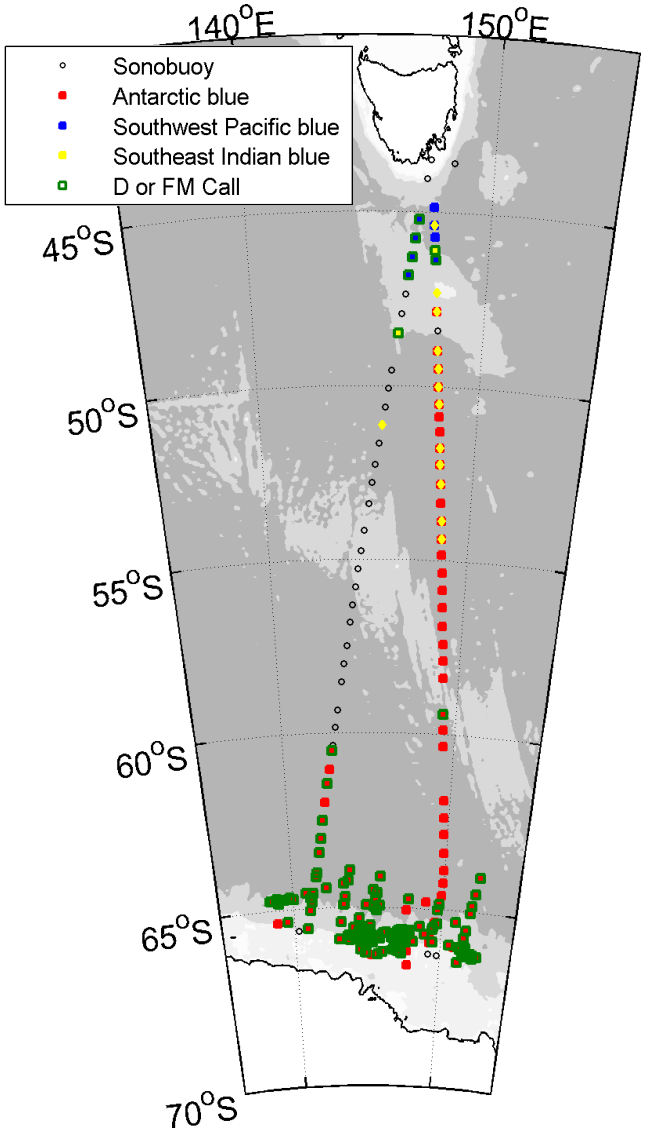


Figure 4.3.2: *Summary of sonobuoy deployments (certain or probable detections only; table) and map of blue whale calls by subspecies/population (certain or probable only).*

Other species detected broadly throughout the study area included fin whales (Figure 4.3.3), humpback and sperm whales (Figure 4.3.4), as well as leopard seals and odontocetes (pilot and/or killer whales; Figure 4.3.5). Minke whales, sei whales, crabeater seals, and Ross seals were detected at only a small number of listening stations (Figure 4.3.5).

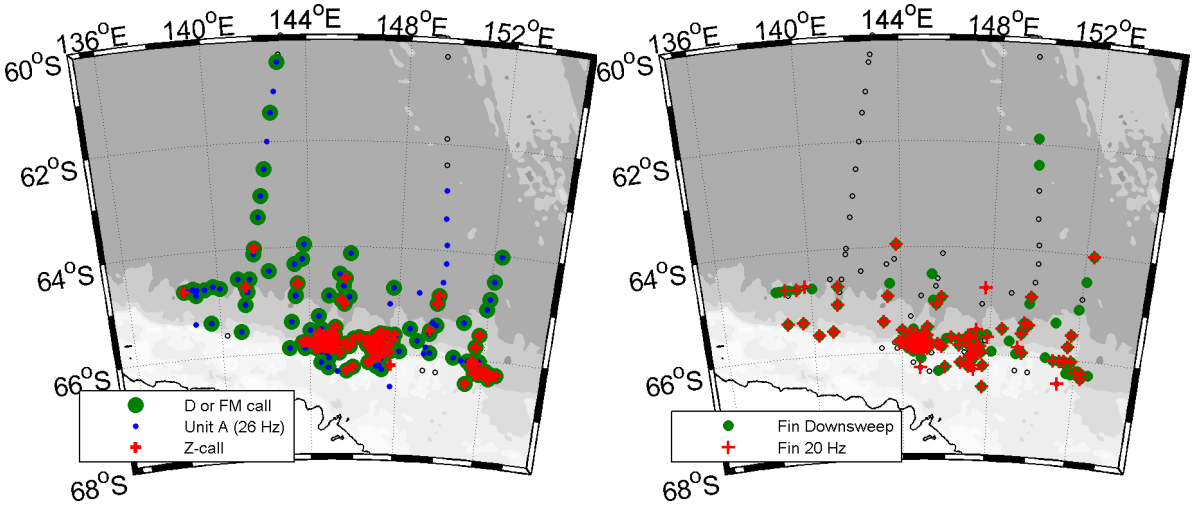


Figure 4.3.3*: Sonobuoys with detections of blue whale sounds (left) and fin whale sounds (right) in the Antarctic study area during 2019 ENRICH voyage.*

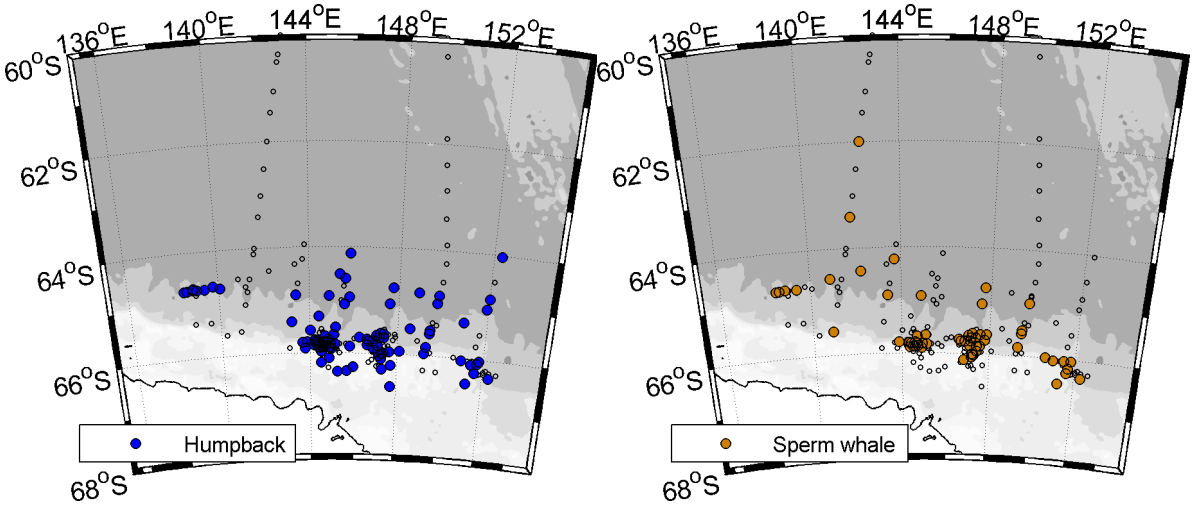


Figure 4.3.4*: Sonobuoys with detections of humpback whale sounds (left) and sperm whale sounds (right).*

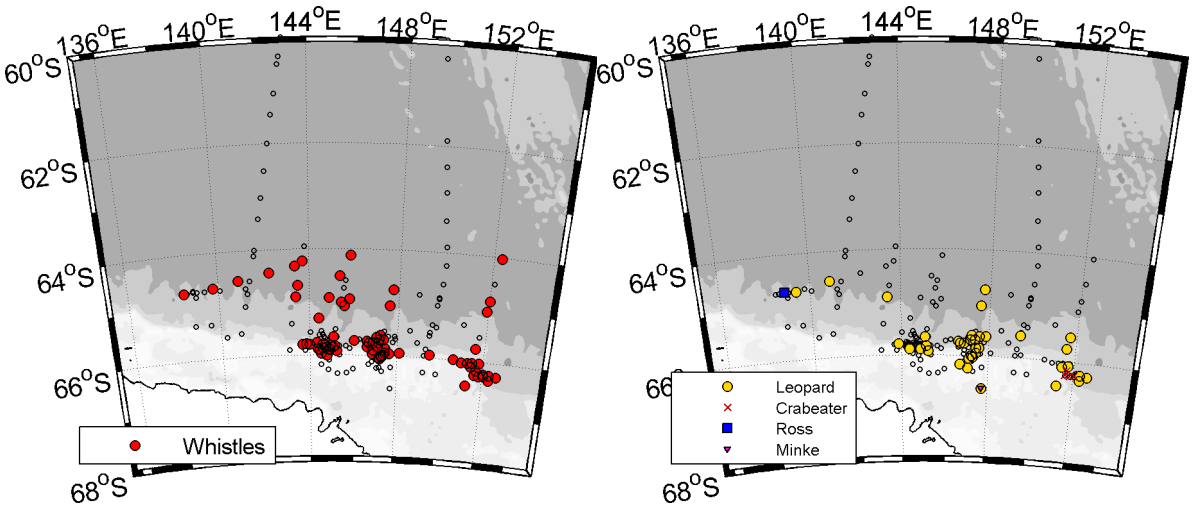


Figure 4.3.5*: Sonobuoys with detections of odontocete whistles (left) and leopard seal and other less commonly detected species of marine mammals (right).*

The acoustic distance sampling survey was conducted from 2-3 February from 22:00 – 10:00. During this time three triplet arrays of hydrophones were deployed along a modified sawtooth survey. The survey was conducted throughout an area where Antarctic blue whales had been heard the previous day. During this survey 549 blue whale calls and 37 fin whale calls were triangulated in real-time with pairs or triplets of sonobuoys (Figure 4.3.6).

During the survey from 04:00-05:00 and from 07:00-09:00 the ship passed close to the triangulated locations. Unfortunately, there were no confirmed visual observations of Antarctic blue whales at any time during the survey, though there were sightings of fin whales and unidentified large baleen whales throughout the survey. Towards the end of the survey at 10:00 a decision was made to use the last remaining daylight to amend the last transect line so that it passed directly through the most recent triangulated position of Antarctic blue whales. Along this last survey line in amongst numerous sightings of fin whales there was eventually a sighting of a blue whale.

With no sightings of blue whales, the survey could not provide a measure of the relationship between the number of blue whales seen and the number of their calls detected in the area. However, such a result might be achievable for fin whales, for which there were numerous sightings though fewer calls that could be triangulated. Thus the concept and design of the survey was demonstrated to be viable -- at least for some density of whales and call rates.

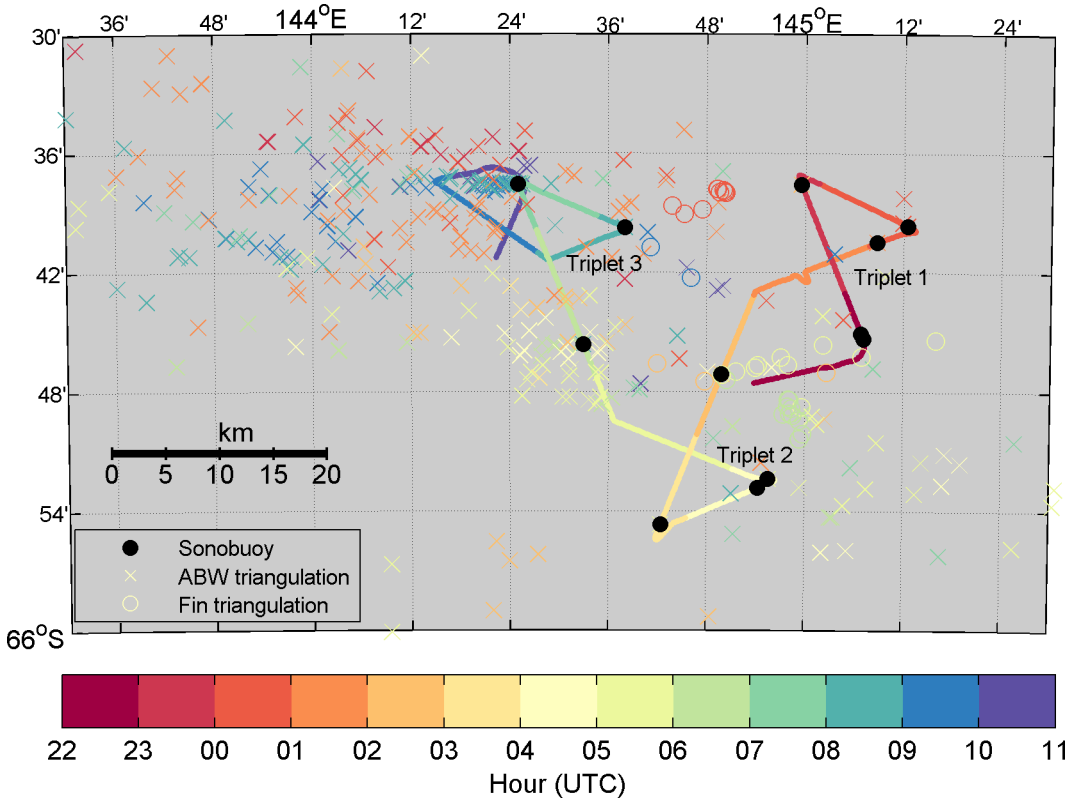


Figure 4.3.6*: Visual and acoustic distance sampling survey conducted from 02 February 22:00 - 03 February 10:00 UTC. The solid line shows the ship's track. Coloured crosses show triangulations of Antarctic blue whale calls. Coloured circles show triangulations of fin whale calls. Colour of ship track and triangulations represents the hour of the survey. Black circles indicate sonobuoy deployment locations.*

Based on preliminary data inspection, the HARP (moored passive acoustic recorder) collected data over 16 days of the deployment; the first four days of data were good quality but due to strumming in the mooring line, data quality started to deteriorate after that and became very poor by the end of the recording. The deteriorating data quality was believed to arise from the realised depth of the HARP and WBAT being much closer to the surface than the intended 350 m. Nevertheless, blue and fin whale calls were abundant through the recording. In addition, killer whale and long-finned pilot whale whistles and echolocation clicks were also regularly detected in the recordings. Initial analysis of the WBAT data indicates variability in the surface backscatter over the duration of deployment. More detailed quantitative analyses of these data sets are underway.

4.3.3 Comments and scientific highlights

The consistent and high-quality passive acoustic data collected during the ENRICH voyage will allow investigation of a number of questions regarding the distribution of Antarctic blue whales and the properties of their acoustic signals. These investigations will focus on the relationship between received level, propagation, and distance to received calls and on environmental correlates of blue whale distribution, krill in particular.

4.3.4 Potential papers

Paper title or scientific question	Likely lead
Distribution of marine mammals from passive acoustic data in East Antarctica (135-150 E) (IWC paper)	Brian Miller
Behavioural ecology of calling Antarctic blue whales (video tracks & sonobuoy data)	Brian Miller
Blue whale call source levels (AKA How loud are Antarctic blue whlaes?)	Brian Miller
Whales and krill from moored recorders	Ana Širović
Association between calling whales (call specific) and krill from targeted small-scale surveys – extension of E Miller paper	TBD
Survey design for assessing the cue rate of calling baleen whales (Shock and awe): fin whales (v blue whales?)	Miller/Kelly

4.4 Visual observations, photo-identification and video tracking

4.4.1 Brief background

The main objectives of the visual observations were

(i) To describe the distribution patterns and estimate the density of the most commonly encountered species (humpback, fin and blue whale) across the study region based on line-transect analyses from broad scale transects

(ii) To relate estimates of Antarctic blue and fin whale numbers from visual observations with passive acoustic data at a range of spatial scales (from individual groups to the whole study region)

(iii) To collect data on behaviour, blow rates and small-scale movements of individual Antarctic blue whales through focal follows (video tracking) to compare with simultaneous acoustic data

(iv) To approach Antarctic blue whales for photo-id and UAV flights

The adaptive nature of the study and the use of passive acoustics to direct the vessel towards aggregations of vocalising whales required a number of different effort modes with different protocols in order to allow data to be analysed appropriately.

These effort modes were:

(a) Line Transect (LT). The vessel was transiting on a pre-determined transect that was independent of any prior knowledge of whale locations. Closing was only undertaken for species identification, with the vessel then returning to the point where the track line had been left.

(b) Acoustic Distance Sampling (VC). The vessel was transiting on pre-determined transect lines which had been selected within an area of vocalising Antarctic blue whales based on passive acoustic data.

(c) Acoustic Bearing (AB). The vessel was searching for blue whales based on passive acoustic localisations. This effort cannot contribute towards a detection function for blue whales since the vessel was often heading directly for an acoustically derived location.

(d) Visual searching (VS). There were occasions when the vessel was not following pre-determined transects and observers were on Deck 05, but also not following any acoustic bearings. This effort is suitable for estimation of detection functions.

(e) Video Tracking (VT). Following a sighting of a blue whale, the vessel would remain 1-2km away to allow behavioural observations and monitoring of small-scale movement patterns. UAV flights were undertaken if the whale came close, prior to switching to photo-id.

(f) Photo-id (PI). The vessel moved in for a close approach 100-300m for photo-identification. It was assumed that the whale's behaviour might be affected by the vessel at these distances.

4.4.1.1 Visual surveys

Visual observations were made from observation boxes on a forward facing platform (Deck05) directly below the bridge (Deck height 16.9m, average observer eye height 18.36 (standing) or 18.07 (sitting). Observers scanned with naked eye or 7x50 binoculars. 25x Big Eye binoculars were used for species identification, but not for scanning. If weather conditions were not suitable for outside observations, systematic watch was kept from the bridge with two observers (average eye height 20.65m).

Effort that could be used to estimate detection functions included Line Transect, Acoustic Distance Sampling and Visual Searching. These all used the same configuration of observers in the observation boxes. Bridge only effort was not used to estimate detection functions because there were few sightings in this mode.

Data on survey effort, sightings and environmental conditions were collected using the Logger software[1] which also collected data from the ship's system (GPS positions and headings).

4.4.1.2 Photographic identification

Obtaining a current estimate of abundance is considered key for the assessment of the status of the Antarctic blue whale population and in monitoring its recovery (Double *et al.* 2015). One of the long-term goals of the IWC-SORP's Antarctic Blue Whale Project is to deliver a precise abundance estimate for Antarctic blue whales (Peel *et al.* 2015). Photo-ID data collected during the voyage are intended for inclusion in a database (the Antarctic Blue Whale Catalogue) that will be used in a capture-recapture approach to estimating abundance. A larger data set will improve previous estimates of abundance of Antarctic blue whales using this method (Olson *et al.* 2018). The data will also be used to examine movement patterns in the Antarctic region.

4.4.1.3 Focal follows (photogrammetric video tracking)

Video tracking involved focal follows of individuals or groups of Antarctic blue whales using video and audio commentary to record behaviour and photogrammetric methods to measure the location of each surfacing relative to the ship. These methods involved using a video camera with a calibrated

[1] The Logger 2000 software was developed by the IFAW to promote benign and non-invasive research

lens to measure the distance to the animal based on the angle of dip from the horizon and a downward pointing still camera to measure bearings (see Leaper and Gordon 2001) for a description of the methodology).

4.4.2 Preliminary results

4.4.2.1 Visual surveys

The total amount of visual effort in the different modes is presented in Table 4.4.1. A number of environmental variables that may affect the probability of detection were recorded, including nautical visibility, cloud cover, glare and sea state. In addition, a single 'sightability' category (1 to 5 scale) was based on an estimate of the ease with which a blue whale blow would be seen in the conditions. The amount of effort in each of these sightability categories is shown in Table 4.4.2. All sightings by species code are presented in Table 4.4.3

Maps illustrating the distribution of visual effort and sightings of blue, fin, and humpback whales can be found in Figure 4.4.1, Figure 4.4.2, Figure 4.4.3, and Figure 4.4.4 respectively. Distance sampling models and histograms of perpendicular distances for these species can be found in Figure 4.4.5.

Table 4.4.1: *Amounts of effort (distance [km] and time [h]) in different effort types (between 2019-01-20 08:51:34 UTC and 2019-02-28 05:33:35 UTC.)*

	Effort [km]	Time elapsed [h]
AB	547.2	44.1
BO	1027.2	66.7
CO	94.5	8.2
LT	2274.8	144.1
PI	192.1	23.0
VC	238.2	17.4
VT	97.4	13.9
Total	**4471.4**	**317.3**

Table 4.4.2: *Amounts of effort (distance [km] and time [h]) in different sightability conditions (between 2019-01-20 08:51:34 UTC and 2019-02-28 05:33:35 UTC.)*

	Effort [km]	Time elapsed [h]
Very Poor	211.6	17.2
Poor	780.9	49.5
Moderate	1002.6	67.6
Good	1378.9	96.7
Excellent	1061.9	82.7
Total	**4471.4**	**317.3**

Table 4.4.3: *All sightings (Bridge and Deck05 combined) by species code. Unid = unidentified.*

Species code	Total Sightings	Total individuals
Antarctic Blue whale	26	36
Antarctic minke	23	30

Crabeater seal	2	9
Cruciger dolphin	4	29
Fin or blue	9	22
Fin whale	124	306
Humpback whale	201	373
Killer whale	5	165
Killer whale type A	1	10
Killer whale type C	2	65
Like Antarctic blue	2	4
Like Antarctic minke	3	3
Like blue whale	4	5
Like fin whale	16	31
Like humpback whale	28	42
Like minke	3	3
Like southern bottlenose	1	5
Long finned pilot whale	1	22
Pilot whale	4	79
Right whale	1	1
Sperm whale	4	4
Unid dolphin	1	1
Unid large baleen	87	121
Unid large whale	9	11
Unid small whale	5	6
Unid whale	5	6

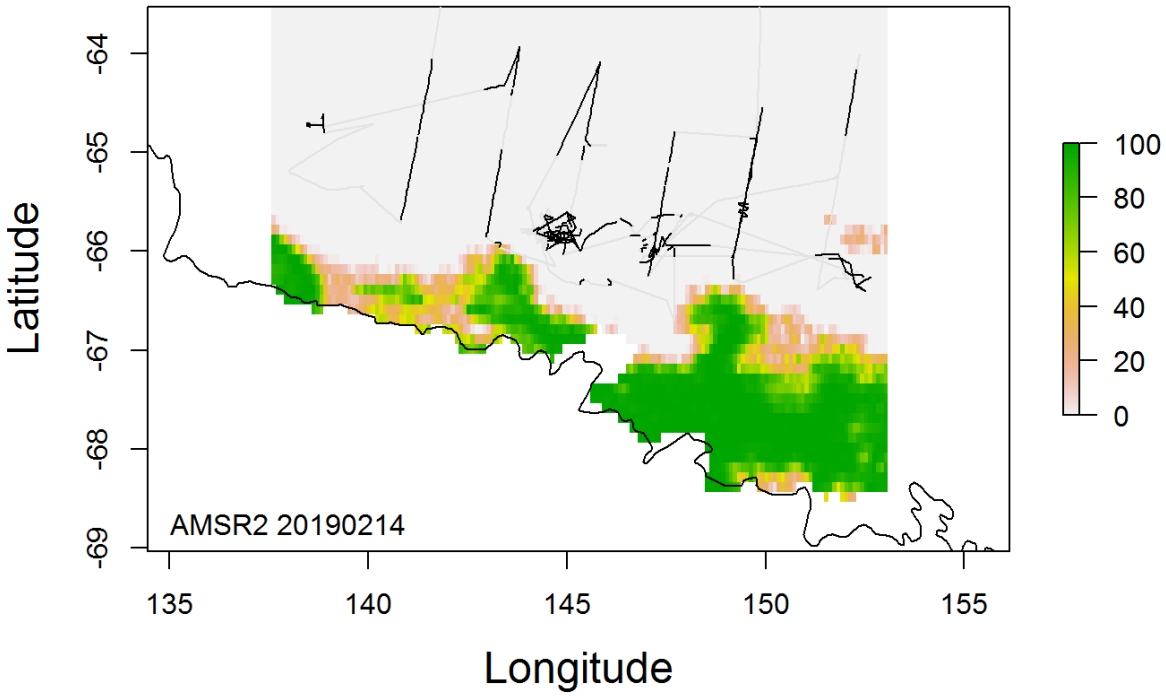


Figure 4.4.1*: Effort in ENRICH survey + AMSR2 sea ice concentration data (scale given in plot legend). Black line indicates primary effort (viz. 'AB', 'LT' and 'VC' effort); grey line indicates off effort. Map has no projection.*

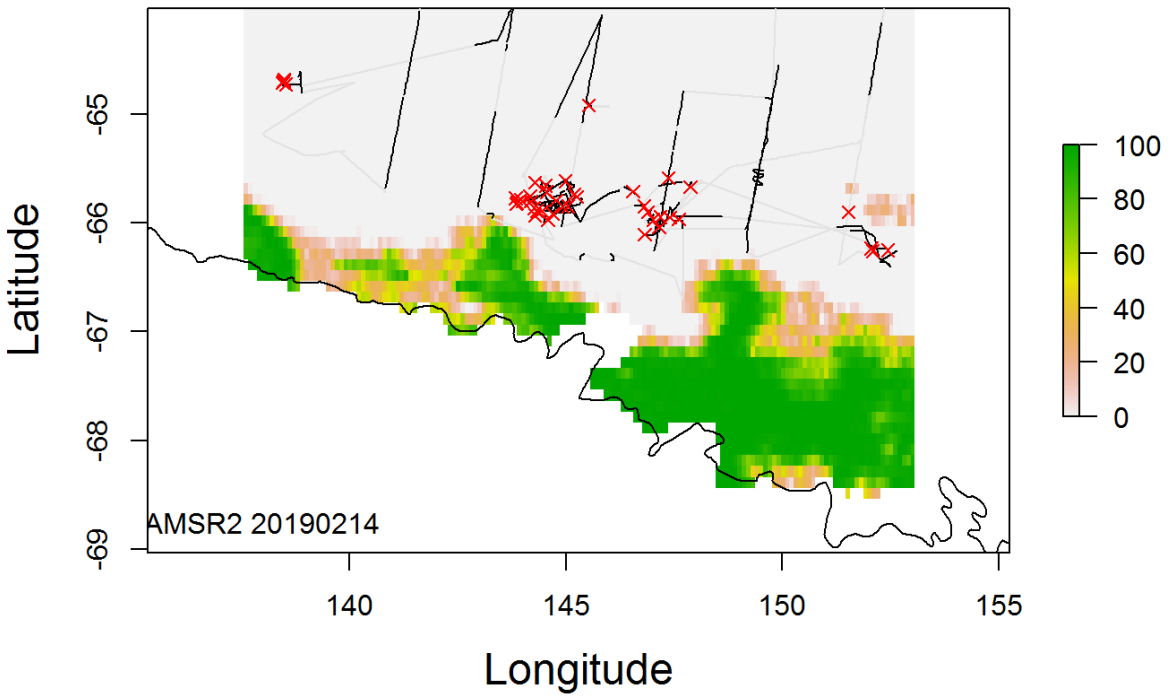


Figure 4.4.2*: Distribution of all blue whale sightings (red crosses).*

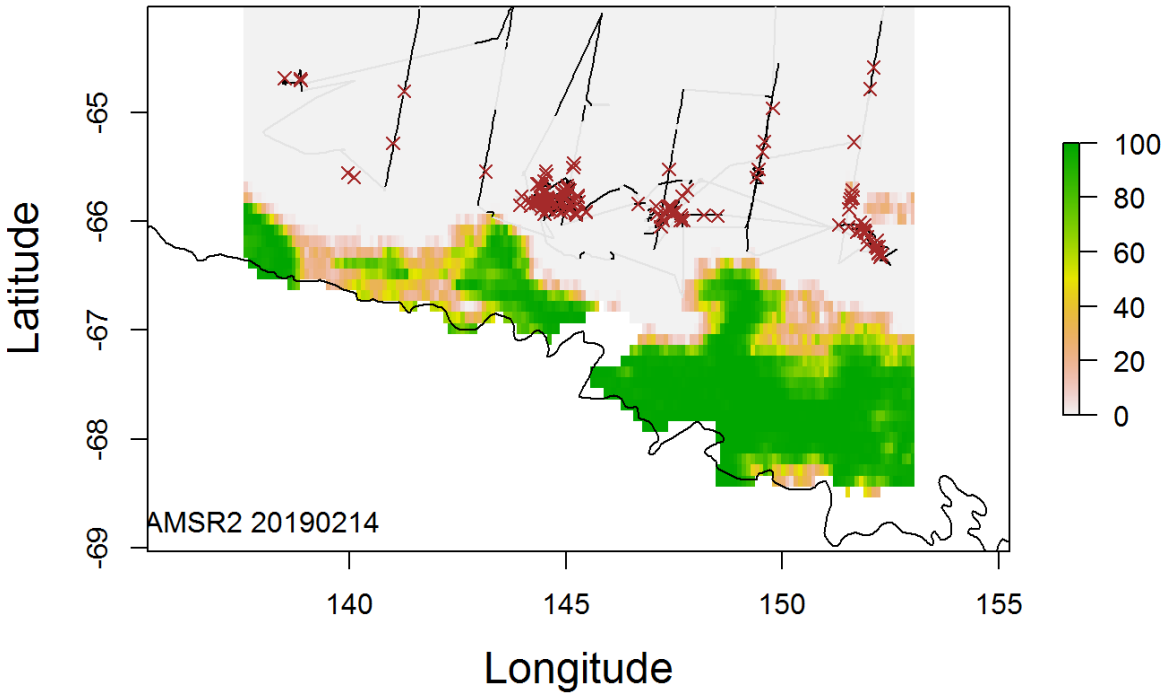


Figure 4.4.3*: Distribution of all fin whale sightings (brown crosses).*

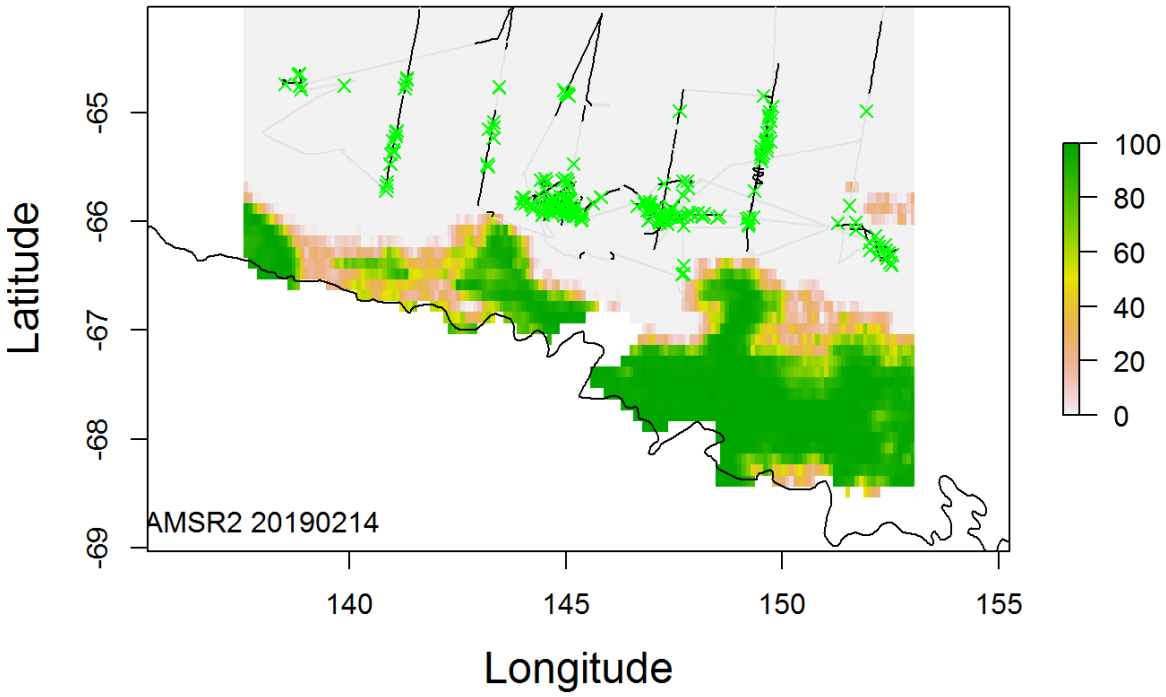


Figure 4.4.4: *Distribution of all humpback whale sightings (green crosses).*

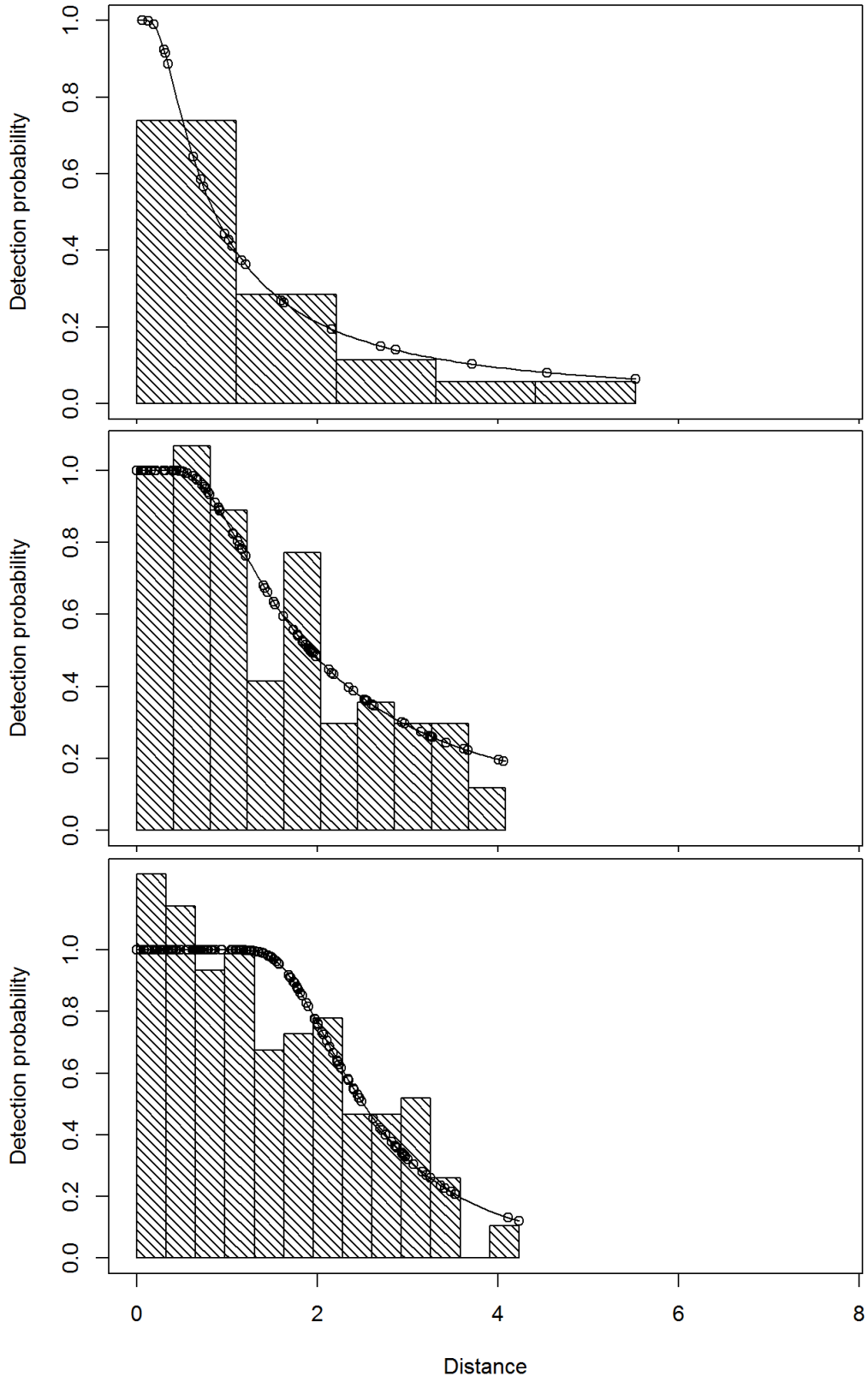


Figure 4.4.5: Distance sampling model fitted assuming detection is certain on the trackline [g(0)=1] and overlaid onto the scaled histogram of perpendicular distances (km). Top panel BLUE whales (and like); Middle panel FIN whales (and like). Bottom: HUMPBACK whales (and like).

4.4.2.2 Photo-identification

Individual blue whales can be identified from the unique patterns of mottled pigment in the area of the dorsal fin on both sides of the body and also from variations in dorsal fin shape (Figure 4.4.6). 19 groups of blue whales were approached for photo-ID, and we were successful in obtaining ID images from 17 of these. Photographs of 25 blue whales were obtained for individual identification from 30 January to 24 February (Table 4.4.4). In addition to the blue whales, six individual fin whales and six individual humpback whales were photo-identified opportunistically. Four groups of killer whales were photographed.

Figure 4.4.6*: Identification photographs of two individual Antarctic blue whales, illustrating the difference in individual mottling patterns (Paula Olson, AAD).*

Table 4.4.4*: Summaries of photo-identified blue whales, fin whales, and humpback whales, and killer whales during the ENRICH Voyage 2019. (u = undetermined).*

Species (common name)	UTC Date	Sighting number	Latite	Longitude	Number whales photo-ID'd
Antarctic Blue	30 Jan	056	-64.72911	-138.601	4
Antarctic Blue	30 Jan	059	-65.63302	-140.3414	4
Antarctic Blue	02 Feb	119	-65.80116	-144.1965	1
Antarctic Blue	03 Feb	166	-65.63494	-144.2878	1
Antarctic Blue	04 Feb	169	-65.7969	-144.7914	1
Antarctic Blue	04 Feb	189	-65.77968	-143.943	1
Antarctic Blue	05 Feb	221	-65.77319	-143.8428	1
Antarctic Blue	05 Feb	222	-65.82431	-143.8474	4
Antarctic Blue	08 Feb	269	-65.83778	-146.7836	1
Antarctic Blue	08 Feb	287	-65.89951	-146.8928	1
Antarctic Blue	09 Feb	296	-65.97265	-147.0104	1
Antarctic Blue	09 Feb	300	-66.05742	-147.0977	2
Antarctic Blue	17 Feb	395	-66.24996	-152.4417	0
Antarctic Blue	18 Feb	468	-65.95614	-147.2067	1
Antarctic Blue	18 Feb	482	-65.97171	-147.5999	1
Antarctic Blue	22 Feb	526	-65.84869	-144.9317	1
Antarctic Blue	23 Feb	579	-65.70022	-146.5187	2
Antarctic Blue	24 Feb	599	-65.95843	-147.4126	1
Antarctic Blue	24 Feb	609	-66.11347	-146.8437	1
Fin whale	29 Jan	053	-64.72679	-138.8763	1
Fin whale	30 Jan	062	-64.68977	-138.4943	1
Fin whale	21 Feb	NA	-65.83902	-144.4292	1
Fin whale	04 Feb	173	-65.78477	-144.8838	2
Fin whale	24 Feb	594	-65.91879	-147.4641	1
Humpback whale	26 Jan	NA	-64.85308	-141.2567	1
Humpback whale	15 Feb	337	-65.98862	-147.6315	3
Humpback whale	15 Feb	349	-65.17678	-149.6447	1
Humpback whale	17 Feb	460	-65.96046	-147.3326	1
Killer whale	02 Feb	086	-66.00783	-144.585	u
Killer whale Type C	09 Feb	288	-65.90595	-146.8946	1
Killer whale Type C	17 Feb	459	-65.9543	-147.3336	u
Killer whale Type C	24 Feb	600	-65.95509	-147.419	u

4.4.2.3 Video tracking

Video tracking was conducted on 24 occasions for a total of 18 hours, mainly with Antarctic blue whales but some fin whales were tracked to obtain surfacing rate data to help improve the line transect estimates (Table 4.4.5). Several of the tracks were in close proximity to sonobuoy deployments and should allow comparison of visual and acoustic behaviour.

Table 4.4.5*: Video tracking during the voyage.*

TrackNumber	StartTime	EndTime	Duration
1	30/01/2019 01:22:45	30/01/2019 02:27:49	01:05:04
2	30/01/2019 07:31:12	30/01/2019 07:48:32	00:17:20
3	01/02/2019 21:33:17	01/02/2019 22:26:21	00:53:04
4	03/02/2019 01:21:10	03/02/2019 02:16:39	00:55:29

5	03/02/2019 05:14:57	03/02/2019 06:25:23	01:10:26
6	03/02/2019 06:33:49	03/02/2019 07:47:48	01:13:59
7	03/02/2019 07:48:48	03/02/2019 08:08:43	00:19:55
8	03/02/2019 09:36:13	03/02/2019 09:39:26	00:03:13
9	03/02/2019 10:01:09	03/02/2019 10:01:49	00:00:40
10	04/02/2019 01:23:53	04/02/2019 02:35:23	01:11:30
11	04/02/2019 19:08:48	04/02/2019 19:52:19	00:43:31
12	08/02/2019 19:11:12	08/02/2019 20:35:02	01:23:50
13	08/02/2019 23:38:00	08/02/2019 23:55:20	00:17:20
14	09/02/2019 00:01:26	09/02/2019 00:01:55	00:00:29
15	09/02/2019 01:54:13	09/02/2019 01:55:05	00:00:52
16	09/02/2019 04:34:54	09/02/2019 04:39:42	00:04:48
17	16/02/2019 23:56:02	17/02/2019 01:00:14	01:04:12
18	18/02/2019 01:52:26	18/02/2019 03:01:17	01:08:51
19	18/02/2019 19:32:35	18/02/2019 20:53:38	01:21:03
20	22/02/2019 01:28:07	22/02/2019 01:56:47	00:28:40
21	22/02/2019 03:50:13	22/02/2019 04:08:37	00:18:24
22	23/02/2019 04:32:05	23/02/2019 06:11:26	01:39:21
23	24/02/2019 02:32:30	24/02/2019 03:43:36	01:11:06
24	24/02/2019 08:12:37	24/02/2019 09:09:46	00:57:09

4.4.3 Comments and scientific highlights

The density of Antarctic blue whales in the study area was low relative to other areas, with only one sighting of a single individual on the broad scale transects. Hence it is not possible to calculate a density estimate from the line-transect data. However some bounds on density may be estimated using a combination of the acoustic data (to indicate distribution patterns), the line-transect data (for an upper bound) and photo-id resights (for a lower bound).

It should be possible to generate density estimates for fin and humpback whales although these will have wide confidence intervals due to the relatively sparse coverage.

Unlike previous voyages (Double *et al.* 2013; Double *et al.* 2015), blue and fin whales were frequently found together (in 2013 only 3% of fin whales sighted were within 5 km of a blue whale), with lower average group sizes of blue whales (1.4 on this voyage compared to 2.1 in 2013) and a higher proportion (67% of single individuals compared to 45% in 2013). Blue whales cannot be distinguished from fin whales just by their blows, making species identification problematic in areas with large numbers of fin whales such that it is not practical to close on every potential sighting. While fin whales can be identified at a distance from the fin, blue whales often show very little body and require a closer approach for positive identification. Some of the blue whales encountered had dive times of over 10 minutes with only 2-3 blows at the surface, making close approaches difficult.

One blue whale was recognized in field as an individual previously photographed in 2013 (ID #1306) due to its distinctive dorsal fin. The distance between the two sighting locations in 2013 and 2019 is 384 km.

The RV *Investigator* proved more effective at getting close enough for photo-ID images than might be expected for such a large ship (93.9 m, 6082 GT). RV *Investigator* is considerably larger than the *Amatal Explorer* (65 m, 1400GT) or *Tangaroa* (70 m, 2291GT) which have been used on previous voyages to work around Antarctic blue whales.

RV *Investigator* conforms to the DNV Silent-R class notation from Det Norske Veritas which specifies the allowable radiated noise for research vessels in the frequency band 10 Hz to 80 KHz. Measurements have also shown that the radiated noise from RV *Investigator* is below the ICES209 standard. The quiet vessel likely contributed to the lack of observed behavioural responses of whales and allowed for photo-ID approaches from such a large vessel.

The video taken from the UAVs contributed to a better understanding of behavioural observations and particularly the ability to identify feeding activity.

4.4.4 Potential papers

Paper title or scientific question	Likely lead
Photo-identification of Antarctic blue whales during the ENRICH research voyage 2019	Paula Olson
Cross-platform calibration of blue whale identification photographs	Paula Olson
Capture-recapture estimates of abundance of Antarctic blue whales	Paula Olson
Movement patterns of Antarctic blue whales	Paula Olson and/or Virginia Andrews-Goff
Review of the spatial and temporal distribution of Antarctic blue and fin whales in the Southern Ocean	Natalie Kelly
Small-scale movement and surfacing patterns of Antarctic blue whales from video tracking: comparisons with other species [results of video tracking from blue whale and other voyages]	Susannah Calderan and Russell Leaper
See section 4.3.4 for papers related to combined analysis of blue whale visual and acoustic data including relationships with krill distribution	

4.5 Unmanned Aerial Vehicles (UAVs)

4.5.1 Brief background

The use of Unmanned Aerial Vehicles (UAVs) (also referred to as Unmanned Aerials Systems (UAS), Remotely Piloted Aircraft (RPA) and/or drones), in scientific research is increasing rapidly due to their ability to provide an often safer and more cost-effective solution to conventional manned aircraft. Specifically, UAVs are becoming increasingly popular in marine wildlife research with applications to marine mammals including behavioural observations, abundance surveys, photo-identification, photogrammetry and blow sampling. One of the greatest advantages of using UAVs is the ability to non-invasively sample marine mammals that are inherently difficult to study because they spend long periods of time underwater. Furthermore, there is an increase in the number and accuracy of data streams that can be collected using UAVs. UAVs are already an integral tool in scientific research across a range of disciplines and their use in the future will only continue to grow as the technology improves.

On the *ENRICH* voyage, a **DJI Inspire 2** (Figure 4.5.1) and **DJI Phantom 4 Pro V1/V2 multi-rotor quadcopter** (Figure 4.5.2) were used to achieve the following objectives:

1. Photogrammetry of whales for length measurements
2. Whale 'blow' sampling for health assessment
3. Photo-identification of whales
4. Surface water sampling for trace metal analysis
5. Visual surveying of whale aggregations/behaviour
6. Video and image collection of whale and scenic imagery

4.5.2 Preliminary results

Over a period of approximately 35 days, 134 UAV flights were undertaken for a range of activities including; photogrammetry, photo-identification, whale 'blow' sampling, surface water sampling, general whale and scenic imagery and surveillance for acoustic mooring retrieval (Table 4.5.1). Of the 134 flights, the media team undertook 113 flights using the **DJI Inspire 2** and the AAD science team 21 flights using the **DJI Phantom 4**. Overall, this meant 33 (25%) science flights (15% AAD science and 9% media team) and 101 (75%) general whale and scenic imagery flights for media purposes. The average flight duration achieved by the media team was 12.16 min, and 15.6 min for the AAD science team.

All objectives for the use of the UAVs were met. Photogrammetry video data was collected for a total of 8 individual Antarctic blue whales and this will be used to obtain length measurements for each whale. One blow sample was successfully obtained from an Antarctic blue whale for post-voyage bacterial microbiome analysis of the pulmonary system (Figure 4.5.2B).

For the first time, the DJI Phantom 4 Pro was equipped with water sampling bottles attached by a nylon line to the UAV, and used to collect water samples (Figure 4.5.2A). Six flights were conducted to collect surface water samples (n=17) for trace metal analysis. Four flights collected 11 samples of surface seawater near two icebergs (<300 m and ~50 m distance from the ship); two flights collected 6 samples close to the RV *Investigator* to undertake a quality control analysis of the influence of the ship on trace metal samples.

Figure 4.5.1*: The DJI Inspire 2 ('media') UAV used to collect scientific data and general whale and scenic imagery. © Peter Shanks, MNF.*

Table 4.5.1*: Summary of UAV flights conducted on ENRICH Voyage*

Date	No. flights	Activity
24-Jan-19	2	Scenic
25-Jan-19	3	Scenic
26-Jan-19	3	Scenic
30-Jan-19	7	Photo-ID
01-Feb-19	5	Scenic
03-Feb-19	3	Photogrammetry
04-Feb-19	10	Scenic
05-Feb-19	9	Photo-ID
09-Feb-19	3	Scenic
11-Feb-19	1	Scenic
15-Feb-19	5	Scenic
16-Feb-19	3	Scenic
17-Feb-19	8	Blow sampling
18-Feb-19	10	Photo-ID/Behaviour/Photogrammetry
19-Feb-19	3	Photo-ID/Behaviour/Photogrammetry
21-Feb-19	12	Water sampling
22-Feb-19	5	Photo-ID/Photogrammetry
23-Feb-19	16	Photo-ID/Photogrammetry
24-Feb-19	18	Photo-ID/Behaviour/Photogrammetry
25-Feb-19	5	Water sampling/Calibrations
04-Mar-19	3	Scenic
TOTAL	**134**	

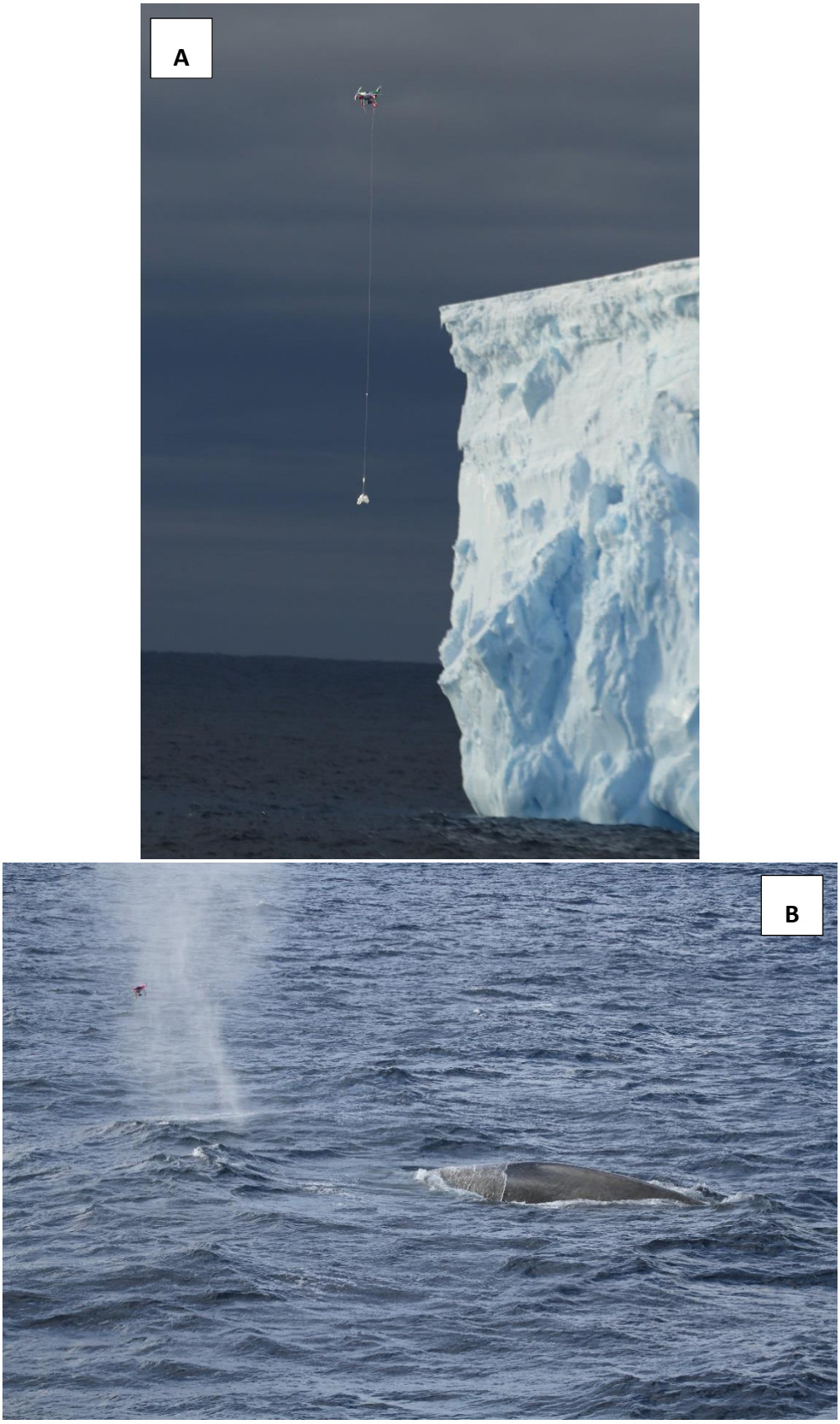


Figure 4.5.2*: The DJI Phantom 4 Pro V1/V2 multi-rotor quadcopter ('science') UAV, A) sampling surface water near an iceberg © Alex Vail; and B) collecting a blow sample from an Antarctic blue whale © Charlotte Boyd, AAD.*

4.5.3 Comments and scientific highlights

The use of UAVs aboard the RV *Investigator* proved highly successful for the collection of scientific data, general whale and scenic imagery, and ship operations involving gear retrieval. Overall, there were approximately 138 successful flights using the DJI Phantom 4 Pro and DJI Inspire 2, involving hand launch and retrieval of the UAVs. Initial testing of the ability to fly the UAVs in P-mode (GPS positioning mode) determined that GPS coverage and positioning was too unreliable to undertake due to proximity to the pole. Consequently, all UAV flights were undertaken in A-mode (Attitude mode) and all obstacle avoidance sensors were disabled to facilitate the launch and retrieval off the bow of the vessel.

A UAV highlight was the successful collaboration between the trace metal analysis team and the UAV science team to achieve one of the first applications of UAVs for the collection of surface seawater samples (see Section 4.5.2).

The biggest impediment to the use of UAVs for scientific purposes during the voyage was the prescribed distance limit. The AAD science and media teams were approved to operate under two different distances from the ship, 400 m and 800 m respectively. This was predominantly due to a requirement by MNF for the UAV teams to adhere to the Civil Aviation Safety Authority (CASA) regulations requiring that the UAV is maintained within Visual Line of Sight (VLOS). However, early advice from the CASA legal team to the AAD science team was that CASA does not have authority in the Southern Ocean (i.e. in the voyage survey area) and therefore UAV operations do not need to adhere to CASA regulations. A strong recommendation following this is that CSIRO/MNF UAV operational procedures need to be aligned with CASA regulation/authority in Antarctica and Southern Ocean waters. Prior to the voyage, it was understood by the AAD science team that CASA had deemed the RV *Investigator* a Commonwealth entity and a Commonwealth entity ReOC was necessary to operate UAVs off the vessel. This highlights the degree of confusion by CASA and applicants to CASA, surrounding the regulations for operating UAVs in Southern Ocean and Antarctic waters.

For future UAV operations in the Southern Ocean and Antarctic waters, it is recommended that special approvals and exemptions are sought for operating Beyond Visual Line of Sight (BVLOS) if it is deemed that CASA has jurisdiction in these areas and that CASA regulations should be adhered to. There needs to be a realistic risk assessment performed and a balance struck between the requirement to have the UAV in visual line of sight and some dependence on the UAV video controller feedback. The distance limitations did impact the collection of scientific data resulting in extremely small sample sizes for activities such as whale blow sampling (n=1). The collection of photogrammetry data necessitated the assistance of the UAV media team because they were able to sample the whales at greater distances from the ship. It is strongly recommended that MNF/CSIRO revisit this distance restriction to ensure that scientific opportunities with UAVs remain internationally competitive and the use of this exciting observation tool is optimised relative to its capability.

The UAV science and media teams worked closely and effectively together. However, the occurrence of two UAV teams operating from the RV *Investigator* with similarly aligned objectives in combination with the differing distance restrictions, did limit the number of opportunities for some

UAV science team operations, e.g. blow sampling. This was because 'with whale' time was at a premium and there were often few opportunities following a one-hour video tracking of whales, to have the whales consistently close (< 400m) to obtain whale blow samples. Furthermore, close approaches by whales were often opportunistic and it was difficult to balance the flights of the UAV media team who were often already in flight to utilise the time when whales were further away (> 400m), with that of the UAV science team when whales opportunistically came close.

4.5.4 Potential papers

Paper title or scientific question	Likely lead
Photogrammetry of Antarctic Blue whales using remotely piloted aircraft	Joshua Smith
Body condition of Antarctic blue whales	Lynette Irvine
The use of remotely piloted aircraft in Antarctic waters	Joshua Smith
Novel drone trace metal sampling methods for surface seawater in proximity to icebergs	Pier van der Merwe/Lavenia Ratnarajah
Using UAS video to identify definite surface feeding behaviour of Antarctic whales	TBD

4.6 Biogeochemistry

4.6.1 Brief background

Our ability to remotely track Antarctic blue whales in real-time (Section 4.3) opens up new possibilities for testing hypotheses regarding their role in Southern Ocean biogeochemical feedbacks. The 'whale pump' describes a recently proposed phenomenon whereby whales and other large mammals release nutrients (iron, carbon, nitrogen and sulphur) from deep, nutrient-rich waters in shallower waters via feeding and excretion (Lavery *et al.* 2014; Nicol *et al.* 2010a; Ratnarajah *et al.* 2014; Roman *et al.* 2014; Roman and McCarthy 2010). Marine mammals primarily feed at depth during short dives followed by extended surface periods during which defecation can occur (Baumgartner and Mate 2003; Croll *et al.* 2001; Roman and McCarthy 2010; Sparling *et al.* 2006).

Southern Ocean phytoplankton biomass and primary production are often limited by low iron (Fe) concentrations (Boyd *et al.* 2007). Laboratory studies suggest that within these high nutrient, low chlorophyll waters, whales could supplement seawater iron concentrations via the consumption and excretion of krill biomass (Lavery *et al.* 2010; Nicol *et al.* 2010a; Ratnarajah *et al.* 2014), potentially stimulating phytoplankton blooms (which are then available for krill consumption). Iron fertilisation likely also stimulates bacteria-driven processes such as nitrogen (N)-fixation (e.g. González *et al.* 2014; Mills *et al.* 2004; Rueter 1988) and biogenic gas production (e.g. dimethyl sulphide (DMS); (Liss *et al.* 2005).

The voyage represented the first *in situ* investigation of whether there is a measurable 'enrichment' effect of whale and krill aggregations, and defaecation, on the persistence of micro- and macro-nutrients in surface waters, primary productivity, microbial biogeochemical (Fe, C, N, S) cycling, and the production of biogenic climate gases. This was achieved via the synchronised collection of

biogeochemistry measurements alongside data on the 3D distribution, density and structure of krill aggregations (Sections 4.1 and 4.2), as well as data within and outside Antarctic blue whale aggregations (Sections 4.3 and 4.4). Seawater incubation experiments were also conducted to determine whether iron released in whale faeces stimulates primary productivity and biogenic gas production, and the timescale of any effects.

Specifically, to investigate 'enrichment' effects there were three main components to the biogeochemical work:

1) **Survey Area investigation:** conducting a matrix of Conductivity-Temperature-Depth (CTD) and Trace Metal Rosette (TMR) deployments within the survey area. The survey area was designed to cover gradients of whale and krill abundances, varying oceanographic features, and varying distances from sea-ice (a known alternative source of iron). The northern extent of CTD and TMR stations was south of the Southern Boundary (with the exception of one station). A suite of laboratory experiments was conducted on water samples collected using Niskin bottles at each station.
2) **Process Station:** a drogue was deployed in a region in which baleen whales (mainly fin whales but also Antarctic blue whales) appeared to be persistent. The same parcel of water was tracked and sampled every 24 hours for 5 days to examine potential temporal changes in the microbial community due to whale/krill enrichment. A suite of laboratory experiments was run on water samples collected from each station.
3) **Leaching and Incubation experiments:** Bottles filled with seawater were spiked with whale faecal material, sea-ice or dust, to determine the relative importance of each as an iron source for the microbial community. Two experiments were conducted, each over a 9 day period (sub-sampling on Days 0, 3, 6 and 9).

The components of the marine microbial community that were examined included phytoplankton (Westwood, O'Brien, Rodriguez-Vives, Brasier), bacteria (O'Brien, Westwood, Bell), and viruses (Bell, O'Brien). Dissolved iron concentrations were also measured, as well as organic ligands which are known to influence iron bioavailability (Ratnarajah, Holmes, Smith).

4.6.2 Preliminary results

A total of 110 deployments were conducted by the biogeochemistry team during the voyage (Figure 4.6.2and Figure 4.6.3). This included the deployment of 28 CTDs at 22 survey stations and 5 Process Station sites (Figure 4.6.2), 35 TMRs at 21 stations, 37 eXpendable BathyThermographs (XBT), and 10 Drifters. Not all CTD and TMR deployments collected water for experimental work.

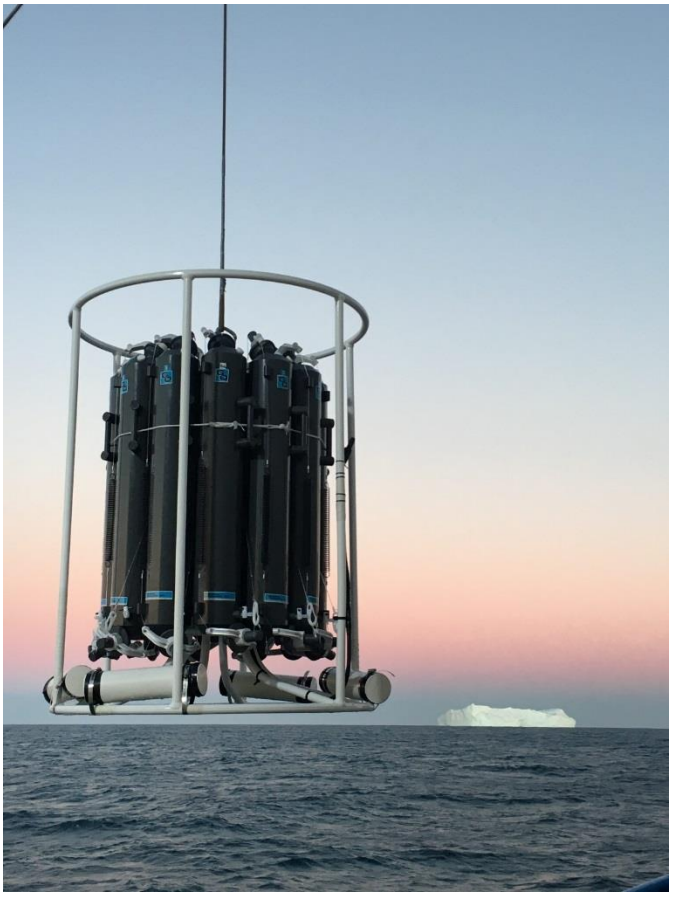


Figure 4.6.1*: CTD deployment © Lavenia Ratnarajah, University of Liverpool*

The resultant data will enable us to describe the surface oceanography within the survey area, as well as to determine macro-and trace metal nutrient availability for the microbial community.

The water samples collected from Niskin bottles on the CTD and TMR were used in a suite of laboratory experiments and/or stored for post-voyage analysis. The events, experiments and sampling are discussed below and summarised in Section 4.6.3.

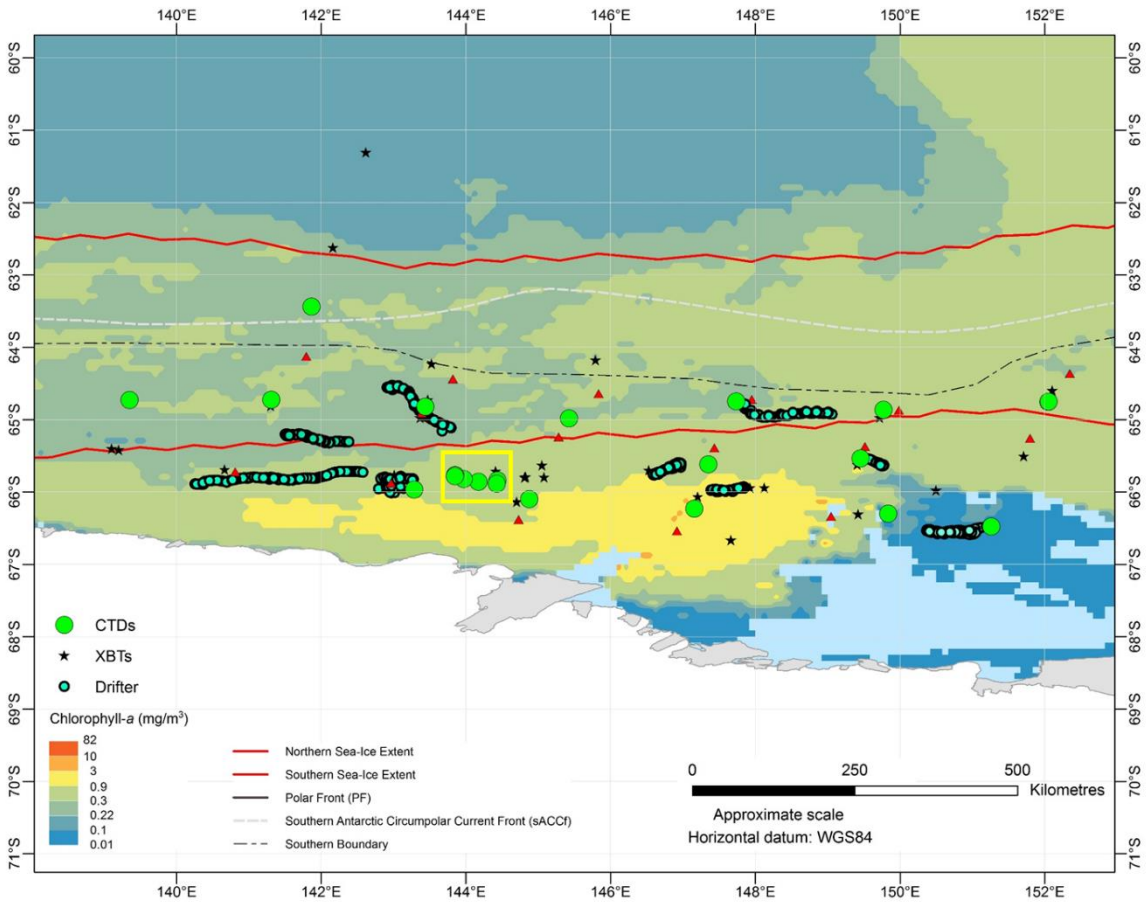


Figure 4.6.2*: CTD (green circles), XBT (black stars) and drifter (turquoise circles) deployments during the ENRICH survey. Red triangles mark the northern-most, middle and southern-most points of each transect line within the survey area. The yellow box delineates the Biogeochemistry Process Station.*

4.6.2.1 Hydrochemistry

Using the CTD, water samples from 12 depths per station were collected for hydrochemical analyses (n=324). Analysis was undertaken by the MNF on board using standard techniques for oxygen, salinity, phosphate, nitrate, nitrite, silicate and ammonia, but the results require quality control before release. It is envisaged that a collaboration will be formed with an oceanographer to interpret the data, write a paper, and to assist with the interpretation of experimental results. See Section 5.2 for details of MNF hydrochemistry data archival.

4.6.2.2 Phytoplankton and primary production

Primary production was measured in water samples collected using the CTD from 6 depths per station ranging from near-surface to 100 m. The sample depths selected were based on downward fluorescence profiles and two of six samples always included both near-surface (approximately 10 m) and the depth of the chlorophyll maximum where applicable. For sampling, 400 ml water was removed from appropriate Niskin bottles and stored in darkened polycarbonate jars in a cooled insulated container, until the commencement of incubations.

Incubations were conducted according to the method of Westwood et al. (2010). 6.327 x 10^6 Bq (0.171 mCi) $NaH^{14}CO_3$ were added to 162 ml of sample to produce a working solution of 39.183 x 10^3 Bq ml^{-1} (1.1 µC ml^{-1}). Seven ml aliquots of working solution were then added to transparent glass scintillation vials and incubated for 1 hour at 21 light intensities ranging from 0 to 1200 µmol m^{-2} s^{-1} (CT Blue filter centred on 435 nm). The temperature of the light incubator was controlled by a water-bath set to within ± 0.1°C of *in situ* values. After 1 hour, 250 µL of 6 M HCl was added to each vial and they were then agitated for 3 hours to ensure that all inorganic carbon was removed. Gaseous $^{14}CO_2$ was trapped in Carbosorb cartridges after being pumped through silica gel to ensure the air was dry. For radioactive counts, 10 ml Ultima Gold LLT scintillation fluid was added to each vial and shaken. Samples were then counted using a PerkinElmer Tricarb 2910TR liquid scintillation counter with the maximum counting time set at 5 min. In addition, Time 0 counts were taken to determine background radiation and 100% counts were used to determine the specific activity of the working solution. For Time 0 counts, 7 ml aliquots of working solution were subjected to acid addition without any exposure to light, and counted after shaking for 3 hours. For 100%'s, 100 µL of working solution from each depth was added to 7 ml NaOH (0.1 M) and immediately counted following the addition of scintillation fluid.

In all, a total of 162 photosynthesis/irradiance curves were produced and photosynthesis was measured under 21 light intensities for each of the six depths (n=3402).

Further samples from the same six depths per station were preserved with $HgCl_2$ for post-voyage analysis of dissolved inorganic carbon (DIC, Dickson *et al.* 2007) (n=162). The data will be used to standardise primary production measurements.

Additional water samples were collected from the 6 depths per station, filtered onto 13 mm GF/F filter paper, and stored in liquid nitrogen for post-voyage High Performance Liquid Chromatography (HPLC) analysis (n=162). The stored samples will be assayed for all phytoplankton pigments (including chlorophyll) at the AAD using the method of Wright et al. (2010). The data will be utilised to map the distribution and abundance of phytoplankton groups at each site, as well as to standardise primary production measurements.

Water samples from two of the six experimental depths per station (n=54) were fixed with Lugol's Iodine. Post-voyage, these samples will be examined using light and scanning electron microscopy to identify the phytoplankton species present and to ground truth HPLC data.

At all stations, samples from two depths (surface and chlorophyll maxima, n = 52) were collected to characterise the eukaryotic phototrophic community using DNA sequencing methods. Cells from 2L of bulk seawater were filtered on to 0.2-micron polycarbonate filters using a peristaltic pump before cryopreservation with liquid nitrogen for laboratory DNA extraction. Extraction of genomic DNA was performed using a Mobio Powerwater® DNA isolation kit in accordance with manufacturer's instructions (https://mobio.com/media/wysiwyg/pdfs/protocols/14900-S.pdf). Quality and quantity of DNA was measured using a Nanodrop 8000 Spectrophotometer before the sequencing of eukaryotic 18S rRNA amplicons using methods described in the data descriptor Brown et al. 2018. Assignment of taxonomy to the amplified sequences using the Protist Ribosomal Reference database (PR2, Guillou *et al.* 2012) will be performed to monitor the relative abundance of eukaryotic phytoplankton communities across all sites.

Total abundances of the phototrophic plankton community will be derived using flow cytometry methods of Porter (2004). Aliquots of 1mL surface and chlorophyll max water were collected at each station (plus an additional depth at southerly stations situated over the continental shelf) and preserved with 2% glutaraldehyde for laboratory analysis (n = 173). Photosynthetic communities will be discriminated by size as pico (<2um) and nano (2-20um) plankton using natural pigment fluorescence (red fluorescence, 488 excitation, 675/30 Emission) and scatter. Cyanobacteria populations of *Synechococcus* and *Prochlorococcus* will be discriminated by exciting Phycoerythrin (orange fluorescence, 488 excitation, 585/40 Emission).

4.6.2.3 Bacteria and bacterial production

Bacterial community composition will be also be characterised using DNA sequencing and flow cytometry techniques. Collection and preservation of samples on board the vessel was described above. Extracted DNA will be amplified using 16S rRNA gene primers described in Brown et al, 2018 and taxonomy will be assigned using the SILVA ribosomal RNA gene database (Quast *et al.* 2012).

Total abundance of bacteria and viruses will be enumerated with flow cytometry by staining cells with a SYBR green nucleic acid stain (1:10,000 final concentration, 15 minute incubation at room temperature) and exciting them with green fluorescence (488 excitation, 530/30 Emission) in accordance with methods described in Brussaard 2004.

Bacterial production was measured in water samples collected from two depths per station (near-surface and the chlorophyll maximum; n=378 samples). Samples were taken from the same jars as for primary production (see above). Bacterial production rates were determined using ^{14}C-leucine and the micro-centrifuge method of Kirchman (2001). All samples were spiked with 70 nM ^{14}C-leucine with three replicates plus two controls per depth. Samples were incubated in the dark at 4 ˚C for 2 h, then killed by adding 100% trichloroacetic acid (TCA). To remove excess isotope that was precipitated, the samples were vortexed then centrifuged for 15 min at 12,200 rpm, and the supernatant removed. This was followed by two rinsing steps; the addition of 5% TCA to the pellet followed by vortexing/centrifugation/supernatant removal, then the addition of 80% ethanol to the pellet followed by vortexing/centrifugation/supernatant removal. For radioactive counts, 1 ml of Ultima Gold scintillation fluid was added to each pellet. Each microcentrifuge tube was then vortexed and placed within a 20 ml glass scintillation vial. As per above, radiation was then measured using a PerkinElmer Tricarb 2910TR liquid scintillation counter. Post-voyage analyses will follow that of (Smith and Azam 1992).

A strong link exists between bacterial production and dissolved concentrations of the phytoplankton secondary metabolite dimethylsulphoniopropionate (DMSP) (Kiene and Linn 2000). Samples were gravity filtered (GF/F, 0.7um pore size) and acid preserved (8.75% final concentration H_2SO_4) from two depths (surface water and chlorophyll maxima) for determination of dissolved DMSP (DMSPd) and total DMSP (DMSPt) (Kiene and Slezak 2006; Galindo et al. 2016). Preserved concentrations of DMSP will be analysed in the laboratory using a purge and trap system coupled with a Gas Chromatograph (Shimadzu GC-2010) equipped with a flame photometric detector (FPD) following protocols described in Levasseur et al., (2006). Particulate concentrations of DMSP (DMSPp) will be derived arithmetically following the formula DMSPp = DMSPt – DMSPd.

Bacterial heterotrophy that is associated with DMSP is largely thought to be linked with the degradation of DMSP into catabolites, such as Methanethiol and dimethylsulphide (DMS) (Landa et

al. 2019). We will measure the rate of production of DMS by running DMSP lyase enzyme assays within the UTS laboratory. Samples were prepared by vacuum filtering 300mL and 600mL of seawater on to 0.2um and 2um polycarbonate filters to represent DMSP lyase enzyme potential of the total community (DLAt) and phytoplankton (DLAp) community respectively. All samples were cryogenically preserved with liquid nitrogen immediately after filtration. Post voyage analysis will follow methods described in Levine et al, 2012 and bacterial DMSP lyase enzyme activity (DLAb) will be calculated through the equation DLAb = DLAt – DLAp.

The ability of bacteria to degrade DMSP is widespread across a diversity of bacterial taxa, particularly amongst the Alphaproteobacteria (Curson *et al.* 2011). Two dominant clades within Alphaproteobacteria, SAR11 and Roseobacter are able to degrade DMSP through two disparate pathways called DMSP cleavage and DMSP demethylation (Curson *et al.* 2011; Sun *et al.* 2016). The total abundance and expression of genes encoding cleavage (ddd) and demethylation (dmdA) will be quantified on triplicate samples of DNA/RNA extracted (following methods described above, n = 318) using quantitative polymerase chain reactions (qPCR) following procedures described in Levine et al, 2012. RNA samples were collected using the same method as DNA samples with exception of an addition of 300uL of RNAlater per sample to preserve the integrity of RNA prior to extraction. Isolation of RNA was performed using a Mobio Powerwater® RNA isolation kit in accordance with manufacturer's instructions (https://mobio.com/media/wysiwyg/pdfs/protocols/14700-S.pdf).

4.6.2.4 Viral production and bacterial mortality

Viruses are the most abundant biological organisms of the world's oceans. There are 10-100 million virus particles in each ml of seawater. Most are bacteriophages and collectively marine viruses are thought to be responsible for up to 50% of bacterial death (review by Suttle 2007). Therefore, viral particle abundance and production was measured in water samples from the same two sample depths (near-surface and the chlorophyll maximum) as the bacterial production assays (Section 4.6.2.1) to investigate the effect of whale/krill enrichment on the viral abundance and corresponding impact on bacterial mortality. In total, 40 experiments were conducted (20 CTD stations, 2 depths per station), based on the dilution technique employed by Wilhelm et al. (2013), to assess the relative importance of lysogenic and lytic viral infection of bacteria. Sub-sampling of these viral experiments generated gluteraldehyde-fixed seawater samples (n=786) that were snap frozen in liquid nitrogen for post-voyage analysis using flow cytometry.

Dissolved DMSP and total DMSP samples were also collected from viral assays (n=314) conducted at the Biogeochemical Process Station to investigate the role of viruses in DMSP production. If results show that bacterial DMSP production is stimulated by viruses, a series of controlled in vitro laboratory experiments will be conducted to confirm the environmental findings and determine which members of the microbial community influence DMSP cycling (i.e., DMSP, DMS, DMSO and regulatory genes).

4.6.2.5 Light intensity and phytoplankton growth

Six experimental incubations were undertaken to test the effects of light intensity and changes in mixed layer depth on phytoplankton growth and bloom initiation in the Southern Ocean. Prior to the research cruise, a thorough preparation of the trace-metal (TM) clean lab equipment was performed following GEOTRACES protocols (Schallenberg *et al.* 2018). The six incubations were taken along the Antarctic ice-shelf, south of the polar front and the Antarctic Circumpolar Current (ACC). The sites

were picked randomly throughout the survey area and the sampling was always done during the night (~11:00-01:00 local time). The water for the incubations was collected from within the mixed layer depth (MLD) using a Trace Metal Rosette (TMR), following the deployment of the 24-bottle CTD rosette. Seawater from two separate Niskins was placed into a TM-clean carboy and mixed prior to filling the bottles. All the bottles were filled inside a TM container and the lights were kept low to ensure that the phytoplankton and chlorophyll pigments were not disturbed. Once the control treatments were filled, the bottles were sealed with clean Parafilm to prevent contamination from the incubator. Three bottles from the six controls were put into a 5% light screening (i.e. mesh bag) and the other three into a 50% light screening. The rest of the bottles were taken to a TM-clean flow hood and desferrioxamine B (DFB; final concentration ~50nM) and $FeCl_3$ in acidified stock (final concentration ~2nM) were added to the respective bottles. Triplicate bottles for all treatments were incubated in an uncovered deck incubator at *in situ* temperature with a continuous water flow for five days. To account for the different light treatments the bottles were placed into mesh bags with different thickness to simulate 5% (for low light treatment) and 50% (for high light treatment) of the total surface photosynthetically active radiation (PAR). The mesh bags were calibrated using a light-meter.

An aliquot from the initial water was taken on day one of the experiment, and several measurements were taken to account for the initial values of: dFe (dissolved iron), macro-nutrients (NO_x, phosphate, silicate, nitrate and ammonia), phytoplankton community using flow cytometry, maximum photochemical efficiency (F_v/F_m) using a Walz WATER-PAM, and chlorophyll *a*. The photosynthetic efficiency was also measured after 24 and 48 hours before the final measurement at the end of each incubation (on day 5). Phytoplankton community, dissolved iron, macro-nutrients and chlorophyll *a* were measured once more at the end of each incubation (on the day 5) for each treatment.

Mixed layer depths were calculated using the method of de Moyer Montégut (2004)de Boyer Montégut for which we applied a temperature difference from the ambient sea surface of 0.2°C (Patel *et al.* 2019). The percentage of total light at collection depth (E_z/E_o) was calculated following the method of Morel (2007).

Chlorophyll *a* was sampled last, because the other sub-sampling methods were more susceptible to iron or light contamination or changes due to temperature. A filtration system with a low vacuum pressure (<5mm Hg) was used to sample the water and collect the pigments with 25mm glass fibre filters (GF/F). The volume of the water filtered was always recorded as was the volume of acetone (90%) added to the final filter. All filters were then incubated in the dark at -20°C for 24 to 72 hours before analysis. The concentration of extracted chlorophyll *a* was measured using the acid (HCl 10%) method on a Turner Trilogy Fluorometer. A standardisation method was applied using a solid standard (Turner Designs, Sunnyvale, California) and measuring blanks for every reading set. Total concentrations of chl-*a* were calculated following methods from Welschmeyer (1994).

4.6.2.6 Underway and FIRe measurements

Salinity (SSS), pCO_2, fluorescence and sea surface temperature (SST) values were measured throughout the cruise from the main underway system on board the RV *Investigator*. The seawater intake was located 6.3 m below sea level (but is referred to here as 'surface') and measurements were done every 5 s using two on board thermosalinographs (SBE-38 CTD and SBE-21 TSG, SeaBird Inc., Bellevue, WA, USA). Partial pressure of CO_2 (pCO_2) values were measured using a General

Oceanics/Neill system as per Moreau et al. (2017). Fluorescence was measured with a WETStar fluorometer (WS3S-443P, Wetlabs, SeaBird Inc., Bellevue, WA, USA).

A Satlantic Fluorescence Induction and Relaxation (FIRe) system was also set up in the underway laboratory to measure the maximum photochemical efficiency (F_v/F_m) of phytoplankton in the surface water. Filtered (GF/F) seawater was used to blank the instrument prior and during the cruise. Measurements were taken every 5 s and blanks were run daily. The flash sequence was set to STF/STRP, induction duration (saturation flash lits) was 40, relaxation duration was 40000, initial flash interval was 50 µs and iteration number (total number of measurements) was 30.

4.6.2.7 eXpendable BathyThermographs (XBT's)

Marine National Facility personnel deployed 37 eXpendable BathyThermographs (XBT's) on behalf of the AAD. These probes measure seawater temperature in real time as they descend from the surface to a depth of 1000 m, and will assist in gaining a higher resolution of water column structure at locations where CTD deployments were unable to be conducted.

4.6.2.8 Drifters

Ten drifters were also deployed as part of the International Global Drifter program (http://www.aoml.noaa.gov/phod/gdp/index.php). The drifters transmit their position, water temperature and air pressure every hour and will assist with interpretation of oceanographic data. One of the drifters was employed as a drogue to mark the body of water chosen as a Biogeochemical Process Station to be followed and sampled over a 5 day period; this drifter/drogue travelled 16 nautical miles in 5 days.

4.6.2.9 Trace Metal Rosette (TMR) sampling

Twenty-one stations were sampled using the Trace Metal Rosette (TMR) (Figure 4.6.3, red dots) and a remotely piloted aircraft (Figure 4.6.3, yellow dots). Samples were processed and stored for post-voyage analyses of dissolved trace metals (n=235) and organic ligands (n=212). Trace metal contamination in the first incubation experiment compromised the original aim. The trace metal incubation experiment was re-run with water collected toward the end of the voyage (Figure 4.6.3, green box). Phytoplankton and bacterial abundance, chlorophyll concentrations, dissolved and particulate metals, and macronutrients will be analysed post-voyage.

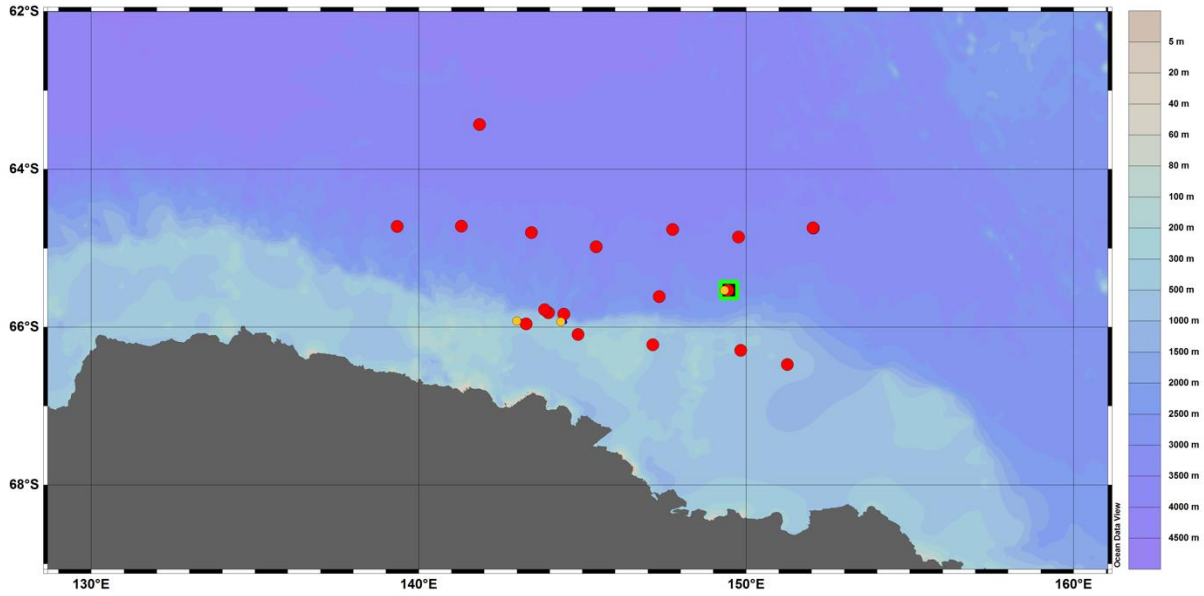


Figure 4.6.3*: Trace metal sampling locations. Red dots represent TMR operations, yellow dots represent trace metal sample collected using the science drone and the green box is the location of the successfully re-run incubation experiment.*

4.6.2.10 Biogeochemistry event and sample summary

Table 4.6.1: Biogeochemistry event and sample summary

Event/parameter	Stations (includes 5 BPS*)	Depths per station	# Experiments	# Samples	Storage	Analysis	Responsible
CTD casts (n=28)	28	20	-	-	-	-	Brasier, Rodriguez Vives, O'Brien
Dissolved oxygen	26	20	-	-	-	OV	Sherrin
Dissolved inorganic carbon	22	6	132	132	Preserved with $HgCl_2$. Stored at ambient temperature	CSIRO Marine and Atmospheric Research	Voyage: Brasier, Rodriguez vives, O'Brien Post-voyage: Kate Berry
Salinity	26	11-12	-		-	OV	Sherrin
Nutrients	26	19-20	-		-	OV	Sherrin
Bacterial production	22	2	44	392		OV; scintillation counter	Westwood
Primary production	22	6	132	372		OV; scintillation counter	Westwood
Total microbial community analysis	26	2	-	52	Lugol's iodine	PV microscopy	Westwood
Pico-nano Eukaryotes,	22	2-3	-	132	2% Gluteraldehyde	PV flow cytometry	Westwood, Bell, O'Brien

Bacteria and viral abundance							
Viral lysis and lysogeny	20	2	40	786	-80°C	PV flow cytometry	Bell, O'Brien
DNA/RNA	22	2-3	-	318	-80°C	PV DNA extraction, qPCR, 18S and 16S rRNA sequencing	O'Brien
DMSP lyase	22	2-3	44	258	-80°C	PV Gas Chromatography	O'Brien
Chl *a* associated with DMSP assays	22	2-3	-	129	-80°C	PV acetone extraction and fluorometry	O'Brien
Total DMSP	22	2-3	-	177	-80°C	PV Gas Chromatography	O'Brien
Dissolved DMSP	22	2-3	-	177	-80°C	PV Gas Chromatography	O'Brien
Metagenomic	2	2	-		-80°C	PV DNA extraction, Next Gen sequencing	O'Brien
HPLC	22	6	-	138	OV: liquid N; PV: -135°C	PV pigment analysis	Westwood
Light-iron incubation experiments (Rodriguez Vives)	2	1	6			PV pigment analysis	Rodriguez Vives
Chl *a* (Rodriguez Vives)	23	1	-	23	-20°C	OV Turner Trilogy fluorometer	Rodriguez Vives
TMR casts	35	12				-	Ratnarajah
Dissolved trace metals	19	12		235		PV trace metal analysis	Smith, Ratnarajah
Organic ligands	19	12		212		PV ligand analysis	Smith, Ratnarajah
Trace metal incubation experiment	2		2			PV	Smith, Ratnarajah
UAV collected trace metal	2	1		22		PV trace metal analysis	Smith, Ratnarajah
NOAA Drifters	10	-	-	-	-	PV Oceanographer required	Westwood. NOAA
XBTs	37	Surface –1000 m	-	-	-	PV Oceanographer required	MNF

**BPS = Biogeochemical Process Station; PV = post-voyage; OV = on voyage*

4.6.3 Comments and scientific highlights

The site chosen for the Biogeochemical Process Station was ideal in that fin and blue whales persisted in the area throughout the five day Process Station period, and were still present a few weeks later when the ship returned to the same area. It was also an area of low chlorophyll because

krill had likely already consumed the phytoplankton. In turn, the whales were in the region to feed on the krill. If our results demonstrate that the phytoplankton were recovering after their decimation then this will be a strong indication that iron recycling and enrichment by whales and/or krill is likely. Moreover, the weather was stable during the 5-day period and the drogue travelled only ~ 16 nmi. This means that potential mixing and dilution problems were limited and that we were therefore sampling the same resident phytoplankton population. This was supported by data from the CTD which showed a similar water column structure on each sampling occasion over the 5 days.

The sampling of surface waters using a drone was also a definite highlight for the trace metal team. When using the TMR the shallowest depth that can be sampled is 20 m, due to iron contamination from the ship nearer to the surface. The scientific drone enabled surface sampling away from ship and the development of this novel technique is exciting. Samples were taken near icebergs to determine their effect on surface iron concentrations.

4.6.4 Potential papers

Paper title or scientific question	Likely lead
A preliminary assessment of whale and krill enrichment effects on primary production in the Southern Ocean (survey and process station data)	Karen Westwood
Distribution of dimethylsulphoniopropionate and DMSP regulatory genes in the Southern Ocean (survey data)	James O'Brien
Abundance and expression of DMSP regulatory genes in a Southern Ocean cetacean hotspot (process station data)	James O'Brien
How bacteriophage infection influences DMSP concentrations in HNLC waters (process station virus experiments with DMSP measurements)	James O'Brien and Elanor Bell
Metagenomic profiling of microbial communities in a Southern Ocean cetacean hotspot	James O'Brien
Viral activity in a cetacean hotspot in the Southern Ocean	Elanor Bell, James O'Brien and Karen Westwood
Detection of whales in the Southern Ocean using eDNA	James O'Brien
Whale faecal material stimulates bacterial growth and production under iron replete conditions	James O'Brien and Karen Westwood
Distribution of dissolved iron	Abigail Smith
Distribution of organic ligands	Abigail Smith
Response of Antarctic microbial communities to natural nutrient fertilisation	Abigail Smith
Characterising the bioavailability of natural nutrient sources to Southern Ocean microbial communities	Abigail Smith
Distribution of other essential trace metals	Thomas Holmes
Summary paper – progressing the quantitative model using trace metal incubation experiment results	Lavenia Ratnarajah

4.7 Continuous Plankton Recorder (CPR)

4.7.1 Brief background

The Continuous Plankton Recorder (CPR) is one of the longest running marine monitoring programmes in the world, initiated in the North Sea in 1931 by Sir Alister Clavering Hardy. The CPR is a unique method for plankton sampling that has remained unchanged since 1948, providing a spatio-temporally comprehensive and consistent record of global marine plankton dynamics.

The CPR component during the ENRICH voyage conducted on-board RV Investigator was the collection of routine samples on both the southward and northward legs of the voyage. Overall, the CPR was deployed eight times from just south of Tasmania to the Mertz sea-ice marginal zone, and return (Figure 4.7.1; Table 4.7.1).

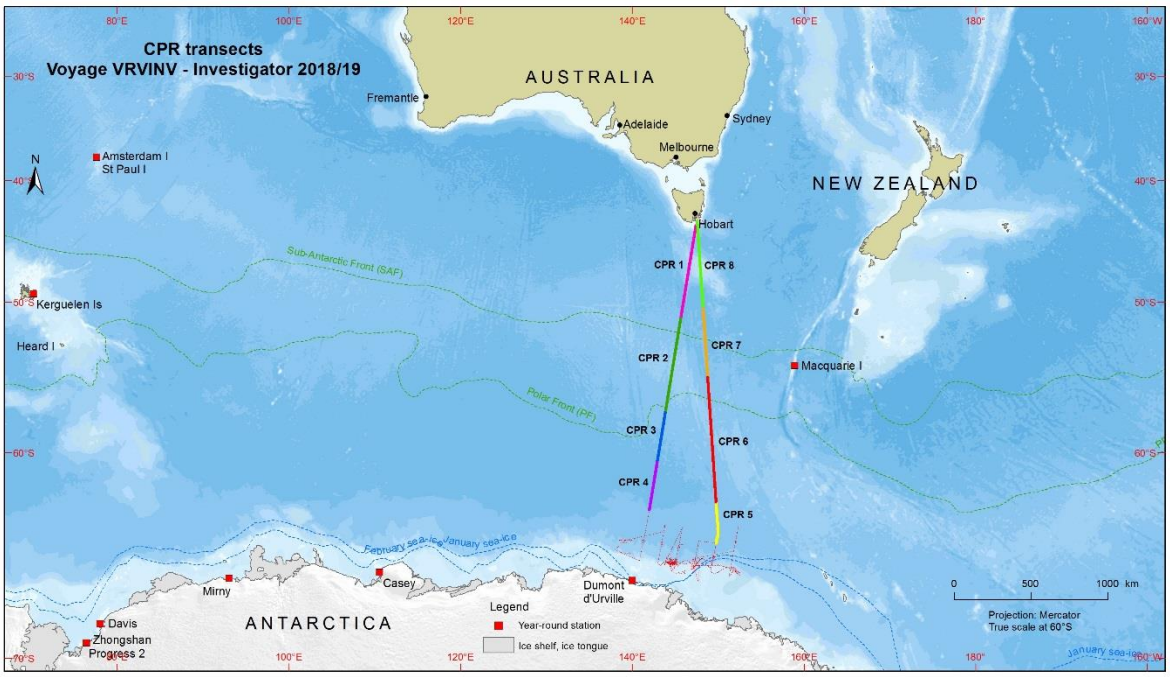


Figure 4.7.1*: Map showing CPR deployments during ENRICH voyage, 2019.*

4.7.2 Preliminary results

The resulting 8 tows, a combined total distance of 2431 nautical miles of continuous plankton recordings, will be post-voyage at AAD headquarters in Kingston.

4.7.3 Comments and scientific highlights

The deployment and retrieval of the CPR using the A-frame went well. The only issues that were experienced were due to user error where the locking tab of the internal mechanism was not fitted correctly during a deployment (Tow 5). Tows 6 and 7 were potentially influenced by propeller shaft damage. MNF engineers repaired the propeller shaft for re-deployment on Tow 8 and the internal mechanism successfully ran on.

The final tow (8.4) contained a lot of fibres, possibly from grey water discharge. Future deployments may want to work with the MNF crew to see if timing can be better coordinated for CPR deployments and grey water discharge.

Table 4.7.1: Deployment / retrieval data for the CPR during ENRICH voyage, 2019. Tows 3 and 8 where broken into stages due to either conflicting operational requirements or equipment problems.

CPR Tow No.	External CPR Unit	Internal CPR Unit	Deployment Date & Time UTC Lat °S Long °E	Retrieval Date & Time UTC Lat °S Long °E	Distance (nmi)	No. of Segments
1	8	5	20-Jan-2019 02:26 43° 52.70' S 147° 23.71' E	21-Jan-2019 21:10 51° 07.81' S 145° 38.21' E	441	88
2	8	4	21-Jan-2019 21:38 51° 11.09' S 145° 37.35' E	23-Jan-2019 09:52 57° 31.57' S 143° 50.29' E	386	77
3.1	8	17	23-Jan-2019 15:28 57° 33.62' S 143° 49.64' E	24-Jan-2019 01:32 59° 20.07' S 143° 16.37' E	108	22
3.2	8	17	24-Jan-2019 03:06 59° 21.67' S 143° 15.54' E	24-Jan-2019 09:21 60° 29.95' S 142° 53.55' E	69	14
4	8	18	24-Jan-2019 15:31 60° 32.71' S 142° 52.14' E	25-Jan-2019 05:53 63° 09.55' S 141° 58.17' E	159	32
5	8	4	26-Feb-2019 10:44 64° 52.48' S 149° 47.73' E	27-Feb-2019 01:29 62° 48.12' S 149° 44.75' E	126	25
6	8	16	27-Feb-2019 01:51 62° 45.48' S 149° 44.36' E	28-Feb-2019 23:04 55° 11.36' S 148° 45.11' E	455	91
7	8	18	28-Feb-2019 23:17 55° 09.11' S 148° 44.85' E	02-Mar-2019 03:25 50° 29.52' S 148° 13.88' E	280	56
8.1	8	4	02-Mar-2019 05:34 50° 16.67' S 148° 12.52' E	03-Mar-2019 09:48 45° 42.73' S 147° 45.13' E	274	55
8.2	8	4	03-Mar-2019 10:20 45° 42.64' S 147° 44.94' E	03-Mar-2019 13:08 45° 22.85' S 147° 43.24' E	20	4
8.3	8	4	03-Mar-2019 13:43 45° 22.39' S 147° 43.25' E	03-Mar-2019 17:30 44° 53.77' S 147° 40.49' E	29	6
8.4	8	4	03-Mar-2019 20:11 44° 53.16' S 147° 40.42' E	04-Mar-2019 06:49 43° 28.88' S 147° 32.57' E	84	17

				Total nmi:	**2431**	

4.7.4 Potential papers

Long term papers may result in the future. The data is stored and publically available from the Australian Antarctic Data Centre (AADC) and it is also shared with IMOS, The Global Alliance of CPR Surveys (GACS), SCAR and SOOS.

Paper title or scientific question	Likely lead
Potential for long-term CPR, collaborative papers through GACS	John Kitchener

5 Summary of data sets & storage

5.1 Summary of data

Summary statistic	Number
9,000 km of active acoustic transect conducted south of 60°S (1,670 km during broad-scale transects)	9000 km
975 krill swarms detected by the EK60 multi-frequency scientific echosounders on large-scale transects	975 krill swarms
41 target trawls conducted to collect live Antarctic krill	41 target trawls
20 IGR experiments conducted with 5472 krill individuals jarred and 1002 that moulted.	5472 krill individuals inspected
474 krill samples for the krill microbiome project	474 krill microbiome samples
300,000 krill eggs collected from gravid females	~300,000 krill eggs collected
10,000 early stage larvae successfully maintained during voyage for use in AAD krill aquarium	>10,000 larval krill returned to AAD
828.8 hours of underwater sound were recorded throughout the voyage using 295 sonobuoys	828.8 hours of underwater sound recorded using 295 sonobuoys
33,435 calls from Antarctic blue whales detected and located in real-time during the voyage	33,435 calls detected from Antarctic blue whales
4471.4 km of visual observations conducted over 317 hours of effort throughout the voyage	4471 km of visual observations
571 sightings of marine mammals (1389 individuals in total)	571 sightings of groups of marine mammals (1389 individuals)
25 identification photographs of critically endangered Antarctic blue whales were taken. 18 hours of video tracking of Antarctic blue whales were conducted characterise their behaviour	25 ID photos and 18 hours of behavioural observations of ABWs

134 UAV flights undertaken over a period of approximately 35 days for a range of activities including: photogrammetry, photo-identification, whale 'blow' sampling, surface water sampling, general whale and scenic imagery and surveillance for acoustic mooring retrieval.	134 UAV flights (30 hours in the air)
110 deployments of oceanographic instruments were conducted by the biogeochemistry team. This included 28 CTDs at 22 survey stations and 5 Biogeochemical Process Stations, 35 TMRS at 21 stations, 37 XBTs and 10 Drifters. Water collected was used in 100's laboratory experiments and 1000's of samples were preserved for post-voyage analyses of hydrochemistry, the microbial community and biogeochemical processes (More details in Table 4.6.1).	110 oceanographic instrument deployments to facilitate 100's experiments

5.2 Data storage

Science theme	Data type	Location and format
Active acoustics	EK60 calibration EK60 ME70 SH90	All acoustic data and associated logs and scripts are digital and stored on NAS drives at AAD and will be archived by the AADC.
Krill biology	Morphometrics 20 IGR experiments 474 Microbiome samples 20 Water samples filtered	Morphometrics and IGR data stored digitally on a harddrive held by So Kawaguchi; raw data in voyage logbooks. Krill samples, microbiome samples and water filtrations stored in the laboratories at AAD. Data will be archived by the AADC.
Passive acoustics	Acoustic recordings Processed acoustic detections Moored active and passive acoustics	All recordings digitized as WAV files. Processed detections stored in PAMGuard binary files & database. Data presently stored at TAMUG
Visual observations	Effort Sightings Video tracking Photo-ID	Effort and sightings stored in the Logger database Video tracks stored as digital video and photos on NAS drives stored at AAD. Processed video tracks also stored at AAD. Photo-ID stored digitally on NAS drives (digital photos and scans of paper log sheets).
UAV	Videos & photos (including photogrammetry photo-ID and behaviour data) Flight logs	.MOV, .m4v, .mp4, .DNG, .jpeg stored on NAS drives, AAD Cloudstor (https://cloudstor.aarnet.edu.au/plus/apps/files/?dir=/&fileid=2716240739), UAS_ENRICH 5TB encrypted external portable hard drive (Murdoch Uni).

	1 whale blow sample	.DAT, .txt stored on NAS drives, AAD Cloudstor (https://cloudstor.aarnet.edu.au/plus/apps/files/?dir=/&fileid=2716240739), UAS_ENRICH 5TB encrypted external portable hard drive (Murdoch Uni). 1x 2 ml cryovial stored Freezer No. 9, Hannan building mezzanine level, AAD.
Biogeochemistry	CTD	MNF/CSIRO: http://www.marlin.csiro.au/geonetwork/srv/eng/search#!00d65d56-d0d4-49a7-9d05-98ec29080598
	Hydrochemistry	MNF/CSIRO: http://www.marlin.csiro.au/geonetwork/srv/eng/search#!232719a6-b3b8-4c2a-9e78-d13c0715192b
	TMR (including samples collected via UAV)	Samples stored at IMAS for analysis. Data on hard drives at IMAS and University of Liverpool. Raw data in logbooks.
	XBT	MNF/CSIRO: http://www.marlin.csiro.au/geonetwork/srv/eng/search#!e4747d4c-4cce-43f4-beec-56ce0d72377b
	Drifter	International Global Drifter program (http://www.aoml.noaa.gov/phod/gdp/index.php).
	Microbial samples	Microbial samples stored at AAD and UTS. Associated metadata stored on hard drives and logbooks and NAS drives. Data will be archived by the AADC.
	Trace metal & ligand samples	Samples stored at IMAS. Associated metadata stored on hard drives and logbooks and in MNF log.
Other	CPR	8 CPR silks preserved and stored at AAD. When analysed data will be held at: https://data.aad.gov.au/metadata/records/AADC-00099 And shared with IMOS, GACS, SCAR and SOOS MNF/CSIRO:
	ADCP	http://www.marlin.csiro.au/geonetwork/srv/eng/search#!c1b2bbd0-2e49-45cd-a7ed-592784e124e3
	Underway data (ship's track, bathymetry, wind speed, air temp,	Underway data stored by MNF (also on NAS drive): http://www.marlin.csiro.au/geonetwork/srv/eng/search#!2c516bf2-2420-44c1-8412-a0f70bb077aa

	water surface temp, etc.)	

6 Acknowledgements

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Sincere thanks to all the scientists on board who worked long hours in often tough conditions to maximise what was achieved. The whole experience was enhanced by their camaraderie, good humour and unerring support of one another.

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8 Appendices

8.1 ENRICH Voyage personnel (IN2019_V01)

	Name	Role	Organisation
IN2019_V01 – CSIRO MNF Staff			
	Lisa Woodward	**Voyage Manager**	CSIRO MNF
	Gary Mitchell	Medical Doctor	CSIRO MNF
	Jay McGlashan	SIT Support	CSIRO MNF
	Will Ponsonby	SIT Support	CSIRO MNF
	Amy Nau	GSM Support	CSIRO MNF
	Matt Boyd	GSM Support	CSIRO MNF
	Peter Shanks	DAP Support	CSIRO MNF
	Steve van Graas	DAP Support	CSIRO MNF
	Kendall Sherrin	Hydrochemist	CSIRO MNF
IN2019_V01 - BBC Media Team			
	Alexander Vail	Media	BBC
	James Cox	Media	BBC
IN2019_V01 – AAD Medical Doctor			
	Lloyd Fletcher	Medical Doctor	AAD
IN2019_V01 – Science personnel			
	Michael Double	**Chief Scientist**	AAD
	Elanor Bell	Deputy Chief Scientist / Biogeochemist	AAD
	Karen Westwood	Voyage Project Manager / Biogeochemist	AAD
	Brian Miller	Cetacean Passive Acoustician	AAD
	Ana Širović	Cetacean Passive Acoustician	Texas A&M University
	Kathleen Stafford	Cetacean Passive Acoustician	University of Washington
	Susannah Calderan	Cetacean Passive Acoustician	AAD
	Joshua Lawrence	Active Acoustician	AAD
	Ailbhe Kavanagh	Marine Mammal Observer	University College Cork
	Russell Leaper	Marine Mammal Observer	AAD
	Lyn Irvine	Marine Mammal Observer / Unmanned Aerial System Operator	AAD
	David Donnelly	Marine Mammal Observer	AAD
	Vanesa Reyes Reyes	Marine Mammal Observer	AAD
	Paula Olson	Marine Mammal Observer	AAD
	Elanor Miller	Cetacean Passive Acoustician	AAD
	So Kawaguchi	Krill Scientist	AAD
	Rob King	Krill Scientist	AAD
	Jessica Melvin	Krill Scientist	IMAS
	Olivia Johnson	Krill Scientist	IMAS
	Haiting Zhang	Krill Scientist	IMAS
	Joshua Smith	Unmanned Aerial System Operator/ Marine Mammal Observer	Murdoch University
	Charlotte Boyd	Marine Mammal Observer	University of Washington
	Lavenia Ratnarajah	Biogeochemist - iron	University of Liverpool
	Thomas Holmes	Biogeochemist - iron	IMAS
	Abigail Smith	Biogeochemist - iron	IMAS
	James O'Brien	Biogeochemist - DMS	University of Technology Sydney
	Clara Rodriguez Vives	Biogeochemist - oceanography	IMAS
	Madeleine Brasier	Biogeochemist - oceanography	University of Liverpool/UTAS
IN2019_V01 – RV *Investigator* crew			

	Name	Role	Organisation
	Adrian Koolhof	**Master**	ASP
	Andrew Roebuck	1st Mate	ASP
	Tom Watson	2nd Mate	ASP
	Sam Edwards	3rd Mate	ASP
	Chris Minness	Chief Engineer	ASP
	Sam Benson	1st Engineer	ASP
	Mike Sinclair	2nd Engineer	ASP
	Damien Wright	3rd Engineer	ASP
	Robert Kinsey	Electrical Engineer	ASP
	James Hogg	Chief IR	ASP
	Peter Taylor	IR	ASP
	Daniel Morse	IR	ASP
	Dennis Bassi	IR 3	ASP
	Rod Langham	IR 4	ASP
	Matt Schmierer	IR 5	ASP
	Paul Langford	IR 6	ASP
	Michael Pawson	Chief Cook	ASP
	Paul Stanley	Cook	ASP
	Gary Hall	Chief Caterer	ASP
	Emma Lade	2nd Caterer	ASP