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Cruise plan for the 2019 IWC-SORP research voyage 'The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean'

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INTERNATIONAL WHALING COMMISSION

Cruise plan for the 2019 IWC-SORP research voyage *'The availability of Antarctic krill to large predators and their role in biogeochemical recycling in the Southern Ocean*'

Double, M.C.[1], Westwood, K. [1], Bell, E.M. [1], Kelly, N. [1], Miller, B.S. [1], de la Mare, W. [1], Andrews-Goff, V.A. [1], Cox, M.J. [1], Kawaguchi, S. [1], King, R. [1], Melbourne-Thomas, J. [1], Davidson, A. [1], Nicol, S. [2], Williams, G. [3], Laverock, B. [4], Ratnarajah, L. [5], Seymour, J. [6], Friedlaender, A. [7], Herr, H. [8], Findlay, K. [9], Iñiguez Bessega, M. [10], Miller, E.J. [11]

1. Australian Marine Mammal Centre, Australian Antarctic Division, 203 Channel Highway, Kingston, Tasmania 7054, Australia

2. Adjunct Professor, Institute for Marine and Antarctic Studies (IMAS), 20 Castray Esplanade, Battery Point, Hobart, Tasmania 7000, Australia

3. Antarctic Climate and Ecosystems (ACE)-CRC, 20 Castray Esplanade, Battery Point, Hobart, Tasmania 7000, Australia

4. School of Science, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand

5. Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Jane Herdman Building, 4 Brownlow Street, Liverpool L69 3GP, United Kingdom

6. University of Technology Sydney, 15 Broadway, Ultimo, NSW 2007, Australia

7. Associate Adjunct Professor, Ecology and Evolutionary Biology, UC Santa Cruz, 115 McAllister Way, Santa Cruz, CA 95060, United States

8. Centrum für Naturkunde - CeNak - Center of Natural History, Universität Hamburg, Martin-Luther-King-Platz 3, Raum 243b, 20146 Hamburg, Germany

9. Centre for Oceans Economy, Department of Biodiversity and Conservation, Cape Peninsula University of Technology, Caper Town, South Africa

10. Fundación Cethus, Gdor Luis Monteverde 3695 (B1636AEM), Olivos, Provincia de Buenos Aires, Argentina

11. Elanor Miller Consulting, Hobart, Tasmania, Australia

Corresponding author: mike.double@aad.gov.au

ABSTRACT

In January to March 2019 the Australian Antarctic Division will lead a 49-day Antarctic voyage on the Australia's Marine National Facility vessel the *RV Investigator*. This voyage will contribute to the IWC-SORP's 'Antarctic Blue Whale Project' and 'Acoustic Trends' themes. The voyage's objective is to determine whether the characteristics of krill swarms can predict the distribution and behaviour of Antarctic predators, particularly Antarctic blue whales. The density, distribution, and fine-scale 3D structure of krill swarms will be described relative to predator density and distribution estimated through visual surveys and passive acoustics. In addition, through measurements of the abundance and speciation of whale faecal iron the voyage will assess the controversial theory of iron-fertilisation by whales and determine whether iron concentrations are higher within aggregations of feeding whales than within krill-only aggregations or than in adjacent areas. The data collected on this voyage will further describe how large Southern

Ocean whales interact with krill in time and space which will inform the development of management tools for both whales and krill.

KEYWORDS: RESEARCH VOYAGE, IWC-SORP, KRILL, ANTARCTIC BLUE WHALE. PASSIVE ACOUSTICS, PHOTO-ID, BIOGEOCHEMICAL RECYCLING, SOUTHERN OCEAN

INTRODUCTION

The aim of this paper is to notify the IWC's Scientific Committee of a 49-day IWC-SORP Antarctic research voyage to be conducted in early 2019 and describe its scientific objectives. The opportunity to conduct this voyage followed an open and competitive application for ship time on Australia's Marine National Facility vessel the *RV Investigator*. The success proposal, was titled the '*Availability of Antarctic krill to large predators and their role in Southern Ocean biogeochemical recycling*' and the resulting project will be led by scientists from the Australian Antarctic Division (AAD).

Science background

Antarctic krill (*Euphausia superba*), hereafter krill, is the primary prey for many squid, fish, bird, and mammal species. Krill distribution in the Southern Ocean (SO) is highly variable; they may be found in large diffuse patches spread over wide areas (Jarvis *et al.* 2010), densely packed into coastal bays (Nowacek *et al.* 2011), or in variously distributed discrete dense swarms (Tarling *et al.* 2009, Cox *et al.* 2011). Prior research has focused around the Antarctic Peninsula, hence the factors that influence the distribution, density, form and behaviour of krill in the eastern Antarctic are not well understood (Jarvis *et al.* 2010).

The distribution and form of krill are hypothesized to influence their availability to predators (Friedlaender *et al.* 2011): dense, large, widely distributed swarms of krill in shallower water may be targeted by fast-moving blue and fin whales that have high engulfment capacity, while more diffuse, small, deep swarms of krill may be suitable for humpback and minke whales that are more manoeuvrable but make slower lunges with smaller engulfment-capacity (Goldbogen *et al.* 2012).

Until very recently SO predator-prey interaction studies have focussed on land-based krill predators (Agnew 1997, Constable *et al.* 2000) but there are now descriptions of the forms of krill preyed upon by minke, humpback and fin whales (Friedlaender *et al.* 2008, Friedlaender *et al.* 2009, Santora *et al.* 2010, Friedlaender *et al.* 2014, Santora *et al.* 2014). Such studies are generally conducted at a relatively local scale so it is less clear whether these whales have targeted a particular form of krill or how widely such forms are distributed in space and time.

Despite their rarity (population abundance <2% of pre-exploitation levels , Branch 2008), recent Australian Antarctic Division-led developments in passive acoustics now provide novel, robust and reliable means of detecting, tracking and mapping the distribution of Antarctic blue whales (ABWs) on their Antarctic feeding grounds (Miller *et al.* 2015). This then allows us to use active acoustics to describe the density, distribution, and fine-scale 3D structure of krill swarms both within the vicinity of ABWs and in control locations that are demonstrably distant from these specialist and extreme krill predators.

The passive acoustic technology involves deploying DIFAR sonobuoys to locate aggregations of ABWs from hundreds to thousands of kilometres away, homing in on them to close these distances, and ultimately triangulating the precise location of calling individuals (Miller *et al.* 2014b, Miller *et al.* 2014c, Miller *et al.* 2015, O'Driscoll and Double 2015). Passive acoustic tracking during recent surveys showed that ABWs clump together in dense vocal aggregations in mid-late summer in eastern Antarctica singing loud songs consistently for weeks on-end, providing not only the ability to approach these aggregations, but a means of remotely tracking their position in real-time for the entire duration of a voyage (Miller *et al.* 2015, O'Driscoll and Double 2015). This method has opened new avenues for monitoring ABW population recovery and conducting designed ecological studies in their vicinity.

During the 2015 NZ-Australia Antarctic Ecosystem Voyage, a preliminary investigation of the distribution and density of krill was conducted around and away from ABWs using multi-frequency echosounders (O'Driscoll and Double 2015). This study found that krill swarms near blue whales tended to have a higher intra-swarm density than those observed elsewhere (Cox *et al.* 2015, Miller *et al.* 2018). These intriguing preliminary findings confirm our ability to successfully integrate multidisciplinary acoustic methods but more data are needed to augment existing information and confirm our findings. The *RV Investigator* is the only Australian ocean-going research vessel capable of water column multibeam sampling; this state-of-the-art equipment would facilitate a detailed 3D analysis of krill swarm structure and allow us to fully test our krill availability hypotheses.

Our ability to remotely track ABWs in real-time also opens up new possibilities for testing hypotheses regarding their role in SO 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 (Nicol *et al.* 2010, Roman and McCarthy 2010, Lavery *et al.* 2014, Ratnarajah *et al.* 2014, Roman *et al.* 2014). Marine mammals primarily feed at depth during short dives followed by extended surface periods during which defecation can occur (Croll *et al.* 2001, Baumgartner and Mate 2003, Sparling *et al.* 2006, Roman and McCarthy 2010).

Southern Ocean phytoplankton biomass and primary production are often limited by low iron (Fe) concentrations (Pollard *et al.* 2009). Laboratory studies suggest that within these high nutrient, low chlorophyll waters, whales can supplement seawater iron concentrations via the consumption and excretion of krill biomass (Lavery *et al.* 2010, Nicol *et al.* 2010, Ratnarajah *et al.* 2014), thus stimulating large phytoplankton blooms (which are then available for krill consumption) and increasing primary production (Blain *et al.* 2007, Lavery *et al.* 2010, Lavery *et al.* 2014). Iron fertilisation also likely stimulates bacteria-driven processes such as nitrogen(N)-fixation (e.g. Rueter 1988, Mills *et al.* 2004, González *et al.* 2014) and biogenic gas production (e.g. dimethyl sulphide (DMS); (Liss *et al.* 2005).

To date, no *in situ* studies have examined whether there is a measurable effect of whale and krill aggregations, and defaecation, on the persistence of nutrients in surface waters, primary productivity, microbial biogeochemical (Fe, C, N, S) cycling, and the production of biogenic climate gases. Thus, this first ever field examination will investigate krill availability to ABWs and, simultaneously, the influence of this predator-prey interaction on local biogeochemical feedbacks. We will achieve this through synchronised collection of data on the 3D distribution, density and structure of krill aggregations, phytoplankton communities and iron both within and far from aggregations of ABWs and other whales, alongside seawater incubation experiments designed to determine whether iron released in whale faeces stimulates primary productivity, N-fixation and biogenic gas production, and the timescale of any effect.

VOYAGE OBJECTIVES

Specifically, the scientific objective of the voyage are to:

1) Characterise the density, distribution and fine-scale 3D structure of 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 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 controversial theory of iron fertilisation by whales and krill. We will test the hypothesis that iron concentrations are higher within aggregations of feeding whales than outside of these areas, and that iron released by whale faeces leads to higher local productivity, microbial biogeochemical (Fe, C, N, S) cycling, and the production of biogenic climate gases (DMS, CO_2, CH_4, N_2O).

REASEARCH VESSEL, SURVEY AREA AND VOYAGE SCHEDULE

The 94-metre *RV Investigator* is managed by Australia's Marine National Facility in Hobart, Tasmania and supports atmospheric, oceanographic, biological and geoscience research in waters around Australia and Antarctica. This vessel can accommodate up to 40 scientists, be at sea for up to 60 days and has an endurance of over 10 000 nautical miles. It has a 1C ice class.

The project's time allocation is 49 days (17 January – 6 March 2019) and its area of operation is to and from Hobart to south of 60°S and between the longitudes of 140°E and 175°W. 29 berths for scientists have been allocated for our project.

PROJECT METHODS

ABW localisation and remote tracking:

DIFAR sonobuoys will be deployed using established adaptive survey methods to efficiently detect, encounter and track vocalising aggregations of ABWs (Miller *et al.* 2015). During transit to the study area, acousticians will continuously monitor for ABW calls, and make calibrated intensity and bearing measurements from single sonobuoys deployed at 30nmi intervals to estimate the distance to vocal aggregations (Miller *et al.* 2014a). Within 30nmi of an aggregation pairs of sonobuoys will triangulate the precise location of calling groups of whales and be used on approach to determine group size and behaviour, and during (krill) transect surveys. Upon leaving the aggregation, single sonobuoys will continue to be deployed at 30nmi intervals to remotely track vessel distance from the aggregation.

Visual observations of ABWs:

Observers will conduct visual observations of whales during all daylight hours to confirm the presence of acoustically tracked or non-vocalising whales. Observers will measure and record distance and angle (from ships heading) to all sightings of whales. Sightings effort, and environmental data will be recorded in a database and linked to the vessel track.

ABW behaviour and relative abundance:

Upon visual sighting ABWs, the vessel will stand-off at ~1-2km and conduct a behavioural focal-follow using a photogrammetric video-tracking system. During ~1h of observation (to include 6-12 surfacing intervals), accurate bearings, locations and behavioural observations will be recorded, including movements, swimming speeds, blow and diving intervals. Simultaneously, observers will estimate the relative number of ABWs and other species present. Continued passive acoustic recordings will provide simultaneous data on vocal behaviour and triangulated underwater movements of vocalising individuals. Faecal plumes observed will be marked using drogued buoys allowing re-sampling to examine the timescale of any biological effects.

Small boat work

The suitability of the *RV Investigator* for the operation of small boat work in the Southern Ocean will be the subject of an operational assessment. At present it appears that small boat work will not be feasible. Whale biopsy samples will be collected opportunistically from the ship using a Larsen gun or PAXARMS biopsy system.

Krill distribution and density:

The EK60 scientific echosounders and the ME70 scientific multibeam (Trenkel *et al.* 2008) will be used to continuously map water column targets, primarily krill, throughout the voyage detailing swarm density, structure, and vertical distribution. All systems will be calibrated and synchronized to avoid interference (Korneliussen *et al.* 2008). We will use Large Scale Survey System (LSSS, Korneliussen *et al.* 2006) software to process and combine EK60 and ME70 data into a single spatially referenced data set to allow efficient description and interpretation of patterns in krill distribution (Korneliussen *et al.* 2009). Krill will be identified using the frequency response approach adopted by CCAMLR (2010) and aggregations delimited using school identification algorithms (e.g. Cox *et al.* 2010, Cox *et al.* 2011). Acoustic mark identification will be validated by target trawling using an RMT 1+8 (Roe and Shale 1979).

Designed transect surveys will be conducted to directly compare patterns in krill at known distances from ABW aggregations. We may replicate the small scale survey design of 8 transects, 6nm long with a 0.75nm transect spacing carried out previously (O'Driscoll & Double 2015). Post-survey, detected krill swarms will be targeted with RMTs (1h trawls, 2kt towing speed) to verify species, measure size composition, condition and Fe-concentration.

Unmanned aerial systems (UAS) operations:

Fixed-wing FX-61 Zeta, multi-rotor S1000+ and kite-based UAS will perform localized aerial mapping and monitoring of whales and faecal plumes. The multi-rotor will also be used to collect 'clean' water and faecal samples from the surface layer. A multi-rotor mounted hyperspectral camera will estimate biological production.

Iron(Fe)-concentration:

Krill samples will be collected from trawls, frozen and analysed ashore to determine Fe-concentration. Water samples for seawater Fe analyses will be collected using a combination of CTD and trace metal rosettes (TMR), *in situ* particle pumps, and sampling from the ship's trace-metal clean seawater supply. Whale faecal material will be filtered through a 0.2µm filter to separate dissolved Fe (dFe) and particulate Fe (pFe). Flow-injection analysis will be used to measure real-time dFe to compare concentrations in areas with whale and krill aggregations, and areas without. Concentrations of pFe will be analysed ashore.

Bacterial and primary productivity:

Seawater from ≥6 depths will be incubated in a modular laboratory (Radiation), to determine the impact of faecal material on bacterial and phytoplankton growth and integrated productivity (Kirchman 2001, Westwood *et al.* 2010). Phytoplankton samples will be size fractionated (to 20µm) prior to incubation (Boyd *et al.* 1984, Jochem *et al.* 1995, Kawaguchi *et al.* 1999, Varela *et al.* 2002). A FlowCAM will provide semi-automated recognition of the phytoplankton community size structure in situ and following experimental treatments (Buskey and Hyatt 2006).

Microbial analyses and biogeochemical rate measurements:

At ≥3 stations with whales and ≥3 without, seawater will be collected from a vertical profile for nutrient concentration, dissolved oxygen (DO), flow cytometry (FCM), and nitrification rate analyses. Replicate water samples from selected depths will be collected for DNA and RNA filtration; DMS/P concentration measurements and lyase activity assays and N2 fixation rate measurements. Abundances of heterotrophic bacteria, viruses and phototrophic picoplankton will be determined using flow cytometry on fixed samples.

At each station, triplicate samples collected from the surface, DCM, and sub-DCM will be filtered (0.22µm) to retain microbial genetic material (DNA and RNA) for molecular analysis ashore. Nitrogen-fixation and prokaryotic nitrification rates will be estimated according to Montoya et al. (1996) and Kitidis et al. (2011) respectively.

Seawater dimethyl sulphide (DMS) concentrations will be measured immediately upon collection using gas chromatography. Samples for dimethylsulfoniopropionate (DMSP, dissolved and particulate fractions) analyses will be stored in 1% sulphuric acid (H2SO4) for analysis ashore. DMSP lyase enzyme activity in both phytoplankton and bacteria will follow Levine et al. (2012).

SCIENCE TEAMS AND PERSONNEL

Up to 29 berths have been allocated to scientific personnel for this voyage of which approximately 10 will be occupied by AAD personnel. The remaining berths will be occupied by scientific collaborators external to the AAD. The science teams, their leader and approximate team size is presented below. Not all berths have been allocated and the final size of each team has yet to be finalised.

Voyage and Science Management Team (3+)	Lisa Woodward (MNF), Michael Double (AAD), Elanor Bell (AAD), Karen Westwood (AAD)
Survey Design	Natalie Kelly (AAD)
Passive Acoustics Team (4)	Brian Miller (AAD), plus 3 external collaborators
Active Acoustics Team (1)	Martin Cox/Natalie Kelly (AAD)
Visual Observations, video-tracking and biopsy (4-6)	Virginia Andrews-Goff (AAD), plus 3-5 external collaborators

Microbial/Biogeochemical Team (6-7)	Karen Westwood (AAD), Bonnie Laverock (Auckland University), Lavenia Ratnarajah (University of Liverpool), plus 3-4 external collaborators
Krill Team (4)	So Kawaguchi (AAD), Rob King (AAD), plus 2 external collaborators
UAS Team (2)	Guy Williams (ACE-CRC), plus 1 external collaborator

FUTURE PLANNING

The full Science Plan and Voyage Plan are currently being drafted, led by Natalie Kelly and Karen Westwood respectively. Two science planning workshops will be held in the second half of 2018 to finalise the Science Plan for this voyage.

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