



**Report on the Status and Trends of Southern Ocean Zooplankton based on the  
SCAR Southern Ocean Continuous Plankton Recorder (SO-CPR) Survey**

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## PREFACE

When the original SCAR Antarctic Climate Change and the Environment (ACCE) Report was first published in 2009 (Turner et al., 2009) there was limited long-term information on Antarctic and Southern Ocean zooplankton to allow comments on the possible effects of climate change. We only had a few time series data sets to examine for long term trends and the few that existed, e.g. JARE NORPAC which had not been fully analysed, and the Southern Ocean Continuous Plankton Recorder Survey was relatively new with not really enough data to reliably identify long term patterns. The new biological SRPs AntEco and AnT-ERA seek to understand the current status of biological systems, and to identify trends in those systems and their degree of resilience. Zooplankton are a crucial link in the Antarctic marine ecosystem and changes in the zooplankton are likely to have substantial flow on effects through the rest of the food web. This report has collated the current knowledge of the status of zooplankton, e.g. what species are known, community structure, biogeography, based on the analysis of the SO-CPR dataset, and 69 publications in peer-reviewed research papers, chapters, atlases, and reviews. This report brings all that information together and highlights achievements over nearly 30 years. The report also includes some new analyses identifying trends (seasonal or long term) in relation to changes in abundance, shifts in distribution, and community composition. This report is a product of the Expert Group on CPR Research. It is hoped that this will be a living report that will include updated information as the SO-CPR datasets continues to grow and be analysed. This report contributes to the efforts of AntEco, AnT-ERA, and hopefully will be a companion to the ACCE report and updates. It also contributes to other reports such as the Ecological Status Report produced by the Global Alliance of CPR Surveys (GACS).

## EXECUTIVE SUMMARY

- The CPR was first used in Antarctic waters by its inventor Sir Alister Hardy during the 1925-27 *Discovery* expedition. It has been used successfully for nearly 90 years to monitor plankton in the North Sea and North Atlantic Ocean.
- The advantage of the CPR is having a consistent design and methodology that has remained relatively unchanged for over eight decades. It has provided sustained long-term observations of inter-annual changes in plankton populations and communities, as well as seasonal patterns of abundance and distribution, phenological shifts and the intrusion of evasive species. For the development of ecological models and the study of trends the errors of uncertainty in the data are constant in an unchanging sampling method.
- In 1991 the CPR returned to Antarctic waters when the Australian Antarctic Division initiated a CPR survey of the Southern Ocean south of Australia and westwards to Mawson. The objectives were to study regional, seasonal, intra-annual and long-term variability in zooplankton abundance, species composition and community patterns, as well as the abundance and distribution of krill larvae produced annually.
- Several nations and ships have participated in the SO-CPR Survey with Australia, Japan, New Zealand, South Africa, France and Brazil currently the most active.
- SCAR established an Expert Group to support the development of SO-CPR Survey and to encourage other nations to participate. SCAR SO-CPR Survey is also a founding member of the Global Alliance of CPR Surveys (GACS).
- The SO-CPR Survey database is an endorsed SCAR Business Product. The database is held at the Australian Antarctic Data Centre, and is distributed to SO-CPR participating members, to other databases such as SCAR's biodiversity.aq, OBIS, GBIF, GACS, and Atlas of Living Australia. It has been used by CCAMLR, and contributes to the objectives of SOOS.
- More than 1000 tows have been conducted by the SO-CPR to date. These have provided 56,942 samples at 5 nautical mile resolution, for 289 zooplankton taxa and developmental stages, representing approximately 284,725 nautical miles covered.
- 69 publications have resulted in peer-reviewed research papers, chapters, atlases, and reviews.

- Approximately 70% of the Southern Ocean has been sampled at some time. The highest concentration of sampling is in the region between south of South Africa and eastwards to the Ross Sea area, and notably in the area south and west of Australia.
- Tows are mainly conducted in the months from September to April when most research and supply ships are operating. A few tows have been conducted in the winter months of May, July and August, but winter sampling is limited due the lack of ships operating in the region in winter.
- Zooplankton data, and hence distribution patterns, can be time and geo- coded to GPS data, and environmental data collected by the ships' underway monitor system, e.g. fluorometry, water temperature, salinity, and meteorological data. The recording of micro-plastics has been initiated.
- Typical CPR tows show very high abundance of zooplankton in the upper 20 m of the Polar Frontal and Permanent Open Ocean Zones between the Sea-ice Zone and the Sub-Antarctic Front. By comparison the surface waters of the SIZ has low species diversity and abundances. We know the SIZ has high zooplankton abundances based on other plankton and RMT8 nets. The absence of zooplankton in CPR samples suggests the near surface waters of the SIZ are not suitable for zooplankton, or other behavioural change that prevent zooplankton vertically migrating into or staying near the surface.
- The CPR provides enough taxonomic resolution to distinguish consistent zooplankton assemblages with strong latitudinal zonation associated with oceanographic fronts. At times, the zooplankton assemblages were better at showing separation of frontal zones than direct measurement of the oceanography by shipboard underway recording systems. The Sub-Antarctic Front is a major biogeographic boundary.
- Both real observation and modelled biogeographic atlas have been published.
- Small calanoid and cyclopoid copepods, notably *Oithona similis*, larvaceans, smaller euphausiid species, thecosome pteropods and foraminifera typically dominate the plankton in the open ocean.
- *Oithona similis* has remained consistently the most abundant species in the samples at approximately 50% of numbers although its real abundance is expected to be substantially much higher considering its relatively small size compared to the CPR mesh.
- Soft bodied gelatinous zooplankton can be difficult to identify because they are easily damaged but advancing genetic/molecular methodologies are proving promising.
- Comparison between CPR and other plankton net system showed the abundances of zooplankton collected by CPR in surface waters are equivalent to, and for some species higher, than a plankton net fitted with the same mesh. The CPR data are also reasonably indicative of species composition deeper in the water column.
- Massive blooms of planktonic foraminifera have been observed over large areas supressing abundances of other plankton. It is not known if such blooms are routine natural events or a response to changing oceanic conditions. Foraminifera and thecosome pteropods are target groups for detecting ocean acidification effects.
- Boosted Regression Tree (BRT) modelling was applied to six key taxonomic groups of zooplankton: Copepoda (Calanoida), Euphausiidae, Foraminifera, *Fritillaria* spp., *Oithona similis*, and pteropods, which predicted the circumpolar distribution of the occurrence and abundance of these taxa, in both areas sampled and into areas not sampled. Later CPR tows in the areas not previously sampled showed the predictive models were true. The BRT modelling identified persistent hot-spots of abundance which appear to match predator patterns or abundances obtained from global net samples. The modelling also showed that between 1997 and 2018 environmental suitability for calanoid and cyclopoid copepods, Foraminifera and *Fritillaria* spp. had improved, whereas for pteropods in some areas, particularly the Ross Sea, the environmental suitability had worsened.
- Generalised Dissimilarity Modelling (GDM) was applied to the whole data set to identify and predict the seasonal biogeographic distributions of whole species assemblages. Species characteristic of each assemblage were identified along with likely dominant species. The

success of the BRT and GDM predictions lies in the sensitivity of zooplankton to align with and reflect the oceanographic conditions of their environment

- There is no evidence to date of any non-indigenous species being recorded by CPR south of the SAF. At the moment SAF is probably a sufficient major biogeographic barrier to prevent incursions, or the waters of the ACC are too cold for northern species.
- However, the CPR has recorded the heterotrophic dinoflagellate HAB *Noctiluca scintillans* in the sub-Antarctic Zone up to 240 km south of Tasmania. This is probably the most southerly oceanic record of *Noctiluca* and the furthest extension into the oceanic realm globally. The East Australian Current is predicted to continue to strengthen and transport more warm water and eddies further south. This may result in viable populations of *Noctiluca* become established in the Southern Ocean in the future with unknown effects for the food web.
- In the eastern Antarctic region south and west of Australia the CPR data show an increasing trend in total zooplankton abundance in the SAZ, PFZ, POOZ and SIZ since 1991. In the same period, the Average Copepod Community Size (ACCS) metric showed a significant positive trend suggesting a shift towards larger copepod species. This is contrary to the paradigm that warming waters should see a shift to warmer copepod species which are usually smaller. Reasons for this change are not known at present.
- No trend in abundance of ACCS was seen in the Ross Sea area to the east, possibly because of the much shorter data collecting period. The Ross Sea region does generally have higher abundances than the eastern Antarctic and show greater inter-annual variation. The Ross Sea region does not show the same patterns as the eastern Antarctic region despite the PFZ and POOZ of the Ross Sea region being “downstream” of the Eastern Antarctic region in relation to the ACC.
- Preliminary analyses has shown that the CPR data can be combined with satellite observations to determine the possible effects of large scale climatic events, notably the Southern Annular Mode (SAM) and El Niño Southern Oscillation (ENSO), on zooplankton presence and abundance distributions.
- Responses to climate modes were found for all 12 major abundant zooplankton, although mechanisms for each species were often difficult to attribute. Increases, in the abundance of some species could be linked with increases in primary productivity as a results of the phases of the climate mode, or with strengthening wind speeds during negative SAM and El Niño/positive SAM events.
- Fully understanding the full relationships between species and climatic modes or local environmental effects is hampered by the lack of sufficient information on the ecology of most zooplankton necessary to understand how species respond to their environment.
- The CPR has proven to be the most efficient and cost effective means of quickly, routinely, and consistently monitoring and studying real zooplankton observations over ocean basin scales using a consistent unchanging sampling method.

## INTRODUCTION

Sir Alister Hardy first trialled his Mark I continuous plankton recorder (CPR) in Antarctic waters during the 1925-1927 voyages of the RRS Discovery and RRS William Scoresby (Hardy, 1936). Initial tows across the southern Atlantic Ocean were not always successful, but satisfactory samples were collected over 2,300 nautical miles, including a very successful series of tows across Drake Passage producing a continuous trace of Antarctic plankton abundances for nearly 300 nautical miles. Sir Alister proved the CPR as a valuable plankton surveying tool and then established the CPR Survey in 1931 in the North Sea and North Atlantic, now the longest running and most successful plankton monitoring programme at an ocean scale. The CPR Survey, formerly the Sir Alister Hardy Foundation for Ocean Science, is now based with the Marine Biological Association, Plymouth UK.

There have been a number of attempts to define and describe the composition, structure and distribution patterns of zooplankton communities around Antarctica, as well as the distribution and life histories of individual species (e.g. Mackintosh, 1934, 1937; Hardy and Gunther, 1935; Baker, 1954; Foxton, 1956; Voronina, 1968, 1972; Hopkins, 1971). Those surveys relied on traditional zooplankton net or midwater trawl sampling methods, and have generally been sporadic in both space and time. More concerted and systematic attempts to describe zooplankton ecological patterns started during the BIOMASS (Biological Investigation of Marine Antarctic Systems and Stocks) programme and have continued for many years after. Several studies have focussed on the Atlantic sector, including the Antarctic Peninsula area, Scotia Arc and southern Weddell Sea, e.g. Boysen-Ennen and Piatkowski (1988), Hubold et al. (1988) and Piatkowski (1989a, b), Siegel and Piatkowski (1990), Siegel et al. (1992), Smith and Schnack-Schiel (1990), Schnack-Schiel and Mujica (1994), Pakhomov et al. (1997), Ward et al. (2005, 2006a, b, 2007). Surveys in the eastern Antarctic sector have covered a wider geographic area including the Lazarev Sea (Pakhomov et al., 1993; Hunt et al., 2011), Cosmonaut Sea (Pakhomov and Pelevin, 1989; Hunt et al., 2007), the Prydz Bay region (Hosie and Stolp, 1989; Pakhomov, 1989; Hosie 1994; Hosie and Cochran, 1994; Hosie et al., 1997, 2000; Yang et al., 2011), from Casey station to the Ross Sea (Chiba et al., 2001), and in the Ross Sea (Pane et al., 2004). However, one region that has consistently remained poorly studied has been the Pacific sector between the Ross Sea and the Antarctic Peninsula, i.e. the Amundsen and Bellingshausen Seas.

All the previous surveys ranged from small to large scale in area with sampling resolutions of 10s to 100s km between sites. They were essential in defining species composition, community structure and distribution patterns, which were ideal foundations for future monitoring, but they lacked both the spatial and temporal resolution to assess long-term variation in the system. To some degree, even up to relatively recent times, monitoring was not seen as real science, certainly not important, and yet Sir Alister Hardy established the longest running marine biological monitoring programme, the CPR Survey, which has provided crucial evidence of significant changes in plankton that are affecting marine systems and fisheries in the North Sea, North Atlantic and far north Pacific.

Japan was the exception in the Antarctic when it established the Japanese Antarctic Research Expedition (JARE) monitoring program during the 1972/73, JARE 14 expedition (Takahashi et al., 1998). NORPAC plankton net samples, along with other oceanographic observations, were collected daily at approximately noon during transits across the Southern Ocean to and from Syowa station in December and March (Takahashi et al., 1998). The JARE monitoring programme, overseen by the National Institute of Polar Research (NIPR), aims to study the processes and mechanisms of variability of Antarctic systems over decade periods, to determine the impact of global change on Antarctic systems and to understand the role of Antarctica in influencing global change (Takahashi et al., 2019a; Takahashi and Odate, 2020a, b). The JARE marine programme still continues and has produced a valuable time series extending 46 years. However, the investigators overseeing the NORPAC sampling acknowledged that the daily sampling is too coarse for monitoring in relation to spatial variation at an oceanographic scale.

In 1991, 65 years after the CPR was first trialled, the Australian Antarctic Division re-employed the CPR in the waters around eastern Antarctica as a more effective method for routine, rapid and repeated surveying and monitoring of zooplankton patterns through the region, in order to detect assess the effects of environmental change (Hosie et al., 2003). Those initial few tows in January 1991 from the RSV *Aurora Australis* has grown substantially into the SCAR endorsed Southern Ocean CPR (SO-CPR) Survey, which has involved 16 ships from 12 nations. The SO-CPR Survey provides the largest comprehensive and systematic Antarctic zooplankton data set, spatially and temporally, using a consistent sampling methodology ideal for the purpose of mapping the seasonal, inter-annual, long-term and spatial variation in plankton diversity, as well as to use plankton as sensitive indicators of environmental changes to monitor the health of the Southern Ocean. This report describes the purpose of the Southern Ocean CPR Survey, why it was developed, what we seek to discover, provides an overview of what we have learnt to date, notably in relation to biogeographic patterns, trends in abundance community composition, as well as identify significant gaps in our knowledge, and provides recommendations for future opportunities. Much of this work has already been published in 68 CPR based research papers, chapters, atlases, and reviews, but the report will also include up to date analyses of the SO-CPR data series. The report will contribute to AntEco, AnT-ERA, and will be a companion to the ACCE report and updates. It will also contribute to other reports such as the Ecological Status Report produced by GACS.

Several acronyms are used in this paper and for convenience have been listed in Table 1.

Table 1. Status Report Acronyms

|            |   |
|------------|---|
| AAD        | Australian Antarctic Division   |
| ACC        | Antarctic Circumpolar Current   |
| ACCS       | Average Copepod Community Size  |
| ACCE       | Antarctic Climate Change and the Environment  |
| AntEco     | State of the Antarctic Ecosystem (SCAR Scientific Research Programmes)                                |
| AnT-ERA    | Antarctic Thresholds-Ecosystems Resilience and Adaptation (SCAR Scientific Research Programmes)       |
| AZ         | Antarctic Zone – between the PF and SACCF   |
| BIOMASS    | Biological Investigation of Marine Antarctic Systems and Stocks                                       |
| BRT        | Boosted Regression Tree   |
| CAASM      | Catalogue of Australian Antarctic and Sub-Antarctic Metadata  |
| CAML       | Census of Antarctic Marine Life   |
| CCAMLR     | Commission for the Conservation of Antarctic Marine Living Resources                                  |
| C-EMP      | CCAMLR Ecosystem Monitoring Program   |
| CPR        | Continuous Plankton Recorder  |
| CPR Survey | Original Continuous Plankton Recorder Survey based at the Marine Biological Association, Plymouth UK. |
| EAC        | East Australian Current   |
| ENSO       | El Niño Southern Oscillation  |
| GACS       | Global Alliance of CPR surveys  |
| GBIF       | Global Biodiversity Information Facility  |
| GDM        | Generalised Dissimilarity Modelling   |
| GLOCHANT   | Global Change in Antarctica   |
| IGMETS     | International Group for Marine Ecological Time Series   |
| IMOS       | Australian Integrated Marine Observing System   |
| ISIZ       | Inner Seasonal Ice Zone   |
| JARE       | Japanese Antarctic Research Expedition  |
| LMR-GOOS   | Living Marine Resources-Global Ocean Observation System   |
| NIPR       | National Institute of Polar Research  |
| NIWA       | National Institute of Water and Atmospheric   |
| NORPAC net | North Pacific standard net  |

|             |   |
|-------------|---|
| OBIS        | Ocean Biogeographic Information System                    |
| OOZ         | Open Ocean Zone   |
| OSIZ        | Outer Seasonal Ice Zone                                   |
| PAR         | Photosynthetically active radiation                       |
| PCI         | Phytoplankton Colour index                                |
| PF          | Polar Front   |
| PFZ         | Polar Frontal Zone – between the SAF and PF               |
| POGO        | Partnership for Observation of the Global Oceans          |
| POOZ        | Permanent Open Ocean Zone                                 |
| RAMS        | Register of Antarctic Marine Species                      |
| RMT         | Rectangular mid-water trawl                               |
| SACCF       | Southern Antarctic Circumpolar Current Front              |
| SAF         | Sub-Antarctic Front                                       |
| SAHFOS      | Sir Alister Foundation for Ocean Science                  |
| SAM         | Southern Annular Mode                                     |
| SAZ         | Sub-Antarctic Zone – between the STF and SAF              |
| SB          | Southern Boundary   |
| SCAR        | Scientific Committee on Antarctic Research                |
| SCAR-MarBIN | SCAR Marine Biodiversity Information Network              |
| SCOR        | Scientific Committee on Oceanic Research                  |
| SIZ         | Sea-ice Zone  |
| SO-CPR      | Southern Ocean Continuous Plankton Recorder Survey        |
| SO-GLOBEC   | Southern Ocean-Global Ocean s Ecosystems Dynamics program |
| SOOS        | Southern Ocean Observing System                           |
| WoRMS       | World Resister of Marine Species                          |

### **Zooplankton Monitoring in Antarctica**

The need to conduct monitoring programs of the Antarctic environments has in fact long been recognised. An earlier version of the constitution of the Scientific Committee on Antarctic Research SCAR noted (Annex 1) that "scientific research and the exchange of information, which encompasses both the provision of baseline data and the monitoring of change, is an essential part of environmental protection." The obligation to conduct monitoring became more formal under the (Madrid) Protocol on Environmental Protection to the Antarctic Treaty 1991. Article 3 (2d, e) of the Madrid Protocol specifically states the requirements to establish programs to monitor existing impacts and activities but also establish monitoring programs for the detection or possible unforeseen impacts (see SCAR Bulletin No. 110).

SCAR has been active in establishing and/or supporting a number of marine monitoring programmes. Both current biological Scientific Research Programs, the Antarctic Thresholds - Ecosystems Resilience and Adaptation (AnT-ERA) and State of the Antarctic Ecosystem (AntEco), both recognise the need for long-term monitoring and observations networks. SCAR was instrumental in the establishment of the Southern Ocean Observing System (SOOS) which strives to develop an effective comprehensive and sustained long-term multi-disciplinary, interlinked network to collect and deliver essential observations on the dynamics and changes the Southern Ocean systems. The CPR was identified as an important tool in the SOOS Initial Science and Implementation Strategy (Rintoul et al., 2012), as well as being a foundation component of AnT-ERA and AntEco. In turn, SO-CPR was a founding member of the Global Alliance of CPR Surveys which seeks to collate plankton information at a global level, thus placing Southern Ocean observations in a global context. Reid et al. (2003) highlighted the need for collective global zooplankton observations to help identify and understands the effects of changes at local, regional and oceanic scales.

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) also recognised in its early years the need to establish an integrated international ecosystem monitoring program. The CCAMLR Ecosystem Monitoring Program (C-EMP) which has two clear objectives: 1) to detect and record significant changes in critical components all the ecosystem, to serve as a basis for the conservation of Antarctic marine living resources; 2) to distinguish between changes due to the harvesting of commercial species and changes due to environmental variability, both physical and biological (Agnew, 1997). It is relatively easy to establish programs to detect change, the difficult objective is distinguishing human induced and natural environmental change. CCAMLR has identified three key prey species, Antarctic krill *Euphausia superba*, the neritic species *Euphausia crystallorophias*, and the fish *Pleuragramma antarcticum*, plus nine of their avian and mammalian predators. The current C-EMP program does have some limitations. For example, it is only looking at a small part a large complex ecosystem. The program is mainly based around Antarctic krill and its predators (Croxall, 1990; Kerry et al., 1993; Agnew, 1997), which is understandable given the importance of the krill as a key species in the ecosystem, and as a target for commercial harvesting. The monitoring of krill and its predators is ideal for detecting effects of localised over-harvesting and a number of C-EMP sites have been set up around Antarctica. However, there are still some difficulties in adequately measuring the abundance of krill as well as surveying some of the predators such as crabeater seals. Further, the C-EMP program may not be sensitive enough to detect changes due to environmental variability and consequently distinguish those changes from harvesting impact (C-EMP second objective). This is where the monitoring of zooplankton can help make that distinction by serving as a reference for the general state of the ecosystem through studies on natural variability and global change. The value of time series studies of zooplankton to monitor the status of oceans and define long term patterns of variation is well recognised (Colebrook et al., 1984; Colebrook, 1986; Roff et al., 1988; Warner and Hays, 1994; Taylor, 1995; Fromentin and Planque, 1996; Sugimoto and Tadokoro, 1997; Reid et al., 1998a; Clark et al., 2001).

The sensitivity of plankton to changes in the ecosystem has been well demonstrated. Their relationship sensitivity to oceanic discontinuities (e.g. fronts) and to changes in the environment makes them useful indicators of water mass types and of ecosystem health. Changes in plankton abundance over time may also act as early warning indicators of critical shifts in ecosystem function. Reid et al. (1998b) reported a sudden major change in the phytoplankton regime in the North Sea and North Atlantic in the late 1980's. The significance of the dramatic change would not have been fully appreciated had it not been for the preceding 30 years of monitoring demonstrating the long-term stability of the previous regime. The decline in North Sea cod has been attributed to long-term fluctuations in plankton (Beaugrand et al., 2003). Such fluctuations can thus have significant ecological and economic impacts. Variations in plankton abundance in the Atlantic have been linked to climatic variability, including the North Atlantic Oscillation, Gulf Stream Index, and changing sea-surface temperatures (Reid et al., 1998b; Beare and McKenzie, 1999). Hare and Mantua (2000) reported much clearer indications of the 1989 North Pacific regime shift in the biological records than expressed by indices of Pacific climate. They noted that monitoring of ecosystems may allow earlier identification of change than is possible from just monitoring climate data. These studies plus Reid et al. (1998a), have revealed that significant biological changes can occur in a very short time scale, i.e. one or two years, in a step wise or threshold fashion rather than responding linearly to climatic/environmental forcing over several years. Plankton data are crucial as baseline information, in the context of long-term changes such as global warming, and should be measured as part of all long-term oceanic field programmes, especially if we are addressing questions about changes in biodiversity with consequent changes in ecosystem functioning.

The Southern Ocean is known for its numerous frontal zones, particularly within the Antarctic Circumpolar Current. Each front marks the limit of unique water masses or zone with distinct changes in temperature, salinity and oxygen properties indicative of each zone (Orsi et al., 1995; Sokolov and Rintoul, 2009a, b). Temperature is one of the major factors controlling Antarctic zooplankton communities at macro- and meso-scale level (Hosie et al., 2000). Many species, including copepods, have wide circumpolar distributions but still exhibit preferences for warmer or colder waters with the



region (Lomakina, 1966; Foxton, 1971; Kittel and Stepnik, 1983; Everson, 1984; Beaumont and Hosie, 1997). For example, *Salpa thompsoni* is generally confined to the warmer waters of the ACC (Foxton, 1966; Smith and Schnack-Schiel, 1990), whereas the copepod *Metridia gerlachei* prefers the colder waters typically found south of the southern boundary of the ACC (Zmijewska, 1983; Schnack-Schiel and Mujica, 1994). Similarly, the region is known for the massive seasonal change in sea-ice cover between winter and summer which drives so much of the oceanographic patterns (Zwally et al., 1983; Comiso and Zwally, 1984), as well as affecting krill and zooplankton distribution, abundance and life cycles (Knox, 1994; Loeb et al., 1997; Tyan, 1998; Nicol et al., 2000). The Antarctic Climate Change and Environment report (Turner et al., 2009), and its supplements, provide a detailed description of the Antarctic marine environment and the issues affecting it.

Antarctica and surrounding waters are expected to be particularly sensitive and vulnerable to climate change and Antarctic zooplankton may respond to climatic forcing in the same fashion as demonstrated in the North Sea, North Atlantic and North Pacific. Further, there is already some indication that both zooplankton and krill may be very sensitive to only slight increases in UV levels and ocean acidification (Malloy et al., 1997; Newman et al., 1999; Kawaguchi et al., 2013). Changes in the environmental conditions, e.g. circulation patterns, position of frontal zones, climate, sea-ice cover, UV, acidification or pollution, would be expected to affect first the phytoplankton/microbial communities, then the zooplankton communities. Antarctic zooplankton have much shorter lives, often one or two years or considerably less for small copepod species (Kane, 1966; Everson, 1984; Marin, 1988; Paffenhöfer, 1993; Atkinson, 1998), and therefore a faster population turnover than their vertebrate predators and hence expected to respond more rapidly to change. In turn, zooplankton such as copepods and salps are important grazers along with krill and form alternative links in the Antarctic chain between phytoplankton and those vertebrates. Zooplankton are also not harvested by humans, noting that adult Antarctic krill are not really zooplankton, hence there is no direct human impacts on zooplankton communities complicating assessment of changes in abundance. Long-term monitoring of the zooplankton communities, including krill larvae, would give a more valuable early warning to changes in the Southern Ocean and Antarctic ecosystem, rather than relying solely on monitoring changes in adult krill abundance or populations of higher vertebrates targeted by C-EMP and other monitoring programs.

Until the establishment of SOOS, there were a few programs had been proposed to conduct monitoring at regional and global scales relevant to the Antarctic, i.e. Living Marine Resources-Global Ocean Observing System LMR-GOOS, Global Change in Antarctica GLOCHANT, and Southern Ocean-Global Oceans Ecosystems Dynamics program SO -GLOBEC (GLOBEC, 1994; SCOR, 1996; El-Sayed, 1998). Surprisingly, despite the value and relative ease of zooplankton monitoring, few large scale sustained zooplankton monitoring programs have been initiated to date other than those from the CPR community. Those surveys are now coordinated through the Global Alliance of CPR Surveys (GACS). Most of the marine monitoring programs such as C-EMP and programs such as the Palmer Long Term Ecological Research site (Ross et al., 1996) or studies around Elephant Island (Siegel et al., 1998) are quite localised. The Seminar on Antarctic Ocean and Resource Variability (Paris 1987) cautioned the results of such localised studies may not be suitable for any extrapolation to either larger regions or the whole Antarctic system (Sahrhage, 1988).

### **Establishing the SCAR Southern Ocean CPR Survey**

The Australian Antarctic Division AAD initiated a long term continuous plankton recorder survey in 1991 to map and monitor zooplankton patterns in the Southern Ocean as a means of assessing the status or health of the region. Specific objectives of the survey are:

1. Map the biodiversity and distribution of zooplankton, including euphausiid (krill) life stages, in the Southern Ocean,
2. To assess the seasonal, annual and long term variability in abundance, species composition and distribution patterns of the Southern Ocean zooplankton communities,

3. Similarly, assess the variability of abundance and development of krill larvae produced each year.

The first objective deals with the issue of first defining the species composition and fine scale distribution patterns in the region before studies of variation (second objective) can be assessed. The various zooplankton surveys of the Sea-ice Zone (SIZ) and Permanent Open Ocean Zone (POOZ) (e.g. Hubold et al., 1988; Pakhomov, 1989, 1993; Siegel and Piatkowski, 1990; Hosie, 1994; Hosie and Cochrane, 1994; Hosie et al., 1997, 2000; Takahashi et al., 1998; Atkinson and Sinclair, 2000), have provided essential information on species composition through the water column but, the large spatial separation of the samples is too coarse to define fine scale patterns around frontal zones. The third objective attempts to use the CPR as a predictive tool for future krill stock variation rather than relying on assessment of current adult krill biomass, which is largely historical data by the time acoustic data and net samples are analysed. Hosie (1991, 1994) identified likely krill larvae dispersal routes with very high abundance of larvae occurring in the Prydz Bay region in March, especially along ship routes where CPR tows were scheduled to be conducted. Abundances in March 1987 often exceeded 100 m<sup>-3</sup>.

#### *Establishment period, 1990-1994*

Two Type II Mark III CPR units (each comprising an external body and three internal recording mechanisms “cassettes”) were obtained from the CPR Survey in Plymouth UK, formerly the Sir Alister Hardy Foundation for Ocean Science (SAHFOS). SO-CPR initially focussed on the SIZ in the eastern Antarctic region, where previous intensive net sampling surveys had defined zooplankton composition and shown stability in the species associations and community structure (Hosie, 1994; Hosie and Cochran, 1994; Hosie et al., 1997, 2000). Any future changes in species association and composition would be indicative of change in the ecosystem. The SIZ is also the main habitat of Antarctic krill (Atkinson et al., 2008; Nicol, 2006; Smetacek et al., 1990). The POOZ, between the Polar Frontal Zone (PFZ) and Sea Ice Zone (AZ), was not considered at this stage as it was assumed to be oligotrophic (Banse, 1996; Atkinson, 1998) and thus less important than the more productive SIZ. Sampling was undertaken only on dedicated science voyages of the RSV *Aurora Australis*. The first official tow commenced on 12 January 1991 north-east of Davis station. While only a few tows were completed in this period they provided valuable data for comparison with later tows, as well as showing that near surface abundances and diversity of zooplankton were considerably less than anticipated from RMT net sampling despite the finer mesh of the CPR. Operating the CPR in even relatively light concentration sea-ice (1 to 2/10) resulted in frequent damage to the phosphor bronze external unit. The decision was made to redesign and manufacture new units that would be more robust for Antarctic service.

#### *Manufacture and trialling period, 1993-1996.*

Two new external units and 7 cassettes were produced using marine grade 316 steel, rather than phosphor bronze. The units were redesigned for simpler loading and unloading of silk and preservative. Units were nickel plated to reduce corrosion. Unlike the older Mark III CPRs with cassettes fitting only their own housing, the new CPRs were AAD designed and made with each cassettes being interchangeable between external housings and have interchangeable parts. The new units were designated as Type II Mark V (Reid et al., 2003) and are functionally identical to previous and current CPRs. Trials were conducted in the 1995/96 and the first part of 1996/97 austral summer season. After minor modifications to the design and changes in the material of some gear components, fully successful trials were conducted on the last voyage of the season in April 1997. During this period, CPR tows were extended into the POOZ.

#### *Fully operational period, April 1997 onwards*

The 1997/98 season was very successful with 21 tows made over 4 voyages. Sampling now extended through the POOZ and was carried out on all voyages of *Aurora Australis*, dedicated science or resupply. These voyages run nearly throughout the year, and over a wide area south and south-west of Australia. A high level of sampling intensity has been maintained since by Australia.

NIPR identified the CPR as a useful method to complement its JARE NORPAC net program and improve the spatial monitoring. The fixed time schedule and route of the JARE voyages was a useful temporal reference for SO-CPR. Japan joined SO-CPR with its first tows in November and December 1999 from *Kaiyo Maru* and *Shirase*. Japan has maintained its annual sampling schedule from both *Shirase* vessels and *Umitaka Maru*, with opportunistic sampling from other vessels. Germany (*Polarsten* 2004) and New Zealand (*Tangaroa* 2002, *San Aotea II*, 2008) followed. Russia (*Akademik Ferderov*), Brazil (*Ary Rogel*), USA (*Yuzhmorgeologiya*) provided addition tows to those by Australia, Japan, Germany and New Zealand for the Census of Antarctic Marine Life (CAML) during International Polar Year producing over 90 tows around Antarctic. Since 2012, Chile has been attempting to establish its program, while South Africa (*SA Agulhas II*) and France (*Marion Dufresne II*) have established their programs south of Africa and around Kerguelen, respectively.

To date, more than 1000 tows have been conducted by the SO-CPR Survey. Tows have been conducted at some time in over about 70% of the Southern Ocean, mainly in the months from September to April when most research and supply ships are operating. However, a few tows have been conducted in winter: May, July and August. Most of the sampling is in the region south and west of Australia, and the Ross Sea region, which represent approximately 40% of the region routinely and consistently monitored.

Separately, the British Antarctic Survey, the CPR Survey (MBA UK) and the South Georgia government have conducted CPR tows in the South Atlantic between the Falklands and South Georgia (SF route). This routine has been suspended since April 2017 due to lack of funding. The SF samples are processed in UK and the data held in the CPR Survey's database and not in the SO-CPR Survey database. While both surveys are separate they are formally affiliated and linked as founding members of GACS. Both data sets are contributed to the GACS database (Batten et al. 2019).

#### *Endorsement by SCAR*

SCAR recognised the importance and value of the CPR data and established an Action Group at the 2006 Hobart XXIX SCAR Business Meeting to investigate the development of a longer term support group, how the survey could be expanded to involve other groups and nations and make the data more available for users, particularly in support of the IPY projects Census of Antarctic Marine Life (CAML) and the Marine Biodiversity Information Network (SCAR-MarBIN). At the subsequent 2008 St Petersburg XXX SCAR Business Meeting Action Group was converted to a long-term Expert Group (EG-CPR) with the following Terms of Reference:

1. Provide guidance to the SCAR Southern Ocean CPR Survey in order to meet the survey objectives.
  - a. Map the biodiversity and distribution of plankton, including euphausiid (krill) life stages in the Southern Ocean.
  - b. Use the sensitivity of plankton to environmental change as early warning indicators of the health of Southern Ocean by studying spatial-temporal variation in plankton patterns.
  - c. Serve as reference on the general status of the Southern Ocean for other monitoring programs.
2. Develop and maintain the SO-CPR Database and to improve access for users.
3. Expand and enhance the SO-CPR Survey to include more ships and repeat transects around Antarctica.
4. Provide appropriate advice on CPR methodology, data and results to SCAR and to the ATS.

SCAR gave its full endorsement to SO-CPR, now the SCAR Southern Ocean CPR Survey, and the SO-CPR Dataset became an official SCAR Business Product. It is became a substantial key dataset within SCAR-MarBIN which is now incorporated into the Antarctic Biodiversity portal biodiversity.aq.

Those terms of reference are still relevant to the Expert Group. However, following the 2016 Kuala Lumpur XXXIV SCAR Business Meeting the focus of the group has transitioned more to developing the database and maintaining data quality, as well as continuing to develop the survey itself. The new Terms of Reference are:

1. Continue to develop and maintain the SO-CPR Database and to improve access for users.
2. To ensure Quality Assurance and Quality Control (QA/QC) of the data through regular training and standardisation workshops.
3. Encourage other nations, especially developing Antarctic nations, to participate in the workshops and subsequently improve spatial and temporal coverage of CPR tows around Antarctica.

## **DESCRIPTION OF KEY ISSUES**

The SCAR Antarctic Climate Change and the Environment (ACCE) Report identified the Antarctic/Southern Ocean region as particularly vulnerable to the impacts of climate change; warming oceans, changing circulation, diminishing sea-ice habitat and ocean acidification (Turner et al., 2009). The ACCE report gives a thorough description of the issues relating to the physical environment. The following are background summaries of the physical issues pertinent to zooplankton and also in relation to the three principal regions or sectors of the Southern Ocean: Atlantic, Indian and Pacific.

The Antarctic region can be split into three major regions. These are the Atlantic sector of the Southern Ocean which includes the Antarctic Peninsula, Weddell Sea, Scotia Arc and the southern Atlantic. To the east is the Indian Ocean (eastern Antarctic) sector extends from south of Africa to south of Tasmania. The third region is the Pacific sector from south of New Zealand to the Antarctic Peninsula incorporating the Ross, Amundsen and Bellingshausen Seas. These sectors correspond with FAO statistical areas 48, 58 and 88, respectively. The report will often refer to these regions, in addition to smaller discrete regions.

### **Climate and Oceanography**

The Southern Ocean is characterised by the Antarctic Circumpolar Current (ACC), the world's largest current, which flows uninterrupted around Antarctica linking the Atlantic, Indian and Pacific Oceans. The Drake Passage is the only chokepoint in the flow of the ACC, where the passage and the bathymetry of the Scotia Ridge restricts the flow and enhances mixing and vertical circulation (Naveira Garabato et al., 2004; Venables et al., 2012). The ACC comprises a number of distinct fronts (Orsi et al., 1995; Sokolov and Rintoul, 2009a). The Sub-Antarctic Front (SAF) is the northern edge of the ACC. Moving southward are the Polar Front (PF), Southern ACC Front (SACCF) which lies close to the northern sea-ice limit, and the Southern Boundary (SB) of the ACC. South of the SB is the westward flowing Coastal Current and Antarctic Slope Front. Each front is distinguished by distinct changes in oceanographic characteristics in relation, temperature, salinity, density, and oxygen concentration (Orsi et al., 1995), each producing distinctive zones, each characterised by discrete plankton assemblages (Hunt and Hosie, 2005; Hosie et al., 2014). The ACC fronts often have a number of sub-branches (Sokolov and Rintoul, 2002, 2009a). There is a southward latitudinal decline in temperature from about +8°C at the SAF to sub-zero temperatures in high latitudes. The vertical water column is equally stratified through wind mixing, horizontal circulation and the annual formation and melting of the sea-ice.

Since the 1930s there has been a systematic and substantial warming of the Southern Ocean with much of this concentrated in the Antarctic Circumpolar Current (ACC) (Aoki et al., 2003; Böning et al., 2008; Gille, 2002, 2008). Surface temperatures have increased by almost a degree, more than a

tenth of a degree per decade, but significant increases are recorded at all depths (Aoki et al., 2003; Gille, 2008). This warming can be attributed to the southward movement of the ACC. Sokolov and Rintoul (2009b) have confirmed there has been a general shift southward of ACC fronts of 0.6° of latitude (+60 km). Increased heat flux and subduction of warmed surface waters are other potential causes of warming waters (Gille 2008; Rintoul et al., 2012), and may be due to human influence (Fyfe, 2006). The warming of the Southern Ocean is not uniform. Ducklow et al. (2007) described the western Antarctic Peninsula as "experiencing the most rapid warming of any marine ecosystem on the planet". Some areas of the western Antarctic Peninsula (WAP) has recorded significant increase in water temperature caused by Circumpolar Deep Water being pushed up onto the shelf (Dinniman et al., 2012). This has led to a decline in sea-ice, a regime shift in the phytoplankton, a change from a krill-centric to copepod dominated food web and changes in abundance and distribution of Adelie and Gentoo penguins (Dinniman et al., 2012; Hofmann et al., 2008; McClintock et al., 2008; Montes-Hugo et al., 2009). At the same time as the warming and southward shift of the ACC, there has been freshening of the upper surface waters of the Southern Ocean (Boyer et al., 2005; Böning et al., 2008; Rintoul et al., 2012). Freshening of the upper waters will lead to increased stratification and potentially a reduction in the input of nutrients into the euphotic zone (Rintoul et al., 2012).

The paucity of picophytoplankton and absence of cyanobacteria are key features of Antarctic waters. A warmer Southern Ocean is hypothesised to favour picoplankton and cyanobacteria resulting in increases in abundance and range extension south of the Polar Front (Marchant et al., 1987; Ishikawa et al., 2002). This would significantly affect the structure and function of the microbial community in the Antarctic marine ecosystem and subsequently the rest of the food chain.

The coccolithophorid *Emiliana huxleyi* is now very abundant, up to 100 cells ml<sup>-1</sup> between 2002 and 2006, in the sea-ice zone in the eastern Antarctic region of 140°E whereas it was absent or extremely rare south of 60°S prior to 1995 (Cubillos et al., 2007). Similar extensions south have been observed along 45°E (Mohan et al., 2008) and may be a function of general warming in the east Antarctic region.

## Sea Ice

The annual growth and retreat of Antarctic sea-ice is one of the major drivers influencing the distribution, abundance and survival biota of the region. At its maximum extent in September (late winter) sea-ice covers 19 to 20 million km<sup>2</sup> before retreating to about 3 to 4 million km<sup>2</sup> in February (late summer) (Comiso and Nishio, 2008). The area affected by the seasonal growth-retreat of sea-ice is referred to here as the Sea-Ice Zone (sometimes referred to as the Seasonal Sea Ice Zone) and is one of the major drivers influencing the distribution, abundance and survival of biota of the region. The Southern Boundary of the ACC lies within the Sea-Ice Zone.

The spatial and temporal variation in sea-ice extent and volume is complex and the reader is referred to Massom and Stammerjohn (2010) for a detailed review. The major concern is that global warming will affect the extent and volume of sea-ice, which in turn will affect the sea-ice organisms such as Antarctic krill. There has been an approximate 25% loss of sea-ice between the 1950s and 1970s (de la Mare, 1997, 2009). Again, the effects of warming are not uniform. Since the late 1970s there has been a slight but statistically significant increase in sea-ice overall of about 0.9% per decade, higher in the Ross Sea of 4.2% but there has been a substantial decline in sea-ice in the neighbouring Amundsen and Bellingshausen Seas area of 5.7% per decade. In the long term, it is predicted that there will be an overall decline of winter sea-ice extent and volume of 24 to 34 % by the end of the 21st Century (Arzel et al., 2006; Bracegirdle et al., 2008; Turner et al., 2009). Turner et al. (2009) noted that a loss of sea-ice habitat will likely cause the disappearance of marine ice algae. In turn that may a decline in the abundance of zooplankton dependent on that algae, leading to a cascade effect through the higher trophic levels with a decline fish that feed on zooplankton, and subsequently the marine birds and mammals that depend on the fish.

Atkinson et al. (2004) has already suggested that there has been a substantial wide-scale decline in the abundance and distribution of Antarctic krill, which is likely to be due to a decrease in sea-ice extent. At the same time salps, which usually prefer warmer waters, have increased their abundance in parts of the region north of the sea-ice/krill habitat. The assessment by Atkinson et al. (2004) was based on dataset collated from numerous surveys which were primarily designed for purposes other than studying long-term trends, e.g. for stock assessment of krill, zooplankton composition, biogeography. Further, while Antarctic krill and salps are major components in the Southern Ocean in relation to biomass, so are numerous species of copepods, other euphausiids and other zooplankton species that could not be considered in the analyses.

Further sea-ice loss due to warming and plus southward movements of the fronts is expected to see a contraction of plankton southward. There is a limit to southward movement due to the presence of the continent. For example, the coastal ice kill *Euphausia crystallorophias*, which prefers sub-zero water will have nowhere to retreat. Mackey et al. (2012) paper hypothesised the potential changes in macro-zooplankton distributions in the southern Atlantic in response to increase ocean temperatures.

## **Ocean Acidification**

The Royal Society Report on Ocean Acidification (2005) has predicted that increased absorption of CO<sub>2</sub> will lead to an under-saturation of aragonite by the end of this century which would result in plankton such as thecosome pteropod snails unable to produce a shell. This could occur as early as 2050 (Orr et al., 2005). Pteropods are very abundant in the Southern Ocean, they are important in the diet of other zooplankton and myctophid fish, and play an important role in the Southern Ocean carbon pump removing carbon from the upper waters to the deep. We can only speculate the consequences on the food web and carbon pump of the loss of plankton with calcium carbonate shells. However, acidification also effects the development of the larvae of Antarctic krill, a key prey species in the Antarctic marine ecosystem. This may lead to a substantial decline in Antarctic krill stocks which will have a substantial impact on the Antarctic food web and survival of higher predators such as birds and marine mammals (Kawaguchi et al., 2013).

Foraminifera are a large group of protists; they also produce a shell made of calcium carbonate. Because calcium carbonate is susceptible to dissolution in acidic conditions, foraminifera may be strongly affected by a changing climate and ocean acidification. Moy et al. (2009) compared the shell weights of the modern foraminifera *Globigerina bulloides* collected from sediment traps in the Southern Ocean with the weights of foraminifera shells preserved in the underlying Holocene-aged sediments. They found that the modern shell weights are 30–35% lower than those from the sediments, consistent with reduced calcification induced by ocean acidification. They noted that “it was unclear whether reduced calcification will affect the survival of this and other species, but a decline in the abundance of foraminifera caused by acidification could affect both marine ecosystems and the oceanic uptake of atmospheric carbon dioxide.”

Pteropods, foraminifera, euphausiid larvae in general, and *Euphausia superba* larvae specifically are target groups within SO-CPR for monitoring possible changes in abundance that may be in response to acidification effects.

## **METHODOLOGY**

### **CPR sampling**

The CPR is a robust near-surface plankton sampling device that is designed to be towed behind merchant ships as they travel at normal cruising speed (Warner and Hays, 1994). The CPR can in fact

be towed by any vessel that has a suitable winch and towing point. The SO-CPR Survey routine uses research vessels, resupply/cargo, military and fishing vessels. Research vessels have an advantage of often having permanently mounted environmental recording systems that can provide simultaneous data on temperature, salinity, fluorometry, as well as meteorological and GPS data. We used a Type II Mark V CPR, based on the design of the original CPR Surveys Type II Mark III CPRs, with minor modifications to the design as described in the above in the section establishing the SO-CPR Survey (see also Hosie et al., 2003). The CPR is towed horizontally, typically at a ship speed of 12-15 knots. CPRs have been towed successfully at 23 knots. The CPR is deployed from the stern with 100 m of wire cable paid out, with the aim of towing the CPR at a depth of 10 m (Hunt and Hosie, 2003; Hosie et al., 2003). This usually occurs within the wash of the ship's propeller which is estimated to mix the top 20 m. Water enters a small aperture of 1.62 cm<sup>2</sup> (1.27 cm x 1.27 cm) at the front of the CPR body, before entering a wider collecting tunnel of 10 x 5 cm in the internal recording mechanism "cassette" (Fig. 1). This reduces the speed of water flow by about 30 times (equivalent to 0.21 to 0.26 ms<sup>-1</sup>) before it hits a slowly moving band of "filtering collecting silk" with an average mesh size of 270 µm. All silk is sourced from the CPR Survey in Plymouth for the purposes of standardisation. The towing of the CPR through the water turns an external propeller that drives the mesh rolls across the tunnel at a rate of approximately 1 cm per 1 nautical mile (1852 m) of tow. The filtering collecting silk is then covered by a second band of "covering silk" of the same mesh, with the plankton sandwiched between the two layers. Both silks are then rolled into a storage tank filled with buffered formaldehyde. The 6 m long mesh is sufficient to sample 450 nautical miles (833 km) as a normal towing distance. In practice however, the length of individual tows may vary due to operational requirements of the vessel, or sea state. For example, greater vertical undulation in heavy seas results in shorter horizontal towed distance, while a following sea or current can lead to longer distances. Note: CPRs have been used successfully during heavy sea conditions in the Antarctic up to Storm (Force 10), but it is not recommended to deploy or retrieve a CPR in such conditions. At the end of tow, usually 30-38 hours of towing depending on ship speed, the CPR is brought on deck, the recording cassette is removed and fresh cassette installed, and the CPR is redeployed. This usually takes 10 minutes. The cassettes are relatively easy to load by both scientific and non-scientific personnel. The ship does not need to stop. With a set of prepared cassettes, or refurbishing the units on board with new silk, continuous series of tows can be made for 1000s of nautical miles.

### *Survey Area*

The principal area of interest for SO-CPR is all the region south of the Sub-Antarctic Front, through to the Antarctic coast. We define the SAF as the northern boundary of the Southern Ocean, although we will frequently make reference to the Sub-Antarctic Zone north of the SAF. Some participating nations elect to conduct sampling soon after leaving a home port, e.g. Cape Town South Africa, Wellington or Timaru New Zealand. France has a particular interest in ecology around Kerguelen and concentrates most of its tows in the sub-Antarctic with some tows extending into the PFZ. Due to the high volume of samples collated and constraints of resources and logistics, Australia and Japan usually aim to commence sampling just north of the SAF in order to capture the SAF. On return routes north, tows are concluded after crossing the SAF as indicated by rising sea surface temperature. For safety and navigational reasons, CPR tows usually cease operation before entering pack ice, and also in the inshore neritic zone because of the presence of persistent sea-ice and icebergs.

□

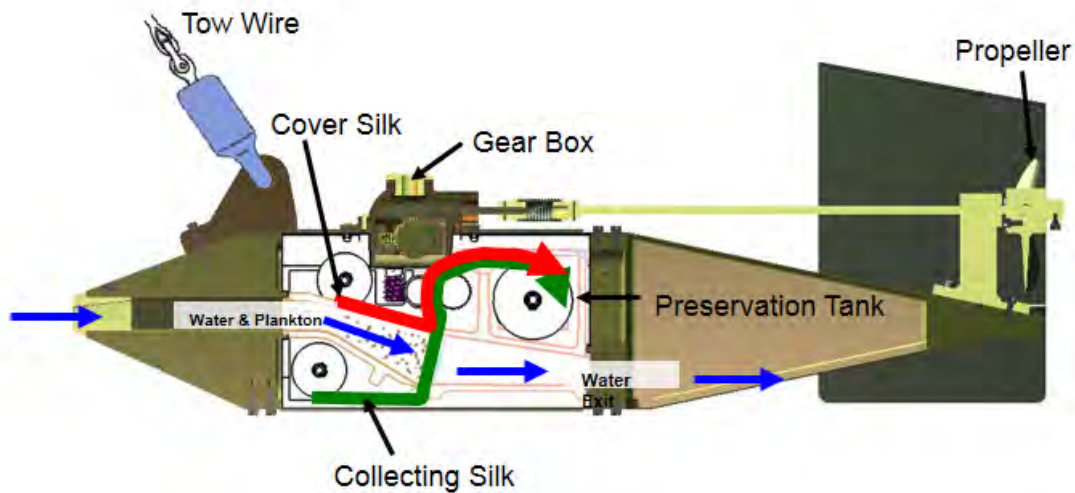


Figure 1. The internal workings of the Continuous Plankton Recorder (CPR).

### Sample processing

In the laboratory, each set of silks (collecting and cover silk) is unrolled and cut into segments representing a 5 nautical mile (9.26 km) of towed distance. A computer programme written in Visual Basic is used to determine the length and number of sections to be cut. The programme enables time-stamped position and selected environmental data, recorded at one-minute intervals on the ships' data-logging systems, to be assembled into data sections of 5 nautical miles each. Environmental data are averaged for the same sections and subsequently linked with the zooplankton data through the time and latitude-longitude which is calculated for the end of each sampling segment. The 5 nautical mile segments were chosen in order to make comparisons at a higher resolution in the vicinity of frontal zones, but the data can be assembled into still larger bins, e.g. ten nautical miles as used by the CPR Survey UK.

While 5 nautical miles is the selected distance, it hasn't always been the case. For early RSV *Aurora Australis* tows it was assumed that the silks advanced at a predetermined rate of 1 cm per nautical mile, whereas each tow had subtle variations in silk advancement often due to traveling with or against a current. True segment length has since been recalculated. At other times, some silks have been incorrectly cut and the true length has again been recalculated.

The last segment at the end of a tow is usually shorter than 5 nautical miles. The following rule is applied: if the last segment is longer than 2.5 nautical miles it remains as a segment in its own right and can be between 2.5 and 4.99 nautical miles in length, whereas if it is less than 2.5 it is added to the penultimate segment making the last segment 5 to 7.49 nautical miles. The segment length is recorded in the database and can be used to standardise species counts. Dividing species counts by segment length will give counts per nautical miles, which can be standardised back to 5 nautical miles, or to a theoretical volume filtered by multiplying the distance travelled by aperture area (12.7 x 12.7 mm or 0.000161 m<sup>2</sup>).

Volume Filtered = Distance (n miles) x 1852 metres x 0.000161 m<sup>2</sup>.



A 5 nautical mile segment theoretically represents 1.49 m<sup>3</sup>.

A Phytoplankton Colour Index (PCI) score is assigned to each 5 nautical mile segment based on Pantone colour charts ([www.pantone.com](http://www.pantone.com)) with scores of 0 = no colour, 1 = very pale green, 2 = pale green and 3 = green. Pantone colour codes corresponding to these indices depend on other coloured material in the water and the dominant phytoplankton group. This PCI measure is indicative of the chlorophyll-*a* concentration and is based on the CPR Survey method (Reid et al., 1998b). Note: in the Southern Ocean there are some diatoms that are quite common on the silks but as they have very low amounts of chlorophyll their colour and presence doesn't register in the PCI analysis.

For each 5 nautical mile segment, large specimens easily identified by eye (e.g. euphausiids) are removed and counted, and the remaining species are then rinsed off the silks and counted and identified under a dissecting microscope using the SO-CPR Survey method (Hosie et al., 2003). This method was developed from the SAHFOS method to be a more comprehensive approach, and to eliminate the need for highly specialised equipment. When zooplankton abundances were very high, subsampling using a Motoda plankton splitter was carried out (Motoda, 1959). Testing of the efficiency of the plankton splitter took place to ensure that each subsample was an accurate representative of the whole sample.

Zooplankton were identified to the lowest taxonomic level possible, ideally to species based on the Register of Antarctic Marine Species (RAMS) developed by the SCAR Marine Biodiversity Information Network (SCAR-MarBIN; De Broyer and Danis, 2009). Some zooplankton are easily damaged, notably gelatinous and soft bodied species, and were only identified to a coarser taxonomic resolution. For copepods, copepodites and adults are combined. Calanoid copepods are usually identified to Species but if not then to Genus. For example, if a specimen of *Clausocalanus* cannot be identified to species, usually *C. brevipes* or *laticeps*, then it will be recorded as *Clausocalanus* spp.. If it cannot be identified to Genus, or Family, it will be grouped in the database as “Small\_calanoid\_copepods”. These are small copepod species difficult to identify after being trapped on mesh or not developed well enough to permit taxonomic identification. Copepodites of larger species can usually be identified to Genus. The nauplii of copepods are counted separately. The nauplii of *Rhincalanus gigas* are distinctive and abundant and are counted separately. Antarctic krill (*Euphausia superba*) and other euphausiids were identified to adult and developmental stage, i.e. nauplius, calyptopis and furcilia. The larval stages of Antarctic krill *Euphausia superba* and the big-eye krill *Thysanoessa macrura* are further classified into the three calyptopis stages (CI, CII, CIII) and six furcilia (FI, FII, FIII, FIV, FV, FVI). If a larvae cannot be classified to a distinct stage then they are grouped as coarser level of calyptopis or furcilia. Antarctic krill is a key species for monitoring. *T. macrura* is a smaller but more abundant species and the larvae are monitored as a proxy for potential environmental effects on krill larvae. Other specimens unable to be identified to species with certainty are grouped at a higher level, e.g. Genus or Family or higher.

The zooplankton taxa for the SO-CPR Survey are regularly checked using the Taxon Match of the World Register of Marine Species (WoRMS: <http://www.marinespecies.org/index.php>) name validation tool. WoRMS is an open-access inventory of all marine species, and is >90% complete (Costello et al., 2013). The tool performs a cross check of the spelling and taxonomic status of species against the WoRMS database, and returns standard taxonomic information with valid names. The database currently holds data for 289 zooplankton taxa and developmental stages (Table 2).

Table 2. Zooplankton species/taxa list for the SO-CPR Survey. C: Calyptopis stage, F: Furcilia stage.

| Order          | Taxa                                   | Order             | Taxa                             |
|----------------|--|-------------------|----------------------------------|
| Amphipoda      | Amphipoda indet                        | Calanoida         | <i>Calanoides acutus</i>         |
| Amphipoda      | <i>Brachyscelus cruscolum</i>          | Calanoida         | <i>Calanoides brevicornis</i>    |
| Amphipoda      | <i>Cylopus lucasii</i>                 | Calanoida         | <i>Calanoides</i> sp.            |
| Amphipoda      | <i>Cylopus magellanicus</i>            | Calanoida         | <i>Calanus australis</i>         |
| Amphipoda      | <i>Dairella californica</i>            | Calanoida         | <i>Calanus propinquus</i>        |
| Amphipoda      | <i>Hemityphis</i> sp.                  | Calanoida         | <i>Calanus simillimus</i>        |
| Amphipoda      | <i>Hyperia</i> sp.                     | Calanoida         | <i>Calanus</i> sp.               |
| Amphipoda      | <i>Hyperia spinigera</i>               | Calanoida         | <i>Calocalanus plumulosus</i>    |
| Amphipoda      | <i>Hyperiella antarctica</i>           | Calanoida         | <i>Calocalanus</i> sp.           |
| Amphipoda      | <i>Hyperiella dilatata</i>             | Calanoida         | <i>Calocalanus styliremis</i>    |
| Amphipoda      | <i>Hyperiella</i> sp.                  | Calanoida         | <i>Candacia bipinnata</i>        |
| Amphipoda      | Hyperiidae indet                       | Calanoida         | <i>Candacia cheirura</i>         |
| Amphipoda      | <i>Hyperoche medusarum</i>             | Calanoida         | <i>Candacia falcifera</i>        |
| Amphipoda      | <i>Hyperoche</i> sp.                   | Calanoida         | <i>Candacia maxima</i>           |
| Amphipoda      | <i>Phronima</i> sp.                    | Calanoida         | <i>Candacia</i> sp.              |
| Amphipoda      | Platysceloidea indet                   | Calanoida         | <i>Centropages aucklandicus</i>  |
| Amphipoda      | <i>Primno macropa</i>                  | Calanoida         | <i>Centropages bradyi</i>        |
| Amphipoda      | <i>Scina</i> sp.                       | Calanoida         | <i>Centropages furcatus</i>      |
| Amphipoda      | <i>Themisto australis</i>              | Calanoida         | <i>Centropages</i> sp.           |
| Amphipoda      | <i>Themisto gaudichaudii</i>           | Calanoida         | <i>Clausocalanus arcuicornis</i> |
| Amphipoda      | <i>Themisto</i> sp.                    | Calanoida         | <i>Clausocalanus brevipes</i>    |
| Amphipoda      | <i>Vibilia antarctica</i>              | Calanoida         | <i>Clausocalanus ingens</i>      |
| Amphipoda      | <i>Vibilia armata</i>                  | Calanoida         | <i>Clausocalanus laticeps</i>    |
| Amphipoda      | <i>Vibilia</i> sp.                     | Calanoida         | <i>Clausocalanus pergens</i>     |
| Branchiopoda*  | Branchiopoda indet                     | Calanoida         | <i>Clausocalanus</i> sp.         |
| Chaetognatha** | Chaetognatha indet                     | Harpacticoida     | <i>Clytemnestra scutellata</i>   |
| Phragmophora   | <i>Eukrohnia hamata</i>                | Harpacticoida     | <i>Clytemnestra</i> sp.          |
| Aphragmophora  | <i>Pseudosagitta gazellae</i>          | Hexanauplia*      | Copepoda indet                   |
| Aphragmophora  | <i>Pseudosagitta</i> sp.               | Hexanauplia*      | Copepoda nauplius indet          |
| Aphragmophora  | Sagittidae indet                       | Calanoida         | <i>Ctenocalanus citer</i>        |
| Aphragmophora  | <i>Solidosagitta marri</i>             | Calanoida         | <i>Ctenocalanus</i> sp.          |
| Choreotrichida | Tintinnina indet                       | Cyclopoida        | Cyclopoida nauplius indet        |
| Cirripedia***  | Cirripedia cyprid                      | Calanoida         | <i>Drepanopus</i> sp.            |
| Cirripedia***  | Cirripedia nauplius                    | Calanoida         | <i>Euaugaptilus</i> sp.          |
| Diplostraca    | <i>Evadne</i> sp.                      | Calanoida         | <i>Eucalanus hyalinus</i>        |
| Diplostraca    | <i>Podon</i> sp.                       | Calanoida         | <i>Eucalanus</i> sp.             |
| Siphonophorae  | Abylidae indet                         | Calanoida         | <i>Euchirella rostrata</i>       |
| Siphonophorae  | <i>Chelophyes</i> sp.                  | Calanoida         | <i>Euchirella rostromagna</i>    |
| Cnidaria**     | Cnidaria indet                         | Calanoida         | <i>Euchirella</i> sp.            |
| Siphonophorae  | <i>Lensia</i> sp.                      | Harpacticoida     | <i>Euterpina</i> sp.             |
| Siphonophorae  | Siphonophorae nectophore               | Cyclopoida        | <i>Farranula</i> sp.             |
| Siphonophorae  | Siphonophorae sp.                      | Calanoida         | <i>Haloptilus oxycephalus</i>    |
| Narcomedusae   | <i>Solmundella bitentaculata</i>       | Harpacticoida     | Harpacticoida indet              |
| Calanoida      | <i>Acartia (Acartia) danae</i>         | Calanoida         | <i>Heterorhabdus austrinus</i>   |
| Calanoida      | <i>Acartia (Acartiura) tranteri</i>    | Calanoida         | <i>Heterorhabdus lobatus</i>     |
| Calanoida      | <i>Acartia (Odontacartia) pacifica</i> | Calanoida         | <i>Heterorhabdus</i> sp.         |
| Calanoida      | <i>Acartia</i> sp.                     | Poecilostomatoida | <i>Heterorhabdus spinifrons</i>  |
| Calanoida      | <i>Aetideus australis</i>              | Calanoida         | <i>Lubbockia</i> sp.             |
| Calanoida      | <i>Aetideus</i> sp.                    | Calanoida         | <i>Lucicutia flavicornis</i>     |

| Order             | Taxa                              | Order             | Taxa   |
|-------------------|-----------------------------------|-------------------|--|
| Calanoida         | <i>Lucicutia</i> sp.              | Calanoida         | <i>Scaphocalanus echinatus</i>               |
| Calanoida         | <i>Mecynocera clausi</i>          | Calanoida         | <i>Scaphocalanus farrani</i>                 |
| Calanoida         | <i>Mecynocera</i> sp.             | Calanoida         | <i>Scolecithricella minor</i>                |
| Calanoida         | <i>Mesocalanus</i> sp.            | Calanoida         | <i>Scolecithricella</i> sp.                  |
| Calanoida         | <i>Mesocalanus tenuicornis</i>    | Calanoida         | Calanoida indet (small)                      |
| Calanoida         | <i>Metridia gerlachei</i>         | Calanoida         | <i>Stephos longipes</i>                      |
| Calanoida         | <i>Metridia lucens</i>            | Calanoida         | <i>Subeucalanus longiceps</i>                |
| Calanoida         | <i>Metridia</i> sp.               | Calanoida         | <i>Subeucalanus</i> sp.                      |
| Calanoida         | <i>Microcalanus pygmaeus</i>      | Calanoida         | <i>Sulcanus conflictus</i>                   |
| Harpacticoida     | <i>Microsetella norvegica</i>     | Calanoida         | <i>Temora turbinata</i>                      |
| Harpacticoida     | <i>Microsetella rosea</i>         | Poecilostomatoida | <i>Triconia antarctica</i>                   |
| Harpacticoida     | <i>Microsetella</i> sp.           | Crustacea****     | Crustacea nauplius indet                     |
| Calanoida         | <i>Nannocalanus minor</i>         | —                 | Nauplius indet                               |
| Calanoida         | <i>Neocalanus gracilis</i>        | Ctenophora**      | Ctenophora indet                             |
| Calanoida         | <i>Neocalanus tonsus</i>          | Cumacea           | Cumacea indet                                |
| Harpacticoida     | <i>Oculosetella gracilis</i>      | Decapoda          | Decapoda (natant) indet juv                  |
| Cyclopoida        | <i>Oithona atlantica</i>          | Decapoda          | Decapoda megalopa indet                      |
| Cyclopoida        | <i>Oithona frigida</i>            | Decapoda          | Decapoda nauplius indet                      |
| Cyclopoida        | <i>Oithona longispina</i>         | Decapoda          | Decapoda phyllosoma indet                    |
| Cyclopoida        | <i>Oithona similis</i>            | Decapoda          | Decapoda zoea indet                          |
| Cyclopoida        | <i>Oithona</i> sp.                | Decapoda          | <i>Munida gregaria</i>                       |
| Poecilostomatoida | <i>Oncaea curvata</i>             | Decapoda          | <i>Nematocarcinus longirostris</i>           |
| Poecilostomatoida | <i>Oncaea mediterranea</i>        | Decapoda          | Sergestidae indet                            |
| Poecilostomatoida | <i>Oncaea</i> sp.                 | Stomatopoda       | <i>Squilla</i> sp.                           |
| Poecilostomatoida | <i>Oncaea venusta typica</i>      | Noctilucales      | <i>Noctiluca scintillans</i>                 |
| Calanoida         | <i>Onchocalanus</i> sp.           | Echinoidea*       | Echinoidea larvae                            |
| Calanoida         | <i>Paracalanus aculeatus</i>      | —                 | Egg indet                                    |
| Calanoida         | <i>Paracalanus indicus</i>        | —                 | Egg mass                                     |
| Calanoida         | <i>Paracalanus</i> sp.            | Euphausiacea      | <i>Euphausia crystallorophias</i>            |
| Calanoida         | <i>Paraeuchaeta antarctica</i>    | Euphausiacea      | <i>Euphausia crystallorophias</i> calyptopis |
| Calanoida         | <i>Paraeuchaeta barbata</i>       | Euphausiacea      | <i>Euphausia crystallorophias</i> furcilia   |
| Calanoida         | <i>Paraeuchaeta biloba</i>        | Euphausiacea      | <i>Euphausia frigida</i>                     |
| Calanoida         | <i>Paraeuchaeta exigua</i>        | Euphausiacea      | <i>Euphausia frigida</i> calyptopis          |
| Calanoida         | <i>Paraeuchaeta</i> sp.           | Euphausiacea      | <i>Euphausia frigida</i> furcilia            |
| Calanoida         | <i>Paraheterorhabdus farrani</i>  | Euphausiacea      | <i>Euphausia hanseni</i> furcilia            |
| Calanoida         | <i>Paralabidocera antarctica</i>  | Euphausiacea      | <i>Euphausia longirostris</i>                |
| Calanoida         | <i>Pleuromamma abdominalis</i>    | Euphausiacea      | <i>Euphausia longirostris</i> calyptopis     |
| Calanoida         | <i>Pleuromamma borealis</i>       | Euphausiacea      | <i>Euphausia longirostris</i> furcilia       |
| Calanoida         | <i>Pleuromamma gracilis</i>       | Euphausiacea      | <i>Euphausia lucens</i>                      |
| Calanoida         | <i>Pleuromamma piseki</i>         | Euphausiacea      | <i>Euphausia recurva</i>                     |
| Calanoida         | <i>Pleuromamma robusta</i>        | Euphausiacea      | <i>Euphausia similis</i>                     |
| Calanoida         | <i>Pleuromamma</i> sp.            | Euphausiacea      | <i>Euphausia similis</i> furcilia            |
| Calanoida         | <i>Pleuromamma xiphias</i>        | Euphausiacea      | <i>Euphausia spinifera</i>                   |
| Calanoida         | <i>Rhincalanus gigas</i>          | Euphausiacea      | <i>Euphausia spinifera</i> calyptopis        |
| Calanoida         | <i>Rhincalanus gigas</i> nauplius | Euphausiacea      | <i>Euphausia spinifera</i> furcilia          |
| Calanoida         | <i>Rhincalanus nasutus</i>        | Euphausiacea      | <i>Euphausia superba</i>                     |
| Calanoida         | <i>Rhincalanus</i> sp.            | Euphausiacea      | <i>Euphausia superba</i> C1                  |
| Poecilostomatoida | <i>Sapphirina nigromaculata</i>   | Euphausiacea      | <i>Euphausia superba</i> C2                  |
| Poecilostomatoida | <i>Sapphirina</i> sp.             | Euphausiacea      | <i>Euphausia superba</i> C3                  |

| Order          | Taxa                                    | Order           | Taxa                              |
|----------------|---|-----------------|-----------------------------------|
| Euphausiacea   | <i>Euphausia superba</i> calyptopis     | Rotaliida       | <i>Turborotalita quinqueloba</i>  |
| Euphausiacea   | <i>Euphausia superba</i> F1             | Thecosomata     | <i>Clio pyramidata</i>            |
| Euphausiacea   | <i>Euphausia superba</i> F2             | Thecosomata     | <i>Clio</i> sp.                   |
| Euphausiacea   | <i>Euphausia superba</i> F3             | Gymnosomata     | <i>Clione limacina antarctica</i> |
| Euphausiacea   | <i>Euphausia superba</i> F4             | Gymnosomata     | <i>Clione</i> sp.                 |
| Euphausiacea   | <i>Euphausia superba</i> F5             | Gastropoda*     | Gastropoda indet                  |
| Euphausiacea   | <i>Euphausia superba</i> F6             | Thecosomata     | <i>Limacina</i> sp.               |
| Euphausiacea   | <i>Euphausia superba</i> furcilia       | Gastropoda*     | Pteropoda indet                   |
| Euphausiacea   | <i>Euphausia triacantha</i>             | Gymnosomata     | <i>Spongiobranchea australis</i>  |
| Euphausiacea   | <i>Euphausia triacantha</i> calyptopis  | Teuthida        | Teuthida indet                    |
| Euphausiacea   | <i>Euphausia triacantha</i> furcilia    | Isopoda         | Isopoda indet                     |
| Euphausiacea   | <i>Euphausia vallentini</i>             | Bivalvia*       | Bivalvia larvae                   |
| Euphausiacea   | <i>Euphausia vallentini</i> calyptopis  | Mysida          | Mysidae indet                     |
| Euphausiacea   | <i>Euphausia vallentini</i> furcilia    | Ostracoda*      | Ostracoda indet                   |
| Euphausiacea   | Euphausiidae calyptopis indet           | Chordata**      | Pisces egg                        |
| Euphausiacea   | Euphausiidae furcilia indet             | Chordata**      | Pisces larvae                     |
| Euphausiacea   | Euphausiidae indet                      | Myctophiformes  | Myctophidae indet                 |
| Euphausiacea   | Euphausiidae metanauplius indet         | Perciformes     | <i>Pleuragramma antarctica</i>    |
| Euphausiacea   | Euphausiidae nauplius indet             | Myctophiformes  | <i>Protomyctophum</i> sp.         |
| Euphausiacea   | <i>Nematoscelis megalops</i>            | Phyllococida    | Alciopidae indet                  |
| Euphausiacea   | <i>Nyctiphanes australis</i>            | Phyllococida    | Iospilidae indet                  |
| Euphausiacea   | <i>Nyctiphanes australis</i> calyptopis | Phyllococida    | Lopadorrhynchidae indet           |
| Euphausiacea   | <i>Nyctiphanes australis</i> furcilia   | Phyllococida    | <i>Maupasia</i> sp.               |
| Euphausiacea   | <i>Thysanoessa gregaria</i>             | Phyllococida    | <i>Pelagobia longicirrata</i>     |
| Euphausiacea   | <i>Thysanoessa gregaria</i> calyptopis  | Phyllococida    | <i>Phalacrophorus pictus</i>      |
| Euphausiacea   | <i>Thysanoessa gregaria</i> furcilia    | Phyllococida    | <i>Phalacrophorus</i> sp.         |
| Euphausiacea   | <i>Thysanoessa macrura</i>              | Polychaeta*     | Polychaeta indet                  |
| Euphausiacea   | <i>Thysanoessa macrura</i> C1           | Polychaeta*     | Polychaeta larvae                 |
| Euphausiacea   | <i>Thysanoessa macrura</i> C2           | Phyllococida    | <i>Tomopteris carpenteri</i>      |
| Euphausiacea   | <i>Thysanoessa macrura</i> C3           | Phyllococida    | <i>Tomopteris</i> sp.             |
| Euphausiacea   | <i>Thysanoessa macrura</i> calyptopis   | Phyllococida    | <i>Travisioopsis levinseni</i>    |
| Euphausiacea   | <i>Thysanoessa macrura</i> F1           | Phyllococida    | <i>Travisioopsis</i> sp.          |
| Euphausiacea   | <i>Thysanoessa macrura</i> F2           | Phyllococida    | <i>Typhloscolex muelleri</i>      |
| Euphausiacea   | <i>Thysanoessa macrura</i> F3           | Phyllococida    | <i>Vanadis antarctica</i>         |
| Euphausiacea   | <i>Thysanoessa macrura</i> F4           | Phyllococida    | <i>Vanadis longissima</i>         |
| Euphausiacea   | <i>Thysanoessa macrura</i> F5           | Radiozoa**      | Radiozoa indet                    |
| Euphausiacea   | <i>Thysanoessa macrura</i> F6           | Appendicularia* | Appendicularia indet              |
| Euphausiacea   | <i>Thysanoessa macrura</i> furcilia     | Doliolida       | Doliolidae indet                  |
| Euphausiacea   | <i>Thysanoessa macrura</i> metanauplius | Copelata        | <i>Fritillaria</i> sp.            |
| Euphausiacea   | <i>Thysanoessa</i> sp.                  | Copelata        | <i>Oikopleura</i> sp.             |
| Euphausiacea   | <i>Thysanoessa</i> sp. furcilia         | Pyrosomatida    | Pyrosomatidae indet               |
| Foraminifera** | Foraminifera indet                      | Salpida         | <i>Salpa fusiformis</i>           |
| Rotaliida      | <i>Globigerina bulloides</i>            | Salpida         | Salpidae indet                    |
| Rotaliida      | <i>Globigerinita uvula</i>              | Salpida         | <i>Salpa thompsoni</i>            |
| Rotaliida      | <i>Globorotalia</i> sp.                 | Salpida         | <i>Soestia zonaria</i>            |
| Rotaliida      | <i>Neogloboquadrina incompta</i>        | Salpida         | <i>Thalia</i> sp.                 |
| Rotaliida      | <i>Neogloboquadrina pachyderma</i>      |                 |                                   |

## Phytoplankton Counts

Phytoplankton cell counts are an important part of the sample processing for the CPR Survey based at the Marine Biological Association, Plymouth UK. While the SO-CPR Survey conducts the same PCI scoring of Antarctic silks, as per the northern CPR Survey, counts of phytoplankton cells are not conducted. The fundamental reason for this, is the samples collected in the Southern Ocean can remain on ship for several weeks or months, in what are often rough conditions, before the samples can eventually be returned to the laboratory. Samples collected by the northern MBA CPR Survey in the North Sea and Atlantic are usually conducted from ships of opportunity on relatively short runs. The CPR units can be back in the Plymouth laboratory within a few days. The CPR units are usually transported back undisturbed and the silks are removed from the sampling cassettes in laboratory. Antarctic vessels, whether research or resupply, are in the Antarctic for considerably longer periods, weeks to months. We therefore aim to conduct as many tows as possible during those voyages to maximise sampling opportunity. Consequently, the completed silks are usually taken off the sampling cassette, while on ship, rolled up and stored in formaldehyde solution so that the cassettes can be refurbished with new silk in order to continue sampling and extend the range or tows. As noted, the sea conditions in the Antarctic region are notably rough. In reality the samples can be shaken for several weeks, plus we are using 270 $\mu$ m mesh. Large diatoms with frustules or long chain forming diatoms can be trapped on the silk, especially on the silk fibrils. However, smaller phytoplankton or protistan cells, notably pennate diatoms, smooth shell dinoflagellates, coccolithophores, and smaller nano- and picoplankton have substantially lower possibility of being properly trapped on the silk, and remaining there as the samples are disturbed in rough conditions. It is common for such cells to be found in the bottom of sample containers containing the rolled silks after the voyage. Nor can any surety be given that the smooth or smaller phytoplankton found on the silk were in fact originally entrapped on that section of silk and not drifted in from another wrap of silk while on board ship. We cannot therefore give complete guarantee of the validity of phytoplankton counts, and consequently counts are not made. These comments do not apply to the northern CPR Survey. Also it does not apply to zooplankton counts. We do not find zooplankton specimens “loose” in the sample containers.

## Limitations of the methodology

There are a number of limitations with the CPR sampling method and subsequently the data (see below), as there are with all zooplankton sampling systems. The limitations of the CPR need to be acknowledged in order to understand what the CPR data can and cannot deliver. These include:

- The CPR is towed horizontally at a constant depth, which means that the diurnal vertical migration of some zooplankton taxa may affect abundance variations in the observed data on a 24 hour scale. Typically, higher abundances of vertically migrating species of zooplankton are recorded at night as these tend to move up in the water column. These diel variations can be informative, as migrations in zooplankton can be ecologically important (Kerfoot 1985; Steinberg et al., 2002). Normally, this can be taken into account (e.g. by selecting night-time data only, using the ship’s light sensor record to distinguish night from day). This maybe unnecessary for some analyses, e.g. for Generalised Dissimilarity Modelling (GDM).
- Towing at 10 m may only represent near surface zooplankton assemblages. Comparative studies have shown that the CPR results are comparable with plankton net systems fitted with the same mesh, and the horizontal CPR tows are indicative of plankton diversity deeper in the water column – see the later section “Comparison with other sampling systems” and Hunt and Hosie (2003).
- The small aperture of the CPR (12.7 x 12.7 mm), is effective enough for capturing mesozooplankton (0.2 – 2 mm size). Larger organisms, however, are unlikely to be sampled well by the CPR, particularly those that are large such as Antarctic krill *E. superba* that form swarms and have strong predator evasion responses. This results in under-estimates of their

abundance compared with large net systems such as the Rectangular Mid-water Trawl, or when compared with biological hydro-acoustic estimates. Nonetheless, krill of all species are collected by the CPR and their smaller developmental stages (larvae) are quite abundant in the CPR samples.

- There are concerns that the small aperture may result in avoidance by zooplankton. The CPR is towed approximately 100 m immediately behind the ship, effectively in the wash of the ship's propeller. At 15 knots, zooplankton have approximately 13 secs after passing the ship's propeller to re-orientate itself, detect and avoid the CPR while still in turbulent water (Owens et al., 2013). Avoidance is unlikely, but as the sampling methodology is consistent, abundances are at least consistently comparative.
- Gelatinous zooplankton are also often poorly sampled by the CPR, e.g. salps and jellyfish. This is due to their large size and also their soft bodies being damaged when trapped between the silks. Genetic methods can be used to identify tissue. Larvaceans are very abundant in CPR samples, and while the body of the larvacean is often damaged or missing, the tails are easy to identify to genus level and counted. Crustaceans and other harder body zooplankton such as foraminifera can usually be identified to a reasonable level and counted. Damaged individuals can only be identified to a coarser taxonomic resolution than intact specimens.
- Some species are too difficult to identify after being trapped on silk, even those that normally are easy to identify. Therefore, many species have to be grouped into coarser taxa.
- Consistency in taxonomic skill and identification across different laboratories is an issue. Analysts work in different labs in around the world. This issue was addressed and tested during the "Southern Ocean CPR Standards Workshop" (Takahashi et al., 2011a, 2017a, 2019b), which noted that a high degree of consistency in taxonomic identification was maintained. The SCAR Expert Group on CPR strives to have regular standardisation workshops. See section below.
- Temporal coverage is primarily restricted to the period when shipping can access the region, nominally September-October to March-April. Consequently, only a few tows have been conducted in the Antarctic winter months.
- Similarly, spatially there is only a limited amount of CPR data, from two tows, in the Pacific sector between the Ross Sea and the Antarctic Peninsula. This is a region infrequently visited with overall poor biological sampling in general.
- The CPR data set is particularly large with the highest density of data coming from the region south and west of Australia, 60–160 °E. When the data set is fragmented into months the data can become somewhat sparse for the early and late seasons months. It is often necessary to group these months. Even so, despite the combination of months the number of tows in the early and late seasons period are still limited and predicting or modelling patterns around Antarctica based on just a few samples is obviously tenuous. Nonetheless, monthly sub-sets of data sufficient for predicting distributions into other regions not sampled by CPR as demonstrated by Pinkerton et al. (2010) and Hosie et al. (2014).

### **Microplastics counts**

Marine plastic pollution has spread across the world's oceans, and recent findings have now demonstrated that microplastics have indeed reached the Southern Ocean (Isobe et al., 2017; Waller et al., 2017). To understand the sources and scale of this pollution, an internationally coordinated effort is needed with standardised identification techniques for microplastics. The CPR analysts from the Australian and New Zealand have been collecting a limited amount of data about microplastics alongside their primary research on CPR since 2008. In late 2016, there was a proposal for a common and appropriate counting methodology from the Global Alliance of CPR Surveys (GACS) community, which coordinated a global CPR program (Table 3). As the counting rule for microplastics in CPR samples, we try to identify to three types of plastic: "Strand," "Bead," and "Flake." The "Strand" type

is further classified into “Monofilament-type” or “Flat-fibre type.” Each type is divided into seven colours (clear/transparent, black, blue, red, yellow, green, and other colours) and four size ranges (“Small”  $\leq 300 \mu\text{m}$ , “Medium”  $> 300 \mu\text{m} \leq 2 \text{ mm}$ , “Large”  $> 2 \text{ mm} \leq 5 \text{ mm}$ , and “Extra Large”  $> 5 \text{ mm}$ ). This work is new and there are no results ready for presentation

Table 3. Microplastic counting rules from GACS program.

| Type   | Color             | Size   | Split-strands type   |
|--------|-------------------|--|--|
| Strand | clear/transparent | S = Small ( $\leq 300 \mu\text{m}$ )                 | Mono = Monofilament-type strand<br>Flat = Flat-type strand |
|        | black             | M = Medium ( $> 300 \mu\text{m} \leq 2 \text{ mm}$ ) |  |
|        | blue              | L = Large ( $> 2 \text{ mm} \leq 5 \text{ mm}$ )     |  |
|        | red               | X = Extra-large ( $> 5 \text{ mm}$ )                 |  |
|        | yellow            |  |  |
|        | green             |  |  |
|        | other             |  |  |
| Bead   | clear/transparent | S = Small ( $\leq 300 \mu\text{m}$ )                 |  |
|        | black             | M = Medium ( $> 300 \mu\text{m} \leq 2 \text{ mm}$ ) |  |
|        | blue              | L = Large ( $> 2 \text{ mm} \leq 5 \text{ mm}$ )     |  |
|        | red               | X = Extra-large ( $> 5 \text{ mm}$ )                 |  |
|        | yellow            |  |  |
|        | green             |  |  |
|        | other             |  |  |
| Flake  | clear/transparent | S = Small ( $\leq 300 \mu\text{m}$ )                 |  |
|        | black             | M = Medium ( $> 300 \mu\text{m} \leq 2 \text{ mm}$ ) |  |
|        | blue              | L = Large ( $> 2 \text{ mm} \leq 5 \text{ mm}$ )     |  |
|        | red               | X = Extra-large ( $> 5 \text{ mm}$ )                 |  |
|        | yellow            |  |  |
|        | green             |  |  |
|        | other             |  |  |

An excellent review on the threats of microplastics in the Ross Sea region has been produced by Dr Olivia Grover-Johnson, University of Canterbury. This has not been formally published as yet, but can be viewed at <https://ir.canterbury.ac.nz/handle/10092/16123>.

### Standardisation and Methodology Workshops

The SO-CPR Survey involves several countries with analyses conducted by experienced and well recognised plankton and Antarctic researchers albeit in several separated laboratories. Most of the analyses currently is centred at the Australian Antarctic Division (AAD, Australia), National Institute for Polar Research (NIPR, Japan), National Institute of Water and Atmospheric (NIWA, New Zealand), previously at the Alfred Wegener Institute (AWI, Germany) and the CPR Survey (UK) oversees the separate survey in the South Atlantic. New SO-CPR laboratories are being developed in Brazil, France, and South Africa. Consequently, we take every opportunity when we meet to run workshops on methods and taxonomy to ensure we are maintaining the highest level of procedures and identification standards for quality control and assurance. These also provide the opportunity to train new personnel (Appendix – list of workshops). We also regularly exchange information and images electronically.

At the 2010 “Southern Ocean CPR Standards Workshop” held at NIPR, Tokyo, lead analysts and researchers from the AAD, NIPR, NIWA and the CPR Survey in Plymouth met to discuss and review current laboratory methodology. An important discussion point was assessing what can and should be identified to species and what still cannot be identified with confidence. For example, damage to the shell of the thecosome pteropod usually prevents accurate identification of *Limacina* to species *helicina* or *retroversa* based on shell structure and consequently the lowest level of identification

remains as *Limacina* spp.. The same applies to *Clio* spp.. There are issue about the accurate separation of *Ctenocalanus citer* and *C. vanus*. These are grouped as *Ctenocalanus* spp., but based on previous net sampling it is most likely it is almost entirely *C. citer*. This workshop produced a consolidated report on identification issues, agreed methods and counting rules for sample processing (Takahashi et al., 2011a, 2017a, 2019b). This report is reviewed and updated periodically, notably when species taxonomy are updated.

New analysts involved with the SO-CPR Survey receive formal training in CPR methodology and plankton identification from experts at AAD and NIPR. Such training from the leading Antarctic CPR and taxonomic experts is essential in ensure correct identifications and data quality. Additional training have also been conducted at various times at NIWA, Federal University of Rio de Janeiro in support of CAML in 2007 with participants from many South American countries, and more recently in March 2015 at the laboratories of the Departmental of Environmental Affairs (DEA) in Cape Town, South Africa. The latter was supported by the DEA and through an inaugural Scientific Committee on Antarctic Research (SCAR) Visiting Professorship awarded to Dr Graham Hosie. We are also developing a similar training programme for India in support of their Southern Ocean CPR work. Dr Kunio Takahashi has already been awarded a Scientific Committee on Oceanic Research (SCOR) Visiting Scholars Program 2015 to help teach the Southern Ocean taxonomy and methods. The timing of the Indian workshop is under negotiation has the support of the Scientific Committee on Antarctic Research (SCAR), the Scientific Committee on Oceanic Research (SCOR), and the Partnership for Observation of the Global Oceans (POGO).

All these workshops and training sessions are supported by SCAR's CPR Expert Group. Analysts are also encouraged to visit the CPR Survey laboratories in Plymouth to enhance their skills, especially in PCI and identification of micro-plastics, and to exchange taxonomic knowledge of Southern Ocean species.

## SO-CPR DATABASE

### Dataset Description

The SCAR SO-CPR Dataset is maintained at and is available from the Australian Antarctic Data Centre (AADC), and can be accessed at <https://data.aad.gov.au/aadc/cpr/>. The metadata record of the dataset (AADC-00099) is available at [http://data.aad.gov.au/aadc/metadata/metadata.cfm?entry\\_id=AADC-00099](http://data.aad.gov.au/aadc/metadata/metadata.cfm?entry_id=AADC-00099), and also from the Catalogue of Australian Antarctic and Sub-Antarctic Metadata (CAASM) at [http://gcmd.nasa.gov/KeywordSearch/Metadata.do?Portal=amd\\_au&MetadataView=Full&MetadataType=0&KeywordPath=&OrigMetadataNode=AADC&EntryId=AADC-00099](http://gcmd.nasa.gov/KeywordSearch/Metadata.do?Portal=amd_au&MetadataView=Full&MetadataType=0&KeywordPath=&OrigMetadataNode=AADC&EntryId=AADC-00099). Copies of the data are held by partners in the SO-CPR consortium for their direct use, and data are also distributed to the Global Alliance of CPR (GACS, [globalepr.org](http://globalepr.org)) Surveys for use within that group and also to the Australian Integrated Marine Observing System (IMOS, [imos.org.au](http://imos.org.au)). Presence records of known species (with no abundance value) are published via DiGIR web services to the SCAR Antarctic Biodiversity website ([www.biodiversity.aq](http://www.biodiversity.aq)), which includes the former SCAR-MarBIN Marine Biodiversity Information Network ([www.scarmarbin.be](http://www.scarmarbin.be)), the Ocean Biogeographic Information System (OBIS, [www.iobis.org](http://www.iobis.org)), Global Biodiversity Information Facility (GBIF, [www.gbif.org](http://www.gbif.org)), and Atlas of Living Australia ([www.ala.org.au](http://www.ala.org.au)). Our datasets also improve the exchange of data with other international agencies and databases/portals such as Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR, [www.ccamlr.org](http://www.ccamlr.org)), Southern Ocean Observing System (SOOS, [www.soos.aq](http://www.soos.aq)), and International Group for Marine Ecological Time Series, (IGMETS, [igmets.net](http://igmets.net)).



These other data portals may not have the most up to date version of the dataset. Users are should source data only from the AADC as the primary source. This data set conforms to the PICCCBY Attribution License (<http://creativecommons.org/licenses/by/3.0/>).

Use of the CPR data is strongly encouraged and access will be provided for genuine research purposes. It is essential to contact the custodians via the AADC before using these data for a number of reasons. Some explanation of the data will be required in addition to the descriptions below. It is important to ensure that there are no conflicts with any existing analyses, especially with current student projects. Data collected or used by students may be quarantined during the tenure of the study. In some cases, the custodians may team you with another analyst if considered that this will improve the results. It is important that due acknowledgement and referencing of the source of data is given and the custodian can provide the current referencing and dataset doi. Instructions are listed in the citation reference provided at [http://data.aad.gov.au/aadc/metadata/citation.cfm?entry\\_id=AADC-00099](http://data.aad.gov.au/aadc/metadata/citation.cfm?entry_id=AADC-00099) when using these data. The custodians and the AAD Publications Database welcomes copies of any publications <http://www.antarctica.gov.au/about-us/publications>.

This dataset itself contains abundance records, number of specimens observed, for each of currently 289 species or broader zooplankton taxa for each 5 nautical mile sample unit. As noted in the section on Methodology/Sample Processing, zooplankton are identified to the lowest possible taxon, usually species, and counted for each segment. Coupled with each sample abundance record are some environmental records collected by shipboard environmental systems. Not all ships had environmental recording system. Data usually collected were sea temperature, salinity, photosynthetically active radiation PAR, and fluorometry averaged over the length of the sample distance. From September 2007, the phytoplankton colour index (PCI) was recorded where possible for each segment. Each sample is time-stamped and coded with the latitude and longitude position for the end of the sample segment.

The fields in the dataset are:

**Tow\_number** - the CPR tow number unique to each tow

**Ship\_name** - the name of the ship on which the tow was conducted

**Season** - two-year Antarctic season based around the austral summer, e.g. "2000-01" runs from July 2000 to June 2001

**Latitude** - the decimal latitude of the segment sample (negative value for South)

**Longitude** - the decimal longitude of the segment sample (negative value for West, positive for East)

**Observation\_date** - UTC date and time of the segment sample in ISO8601 format (yyyy-mm-ddTHH:MMZ)

The time and date field is also replicated be in the following useful search fields.

**Observation\_date\_year** - the observation date year

**Observation\_date\_month** - the observation date month

**Observation\_date\_day** - the observation date day

**Observation\_date\_hour** - the observation date hour

**Observation\_date\_minute** - the observation date minute

**Observation\_date\_time\_zone** - the observation date time zone (0=UTC)

**Segment\_number** - the individual segment number within each tow

**Segment\_length** - the distance travelled by the CPR during this segment (nautical miles). As noted in the Methodology-Sample Processing the last segment of each tow is rarely exactly 5 nautical miles. Users of the data can elect to dismiss the last segment. The "Segment Length" field can be used to standardise species counts.

**Total\_abundance** - total count of all zooplankton in a segment. It has been included as a data field for convenience. This is the only duplicate field, there are no others.

**Phytoplankton\_colour\_index** - visual estimation of the green colour of the silk mesh providing a quick subjective assessment of the amount of phytoplankton trapped on the silk, using on the PCI method of CPR Survey ([www.cprsurvey.org](http://www.cprsurvey.org)).

**Fluorescence** - water fluorescence measured by the vessel, averaged for the segment (arbitrary units). See Quality notes for more information.

**Salinity** - water salinity measured by the vessel, averaged for the segment (psu). See Quality notes for more information.

**Water\_temperature** - water temperature measured by the vessel (degrees Celsius). See Quality notes for more information.

**Photosynthetically\_active\_radiation** - photosynthetically active radiation measured by the vessel ( $\text{micro-Einsteins m}^{-2} \text{s}^{-1}$ ). This is not available on some vessels but has been included as a useful parameter to help differentiate data from night and day.

The remaining fields (currently "Abylidae" through to "*Vibilia\_sp*") are zooplankton taxon names. The entries in these columns are the counts of each taxon in the segment, including developmental stages for euphausiids. "Total Abundance" is the sum of all zooplankton on a segment and has been included as a data field for convenience. This is the only duplicate field, there are no others. All other fields are unique. For example, a segment with the counts of:

*Euphausia superba* FII – 2

*Euphausia superba* FIV – 6

*Euphausia superba* furcilia – 8

means that there were 2 specimens of stage furcilia II larvae, 6 of stage furcilia V and 8 furcilia larvae that could not be identified to a specific stage; there being a total of 16 krill larvae on that segment.

### Environmental Data Quality

Caution: Fluorometry is measured differently on each vessel and are thus not directly comparable between vessels. Most values come from Turner fluorimeters. The *Aurora Australis* has a Turner TD10, and the units are arbitrary even though the fluorometer is routinely calibrated. Further, a value of 200 on one voyage is not necessarily the same as 200 on another voyage, even with daily calibration and cleaning of the sensor. The fluorometry values are really only of value within a voyage in as much that 200 is double the fluorescence activity of 100. On *Tangaroa*, *Kaiyo Maru*, *Umitaka Maru* fluorometry has been expressed as a concentration of chlorophyll *a*.

Notes on salinity: The salinity measurement comes from the various thermosalinograph units mounted in the underway systems of the ships. Salinity on the *Aurora Australis* has been calculated by thermosalinograph. Salinity values are expressed in practical salinity units.

Notes on water temperature: Temperature on the *Aurora Australis* is measured by a high resolution thyristor near the entrance of a dedicated seawater line to avoid the problems of heating of the water by the ship as it passes through pipes. Again the data may not be fully comparable with other vessels.

## RESULTS

More than 1000 tows have been conducted by SO-CPR to date. These have provided 56,942 samples at 5 nautical mile resolution, for 289 zooplankton taxa and developmental stages, representing approximately 284,725 nautical miles covered. As of March 2018, 42,104 samples have been verified and added to the database. Tows have been conducted at some time over about 70% of the Southern Ocean, mainly in the months from September to April when most research and supply ships are operating (Fig. 2). A few tows have been conducted in the winter months of May, July and August, but winter sampling is limited due the lack of ships operating in the region in winter. The highest concentration of tows and sampling collection is in the region south and west of Australia (McLeod et al., 2010), and the Ross Sea region (Robinson et al., 2014). Collectively, these two areas represent approximately 40% of the region which is routinely and consistently monitored. Table 4 shows the nautical miles sampled and number of samples by month and by year currently contained in the SO-CPR Database.

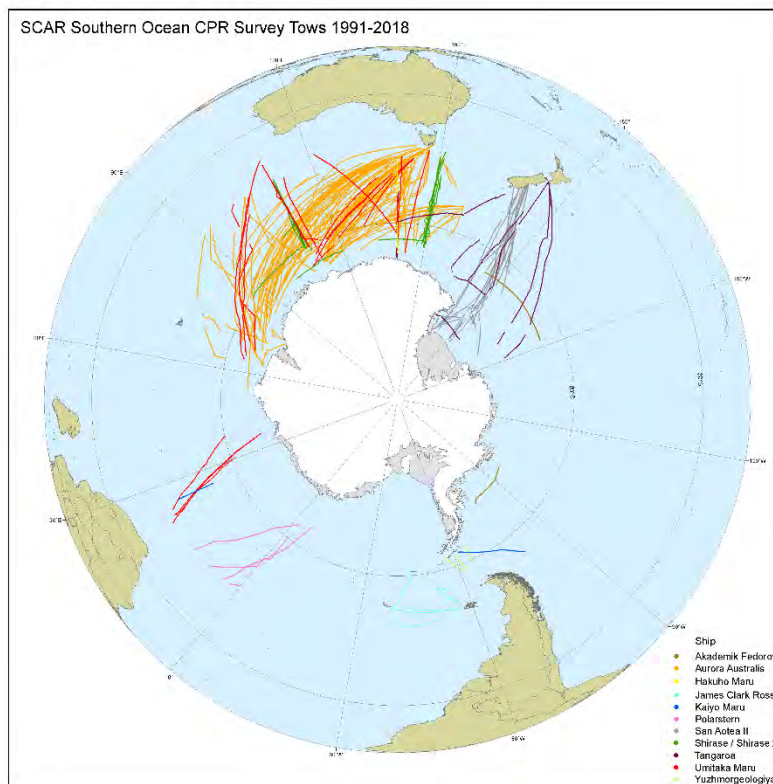


Figure 2. Map of tows of the SCAR Southern Ocean CPR Survey.

Table 4. Nautical miles sampled and number of samples currently contained in the SO-CPR Database, by month and by year up to 2018. Note: year is expressed as the period from July to June as most sampling straddles the Antarctic summer period.

|              | Nautical Miles in Database    |            |             |              |              |              |              |              |              |             |             |          | Total         |
|--------------|-------------------------------|------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|----------|---------------|
|              | July                          | August     | September   | October      | November     | December     | January      | February     | March        | April       | May         | June     |               |
| 1990-1991    |                               |            |             |              |              |              | 130          |              | 943          |             |             |          | 1073          |
| 1992-1993    |                               |            |             |              |              |              | 396          | 85           |              |             |             |          | 481           |
| 1993-1994    |                               |            |             |              |              |              |              | 343          |              |             |             |          | 343           |
| 1995-1996    |                               | 459        |             |              |              |              |              |              |              |             |             |          | 459           |
| 1996-1997    |                               |            |             |              |              |              |              |              |              | 1525        |             |          | 1525          |
| 1997-1998    |                               |            | 777         | 136          | 512          | 874          | 1520         | 3012         |              |             | 904         |          | 7735          |
| 1998-1999    | 427                           |            |             |              | 725          |              |              |              | 885          | 1220        |             |          | 3257          |
| 1999-2000    | 903                           | 216        | 249         | 830          | 586          | 979          | 2068         | 2863         | 1828         |             |             |          | 10522         |
| 2000-2001    |                               |            |             | 1264         | 275          | 2914         | 1900         |              | 4123         |             |             |          | 10476         |
| 2001-2002    |                               |            |             | 1885         |              | 1233         | 3367         | 2961         | 3768         |             |             |          | 13214         |
| 2002-2003    |                               |            |             | 398          | 2041         | 3225         | 2053         |              | 2754         |             |             |          | 10471         |
| 2003-2004    |                               |            | 370         |              | 2498         | 636          | 526          | 3944         | 1163         | 2062        | 266         |          | 11465         |
| 2004-2005    |                               |            |             | 955          | 849          | 2042         | 3170         | 3139         | 1874         |             |             |          | 12029         |
| 2005-2006    |                               |            |             | 870          | 1595         | 3149         | 4260         | 1599         | 4596         |             |             |          | 16069         |
| 2006-2007    |                               |            |             | 832          | 1372         | 900          | 857          | 651          | 3392         |             |             |          | 8004          |
| 2007-2008    |                               |            | 882         | 851          | 1791         | 3839         | 5690         | 4416         | 4516         |             |             |          | 21985         |
| 2008-2009    |                               |            |             | 1137         | 1043         | 897          | 5491         | 2598         | 1053         |             |             |          | 12219         |
| 2009-2010    |                               |            |             |              | 1466         | 2706         | 1221         | 6545         | 2684         |             |             |          | 14622         |
| 2010-2011    |                               |            |             | 1234         | 964          | 3501         | 1984         | 3181         | 3054         |             |             |          | 13918         |
| 2011-2012    |                               |            |             | 1774         | 478          | 3358         | 2107         | 2394         | 3008         |             |             |          | 13119         |
| 2012-2013    |                               |            | 1323        |              | 3314         | 2778         | 3446         | 4371         | 594          |             |             |          | 15826         |
| 2013-2014    |                               |            |             | 1787         | 40           | 2602         | 2905         | 3775         | 851          |             |             |          | 11960         |
| 2014-2015    |                               |            | 505         | 3365         |              | 3080         | 5355         | 5980         | 2455         |             |             |          | 20740         |
| 2015-2016    |                               |            |             | 775          | 1680         | 2945         | 4620         | 2470         | 850          |             |             |          | 13340         |
| 2016-2017    |                               |            |             | 1160         | 545          | 2745         | 6455         | 645          | 850          |             |             |          | 12400         |
| 2017-2018    |                               |            |             | 420          | 2400         | 2320         | 6465         | 900          | 965          |             |             |          | 13470         |
| <b>Total</b> | <b>1330</b>                   | <b>675</b> | <b>4106</b> | <b>19673</b> | <b>24174</b> | <b>46723</b> | <b>65986</b> | <b>55872</b> | <b>46206</b> | <b>4807</b> | <b>1170</b> | <b>0</b> | <b>210772</b> |
|              | Number of Samples in Database |            |             |              |              |              |              |              |              |             |             |          | Total         |
|              | July                          | August     | September   | October      | November     | December     | January      | February     | March        | April       | May         | June     |               |
| 1990-1991    |                               |            |             |              |              |              | 26           |              | 186          |             |             |          | 212           |
| 1992-1993    |                               |            |             |              |              |              | 91           | 17           |              |             |             |          | 108           |
| 1993-1994    |                               |            |             |              |              |              |              | 94           |              |             |             |          | 94            |
| 1995-1996    |                               | 87         |             |              |              |              |              |              |              |             |             |          | 87            |
| 1996-1997    |                               |            |             |              |              |              |              |              |              | 284         |             |          | 284           |
| 1997-1998    |                               |            | 147         | 25           | 82           | 183          | 302          | 558          |              |             | 177         |          | 1474          |
| 1998-1999    | 71                            |            |             |              | 132          |              |              |              | 177          | 243         |             |          | 623           |
| 1999-2000    | 180                           | 43         | 49          | 166          | 116          | 196          | 413          | 613          | 365          |             |             |          | 2141          |
| 2000-2001    |                               |            |             | 253          | 55           | 583          | 381          |              | 825          |             |             |          | 2097          |
| 2001-2002    |                               |            |             | 378          |              | 240          | 676          | 594          | 759          |             |             |          | 2647          |
| 2002-2003    |                               |            |             | 79           | 407          | 644          | 411          |              | 551          |             |             |          | 2092          |
| 2003-2004    |                               |            | 74          |              | 499          | 127          | 105          | 788          | 232          | 412         | 53          |          | 2290          |
| 2004-2005    |                               |            |             | 190          | 170          | 409          | 632          | 629          | 375          |             |             |          | 2405          |
| 2005-2006    |                               |            |             | 174          | 318          | 631          | 852          | 319          | 920          |             |             |          | 3214          |
| 2006-2007    |                               |            |             | 167          | 275          | 180          | 171          | 130          | 678          |             |             |          | 1601          |
| 2007-2008    |                               |            | 177         | 170          | 358          | 766          | 1137         | 882          | 904          |             |             |          | 4394          |
| 2008-2009    |                               |            |             | 227          | 209          | 179          | 1096         | 521          | 210          |             |             |          | 2442          |
| 2009-2010    |                               |            |             |              | 293          | 543          | 244          | 1311         | 539          |             |             |          | 2930          |
| 2010-2011    |                               |            |             | 247          | 192          | 700          | 398          | 635          | 611          |             |             |          | 2783          |
| 2011-2012    |                               |            |             | 355          | 95           | 671          | 421          | 479          | 602          |             |             |          | 2623          |
| 2012-2013    |                               |            | 264         |              | 664          | 556          | 689          | 875          | 119          |             |             |          | 3167          |
| 2013-2014    |                               |            |             | 357          | 8            | 521          | 583          | 756          | 171          |             |             |          | 2396          |
| 2014-2015    |                               |            | 101         | 673          |              | 616          | 1071         | 1196         | 491          |             |             |          | 4148          |
| 2015-2016    |                               |            |             | 155          | 336          | 589          | 924          | 494          | 170          |             |             |          | 2668          |
| 2016-2017    |                               |            |             | 232          | 109          | 549          | 1291         | 129          | 170          |             |             |          | 2480          |
| 2017-2018    |                               |            |             | 84           | 480          | 464          | 1293         | 180          | 193          |             |             |          | 2694          |
| <b>Total</b> | <b>251</b>                    | <b>130</b> | <b>812</b>  | <b>3932</b>  | <b>4798</b>  | <b>9347</b>  | <b>13207</b> | <b>11200</b> | <b>9248</b>  | <b>939</b>  | <b>230</b>  | <b>0</b> | <b>42104</b>  |

### Comparison with other sampling systems

Plankton vary in size from the microbial to large ctenophores and jellyfish, from robust to those extremely fragile and almost impossible to catch without damage. Plankton have extremely diverse behavioural patterns, daily and seasonal vertical migration, and different feeding, reproductive, survival and escape strategies. Even within the crustacean mesozooplankton abundances vary in four dimensions. Consequently, it has been necessary to develop numerous types of sampling systems and

different mesh sizes have been used, in order to capture or observe the various components of plankton. Wiebe and Benfield (2003) listed more than 200 systems and that is not exhaustive. Each system has a different purpose and all systems underestimate parts of or all the plankton. It is virtually axiomatic in plankton research that no plankton sampler, or combination of plankton samplers, can provide a true estimate of abundance for all components of the plankton at anytime (Owens et al., 2013). Researchers need to choose the system most suited to their study, and of course it should be justified.

Amongst the numerous systems listed by Wiebe and Benfield (2003), the CPR has probably had the most scrutiny and required to give more justification than any other plankton sampling system despite more than 1000 publications using CPR data, with a very respectful number in *Nature*. Within the Antarctic community, the Rectangular Midwater Trawl (RMT 8) has long been accepted as the principal net sampling system for krill and macro-zooplankton research and its limitations acknowledged, notably in relation to its large 4.5 mm mesh size. Thankfully, the CPR has a much greater acceptance with the Antarctic community. Elsewhere, there is regular comment that the CPR under-samples zooplankton. Yes, it does, but so does all zooplankton sampling systems as noted above (Owens et al., 2013; Skjoldal et al., 2013). When the 270  $\mu\text{m}$  mesh of the CPR is compared with a net with 200  $\mu\text{m}$  mesh then yes there will be apparent under-sampling. The same applies when comparing a net with 200  $\mu\text{m}$  with another fitted with 100  $\mu\text{m}$ . The CPR has its limitations and users of the data are advised of those limitations. The advantage of the CPR is having a consistent design and methodology that has remained relatively unchanged for eight decades. It has provided sustained long-term observations of interannual changes in plankton populations and communities, as well as seasonal patterns of abundance and distribution, phenological shifts and the intrusion of evasive species. For the development of ecological models and the study of trends the errors of uncertainty in the data are constant in an unchanging sampling method.

Nonetheless, there has always been a desire to understand the differences between each system and to quantify the degree of underestimation. It is essential to help understand and interpret plankton ecology and CPR is no exception. Differences in sampling protocols and nets have made it difficult for large-scale comparisons of plankton abundance and biomass. This issue has become more important as the demand grows for accurate estimates of plankton abundance for estimating productivity and applying biogeochemical, food web and marine ecosystem models. We cannot accurately measure true plankton abundance in the water with certainty against which we can calculate the error of an estimate from a net sample. At best, we are left with comparing one system against another to record the differences, but such comparisons must be conducted in an extensive systematic manner under the same conditions (see Skjoldal et al., 2013), and recognising that the systems being compared each have their own deficiencies.

Under those criteria there have been a number of attempts to compare CPRs with other plankton nets. Clark et al. (2001) and John et al. (2001) working in the North Sea and English Channel compared abundance estimates between the CPR and a commonly used plankton net the WP-2 net fitted with 200  $\mu\text{m}$  mesh. The differences in mesh size expectedly showed differences in abundance and composition, but those studies did serve to quantify how much the CPR misses small copepod species compared to a finer mesh. Hunt and Hosie (2003) compared CPR data against data collected by a NORPAC net along the same sampling track with both systems fitted with the same 270  $\mu\text{m}$  mesh. It was an attempt at a like-versus-like comparison using the same mesh to avoid the issue of different mesh sizes and thus determine any difference due to design and methodology. Vertical NORPAC net samples were collected at 19 stations in four stratified layers (0–20, 20–50, 50–100, 100–150 m) on a southward run along  $\sim 140^\circ\text{E}$  in the Southern Ocean between  $47$  and  $61^\circ\text{S}$  in 9–22 November 2001. CPR tows were conducted on the return northward run along the same transect in 7–11 December 2001. The 0–20 m NORPAC sampling layer was specifically chosen to correspond with the CPR sampling depth.

Despite the differences in timing the CPR produced abundances comparable to those from the 0 to 20 m NORPAC net, with similar species composition (Fig. 3, Table 5). Specifically, for 22 of the 30 taxa compared there were no significant differences in abundance, five were slightly higher in the NORPAC net and three taxa, *Oithona similis*, larvaceans and nauplii of *Rhincalanus gigas* had substantially higher abundances in the CPR than the NORPAC net resulting in the CPR catching more plankton overall. The higher abundances in the CPR may have been due to the time difference between the NORPAC and the CPR sampling, possibly due to natural population increases or growth in size increasing catchability. However, overall the two systems using the same 270  $\mu\text{m}$  mesh produced similar abundances. Similarly, Skjodal et al. (2013) confirmed that different net systems fitted with the same mesh produce similar abundance estimates. This suggests that the design of the CPR and its method of sampling are not a major source of underestimation in abundances relative to different mesh sizes, it is most likely the different meshes themselves.

In relations to the comparison with deeper layer, species richness and diversity increased with depth, and were lowest for CPR samples, conversely, dominance decreased with depth and was highest for CPR samples. Evenness was similar for all sample groups, indicating that all assemblages had a similar distribution of abundance amongst species. While diversity did change with depth, the CPR samples still provided a reasonable representation of species in the 100-150 m layer, primarily due to the vertical migration of many species into the upper surface layer at night. Both the NORPAC nets and CPR surveys identified distinct communities to the north and south of the Southern Sub-Antarctic Front. Despite under-sampling some components of the zooplankton, while catching more of others, the CPR provided sufficient taxonomic resolution to identify biogeographic zones in the Southern Ocean.

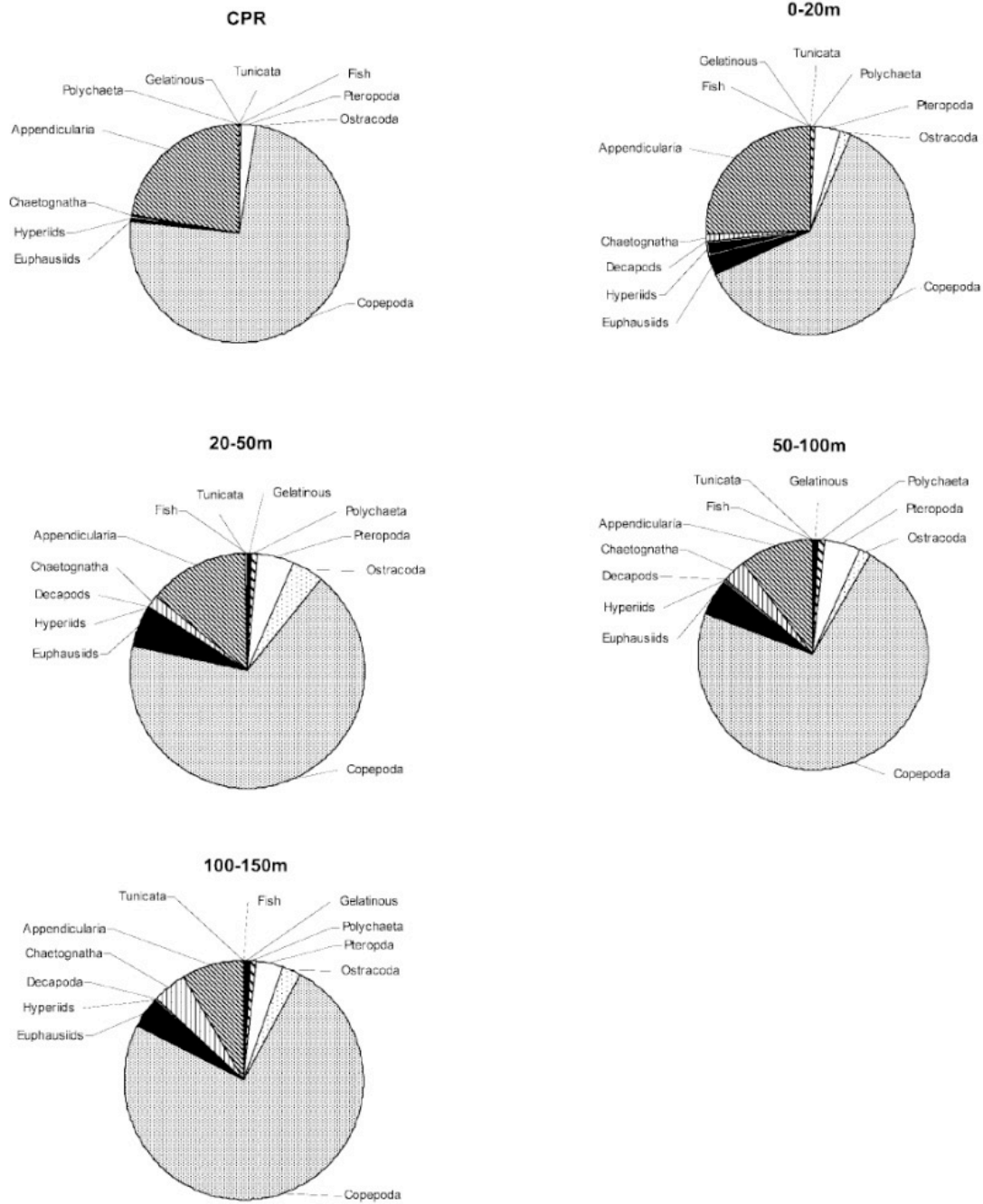


Figure 3. Percentage contribution of major zooplankton groups to total abundance in CPR samples and the four NORPAC net depth zones (0–20, 20–50, 50–100 and 100–150 m). (From Hunt and Hosie, 2003)

Table 5. Abundance of zooplankton species occurring in more than four samples in any of the five sample groups (From Hunt and Hosie, 2003).

| Species                             | P   | Average abundance (individuals m <sup>-3</sup> ) |                 |                 |                 |                 |
|-------------------------------------|-----|--|-----------------|-----------------|-----------------|-----------------|
|                                     |     | CPR  | 20 m            | 20–50 m         | 50–100 m        | 100–150 m       |
| <i>Pelagobia longicirrata</i>       | ns  | 0.214  | 0.264           | 0.199           | 0.139           | 0.264           |
| <i>Clio pyrimidata antarctica</i>   | ns  | 0.125  | 0.018           | 0.074           | 0.335           | 0.353           |
| <i>Limacina helicina antarctica</i> | *** | <b>x</b> 0.000                                   | <b>z</b> 0.036  | <b>z</b> 0.025  | <b>y</b> 0.003  | <b>y</b> 0.001  |
| <i>Limacina retroversa</i>          | ns  | 1.536  | 1.652           | 2.562           | 2.290           | 1.755           |
| <i>Spongiobranchea australis</i>    | *** | <b>x</b> 0.000                                   | <b>x</b> 0.000  | <b>y</b> 0.032  | <b>x</b> 0.010  | <b>y</b> 0.032  |
| Ostracod sp.                        | *** | <b>x</b> 0.143                                   | <b>xy</b> 0.659 | <b>z</b> 2.552  | <b>yz</b> 0.929 | <b>y</b> 1.450  |
| <i>Aetideus armatus</i>             | *** | <b>x</b> 0.000                                   | <b>x</b> 0.000  | <b>x</b> 0.000  | <b>x</b> 0.000  | <b>y</b> 0.056  |
| <i>Calanoides acutus</i>            | *** | <b>x</b> 0.006                                   | <b>x</b> 0.069  | <b>x</b> 0.085  | <b>y</b> 0.195  | <b>y</b> 0.219  |
| <i>Calanus similimus</i>            | *** | <b>x</b> 0.179                                   | <b>yz</b> 3.037 | <b>z</b> 3.907  | <b>yz</b> 2.718 | <b>y</b> 2.091  |
| <i>Clausocalanus brevipis</i>       | *** | <b>x</b> 0.304                                   | <b>xy</b> 0.658 | <b>xy</b> 0.956 | <b>y</b> 1.126  | <b>y</b> 1.236  |
| <i>Clausocalanus laticeps</i>       | ns  | 1.000  | 1.152           | 1.508           | 1.056           | 1.165           |
| <i>Ctenocalanus citer</i>           | ns  | 16.577   | 11.043          | 21.590          | 22.543          | 21.889          |
| <i>Eucalanus longiceps</i>          | *** | <b>x</b> 0.006                                   | <b>x</b> 0.021  | <b>x</b> 0.038  | <b>y</b> 0.122  | <b>y</b> 0.176  |
| <i>Haloptilus oxycephalus</i>       | *** | <b>x</b> 0.036                                   | <b>y</b> 0.201  | <b>yz</b> 0.370 | <b>zp</b> 0.536 | <b>p</b> 0.549  |
| <i>Metridia lucens</i>              | ns  | 0.077  | 0.263           | 0.070           | 0.139           | 0.271           |
| <i>Oithona frigida</i>              | *** | <b>x</b> 0.042                                   | <b>x</b> 0.109  | <b>x</b> 0.058  | <b>y</b> 0.946  | <b>z</b> 3.573  |
| <i>Oithona similis</i>              | *** | <b>y</b> 49.244                                  | <b>x</b> 9.652  | <b>x</b> 7.276  | <b>x</b> 4.276  | <b>x</b> 4.197  |
| <i>Oncaea</i> sp.                   | *** | <b>x</b> 0.000                                   | <b>x</b> 0.062  | <b>x</b> 0.100  | <b>y</b> 2.074  | <b>y</b> 4.236  |
| <i>Rhincalanus gigas</i>            | *** | <b>x</b> 0.048                                   | <b>z</b> 0.377  | <b>yz</b> 0.321 | <b>p</b> 1.183  | <b>q</b> 2.125  |
| <i>Rhincalanus gigas nauplii</i>    | *** | <b>y</b> 8.423                                   | <b>x</b> 0.066  | <b>x</b> 0.074  | <b>x</b> 0.026  | <b>x</b> 0.040  |
| <i>Scolecithricella minor</i>       | *** | 0.137  | 0.000           | 0.077           | 0.292           | 0.907           |
| <i>Themisto gaudichaudii</i>        | ns  | 0.185  | 0.903           | 0.158           | 0.219           | 0.115           |
| <i>Euphausia frigida</i>            | ns  | 0.131  | 0.035           | 0.036           | 0.105           | 0.133           |
| <i>Euphausia triacantha</i>         | *** | <b>x</b> 0.000                                   | <b>xy</b> 0.067 | <b>xy</b> 0.057 | <b>y</b> 0.120  | <b>xy</b> 0.061 |
| <i>Euphausia vallentini</i>         | ns  | 0.042  | 0.130           | 0.559           | 0.079           | 0.079           |
| <i>Thysanoessa</i> sp. (v/m)        | *** | <b>x</b> 0.762                                   | <b>x</b> 1.040  | <b>y</b> 2.303  | <b>y</b> 2.292  | <b>y</b> 2.069  |
| Sergestidae                         | *** | <b>x</b> 0.000                                   | <b>x</b> 0.002  | <b>x</b> 0.004  | <b>y</b> 0.072  | <b>y</b> 0.100  |
| <i>Eukrohnia hamata</i>             | *** | <b>x</b> 0.071                                   | <b>y</b> 0.403  | <b>z</b> 0.756  | <b>p</b> 1.566  | <b>q</b> 2.551  |
| <i>Sagitta gazellae</i>             | ns  | 0.018  | 0.010           | 0.041           | 0.079           | 0.062           |
| Appendicularia                      | *** | <b>y</b> 32.101                                  | <b>x</b> 11.325 | <b>x</b> 7.681  | <b>x</b> 5.463  | <b>x</b> 4.986  |

P levels were determined by one-way ANOVA. Significant ( $P < 0.005$ ) differences between sample groups were determined by Newman-Keuls multiple range tests and are indicated by different letters. Highest abundance levels are shaded. ns, not significant.

## Clogging and Flow Rates

Clogging is an issue that applies to all meshed based sampling systems. As the mesh becomes clogged, often due to filamentous diatoms, the effective mesh size decreases and increases the catchability of smaller specimens. Filtration efficiency is also affected and rarely is this quantified. One of the useful performance experiments conducted by SO-CPR in eastern Antarctica in February/March 2003 was fitting the CPR mechanism with 125, 224, and 270  $\mu\text{m}$  mesh, to attempt to understand the factors affecting the volume of water filtered by a CPR (Hunt and Hosie, 2006a). Three tows were conducted, one each using one of three mesh sizes. The volume filtered was measured at 3 second intervals with a Valeport electromagnetic flow meter attached to the flow outlet of the CPR. Ship speed, photosynthetically active radiation (PAR) and fluorescence were measured every minute. The finer 125, 224 mesh to the standard 270  $\mu\text{m}$  mesh were used to imitate the effects of clogging. Tows were conducted in the POOZ where phytoplankton were expected to be less abundant. However, higher than expected concentrations of phytoplankton actually occurred, similar to abundances expected in the SIZ, which further enhanced the clogging of the mesh. This resulted in



the greater retention of smaller copepod species and early stages of copepods, particularly of *Oithona similis*, which are not normally caught. There was substantial variation in measured volume filtered (MVF) recorded on each transect. Ship speed was positively correlated with MVF and caused up to 30% reductions in MVF. Clogging, predominantly by phytoplankton, resulted in up to 60% reductions in MVF. A maximum 78% reduction in MVF resulted from the combined effects of clogging and ship speed. The substantial impact of clogging on observed zooplankton densities highlights the need for flow meter measurements to further quantify CPR data. However, observations from this study show that the CPR flow meter currently in use may itself have caused the positive correlation between MVF and ship speed because of its mass affecting the attitude of the CPR. The battery pack of the flowmeter alone was 17 kg in air. This highlighted the need for improved flow meter design, a much smaller and lightweight flow meter that can be fitted unobtrusively to the outlet of the CPR. The continuing miniaturization and improved resolution of distance loggers for attachment to marine vertebrate predators is still perhaps the best opportunity in this area.

### **General species composition and community structure**

As noted above, the original target region for CPR monitoring was the SIZ of the eastern Antarctic region, this being a primary habitat of Antarctic krill, and where numerous previous net based studies had shown high abundance of zooplankton (Hosie, 1994; Hosie and Cochran, 1994; Hosie et al., 1988, 1991; Ikeda et al., 1986; Pakhomov, 1989). It was where zooplankton species composition was well known and community structure and distribution patterns were defined. High numbers of krill larvae were also observed late in the summer season (Hosie, 1991, 1994). The paradigm at the time was the SIZ and PFZ were the regions of higher productivity and zooplankton abundance whereas POOZ was considered oligotrophic. The extension of tows into the POOZ quickly dispelled that paradigm. While the open-ocean zone maybe oligotrophic it does supports a large diversity and high abundances of zooplankton, many of which could be considered omnivorous, or specifically heterotrophic and phagotrophic feeders. Zooplankton abundances are consistently low north of the SAF. However, abundances increase substantially south of the SAF, and remain high through the POOZ (Fig. 4) (Hosie et al., 2003). Typically, abundances decline abruptly just inside the SIZ (regardless of the presence or absence of ice) at around 60-62°S (Fig. 5) (Hosie et al., 2003), and remain very low throughout the SIZ. The decline is highlighted in Fig. 5d where zooplankton is notably absent when a peak in abundance is normally concurrent with a period of dark. At the time of sampling in Figure 5, the sea-ice had already retracted to its minimum extent. This decline is regularly observed across the eastern Antarctic region and approximately coincides with the position of the Southern ACC Front (SACCF), although there appears to be no physical link. We know the SIZ has high zooplankton abundances based on RMT8 and other nets. The absence of zooplankton in CPR would suggest the near surface waters of the SIZ are not suitable for zooplankton or there is some other behavioural change that prevent zooplankton vertically migrating into or staying in the upper layer.

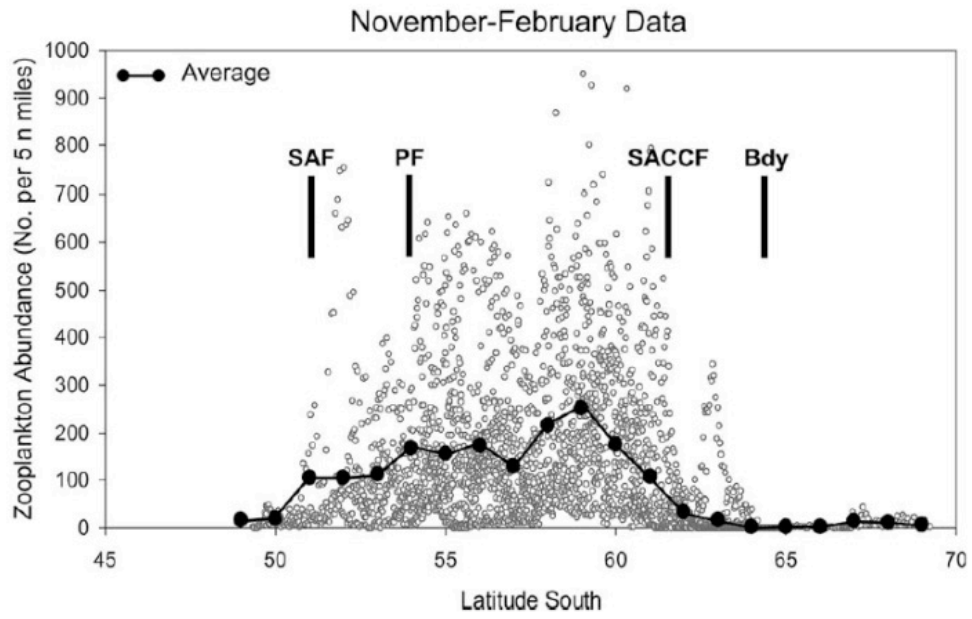


Figure 4. Latitudinal change in total abundance during the peak months of November to February. Each value represents individual 5-nautical-mile sections. Approximate position of frontal zones as defined by Orsi et al. (1995) are shown. (from Hosie et al., 2003).

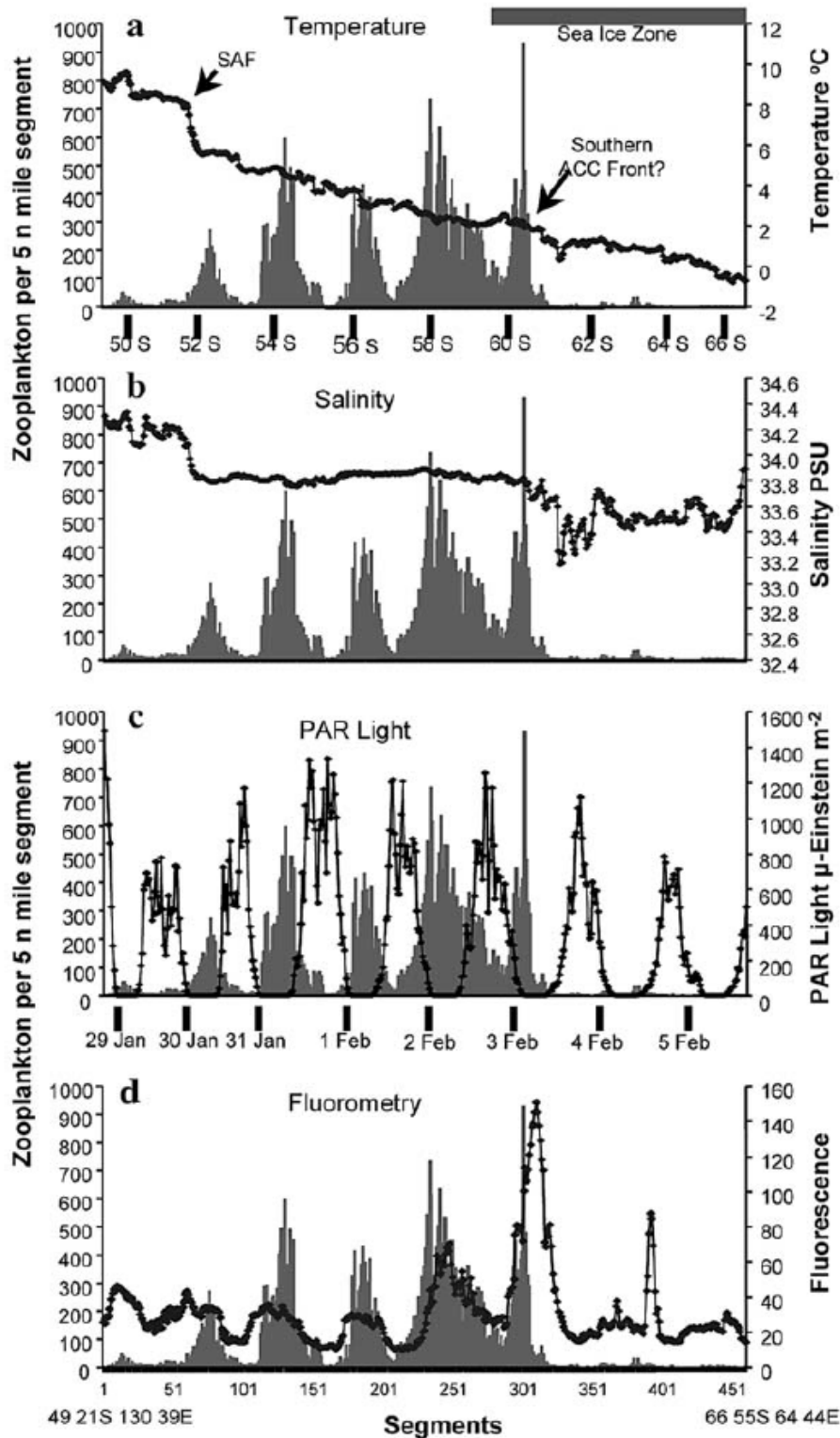


Figure 5. CPR tow from Aurora Australis 29 January–6 February 1998. Total abundance per 5 nautical mile segment and environmental data averaged over the same segment: (a) seawater temperature and latitude shown; (b) salinity; (c) photosynthetically active radiation PAR as  $\mu\text{-Einstein m}^{-2}$ , date is shown for 1200 hours GMT; (d) fluorescence, arbitrary values. Region influenced by sea ice is shown in 5a, maximum northern extent in October and minimum in January–February at time of sampling. (from Hosie et al., 2003)

In relation to species composition, the Southern Ocean near surface zooplankton typically comprises, the ubiquitous *Oithona similis*, small calanoid copepods *Calanus simillimus*, *Ctenocalanus citer*, *Clausocalanus brevipes*, *Clausocalanus laticeps*, the larger copepod species *Rhincalanus gigas*, foraminifera, larvaceans, the pteropod *Limacina* spp. and at times chaetognaths (Hunt and Hosie, 2005, 2006a, b; Mcleod et al., 2010; Takahashi et al., 2010a, b, 2011b, 2017b; Hosie et al., 2014). Hunt and Hosie (2005, 2006b, c) referred to this collection as the main summer taxa, with notably *O. similis*, foraminifera and larvaceans representing the consistent “core taxa”, occurring in >97% of samples and usually contributing an average of 75% to total sample abundance.

*Oithona similis* has remained consistently the most abundant species in the samples at approximately 50% of numbers. Its real abundance is expected to be substantially much higher considering it is a relatively small species of about 1 mm and the 270  $\mu\text{m}$  mesh of the CPR retains significantly less numbers of *Oithona* compared to a WP-2 net with 200  $\mu\text{m}$  mesh (Clark et al., 2001), which in turn the WP-2 probably collects <10% of mesozooplankton numbers (Gallienne and Robins, 2001). Gallienne and Robins (2001) asked whether *O. similis* was the most important copepod in the world’s ocean. They suggested this species true numbers, biomass and its contribution to secondary productivity estimates and ecosystem dynamics was substantially underappreciated.

Adults and larvae of small euphausiids such as *Euphausia frigida*, *E. vallentini* and especially *Thysanoessa macrura* are also common in samples. Notably the large copepods typical of the region and common in net hauls, *Calanus propinquus* and *Calanoides acutus* are not as abundant in CPR samples nor the adults and specifically the larvae of Antarctic krill *Euphausia superba*. The low numbers of adult krill larvae in the CPR samples can be attributed to the aperture size. The paucity of larvae when they should be abundant in late summer (Hosie, 1991, 1994), suggests that either the dispersal patterns have changed, or the larvae do not migrate to the near surface where they can be caught by the CPR. Most of the krill larvae caught in the CPR have been in February (1188 total, as of May 2018) and March (1716). These numbers are actually too small compared to the total volume of samples is to gain a clear understanding of any pattern so far. By contrast, the larvae of the euphausiid *Thysanoessa macrura*, are much more abundant (28,818) and it is possible to use this species as a proxy for Antarctic krill to determine if any environmental changes are causing changes (e.g. phenological) in the development of *T. macrura* that may also affect *E. superba*.

Despite their large size at time, salps such the Antarctic species *Salpa thompsoni* and the warmer water *S. fusiformis* are often collected by the CPR. However, the neritic euphausiid *Euphausia crystallorophias* is rarely sampled. There is concern about this species, important in the diet of penguins and fish, because of its restricted neritic distribution around the Antarctic continent. This species has a preference for cold water, usually < -1.5°C (Hosie, 1994), and usually avoids the warmed surface waters of the neritic zone where the CPR would sample. Warming of Antarctic waters will affect this species and because of its existing distribution cannot move further south. Operating a CPR within the neritic distribution on *E. crystallorophias* is usually difficult. This is a region often with residual pack-ice and frequent icebergs which makes normal ship operations challenging. For safety and logistical reasons CPR tows usually cease near the northern edge or just inside the neritic zone. Unfortunately, this prevents us from monitoring changes in this species.

Several species of larvaceans have reported in the Southern Ocean (O’Sullivan, 1983), however, this is a group notoriously difficult to identify to the species level even when freshly caught. Based on scanning electron microscopy (SEM) and examination using stereo-dissecting microscopes, Lindsay and Williams (2010) identified two principal species in the rectangular midwater trawl (RMT1, 300 $\mu\text{m}$ ) and CPR samples as *Oikopleura gaussica* and *Fritillaria drygalski*. DNA analysis is required to further confirm these identifications. The CPR database records larvaceans as *Oikopleura* spp. or *Fritillaria* spp., but the aforementioned species are likely to be the predominant species. Larvaceans can at times be exceptionally abundant throughout all zones of the Southern Ocean, occurring in several hundred individuals per  $\text{m}^3$  and representing a large portion of the total zooplankton abundance (Takahashi et al., 2002; Hunt and Hosie, 2003, 2005, 2006b, c; Tsujimoto et al., 2006).

Given the reported high abundances, the importance of larvaceans in concentrating organic matter in oligotrophic water by 100 to 1000 times background levels (Davoll and Silver, 1986; Bedo et al., 1993) and their contribution to the production of marine snow (Alldredge and Silver, 1988), Lindsay (2012) embarked on a detailed analysis of larvaceans in net and CPR samples from the Southern Ocean. While she did not observe the same level of abundances previously observed, a majority of samples (55%) often had no specimens, the abundances were still high enough overall to note that this group is a major component of the zooplankton and warrants further investigation as to their role in the Antarctic marine ecosystem, especially in oligotrophic areas. She also noted that the CPR consistently caught higher abundances of larvaceans than other net systems.

As of May 2019, there are 289 species, some developmental stages and other taxa in the database (Table 2). The number continues to grow as new species known to occur in the region are identified for the first time in the samples, other species appear to be moving into the region (McLeod et al., 2012, see “Distributional changes and invasive species” below), and we improve our skills in identification of groups to the species level, notably with foraminifera (Meilland et al., 2016). With each species count/identification linked to a time and position, users can search the database to determine the time and place a species was first observed by the CPR.

### *Enhancing species identifications*

As has been noted in many forums, the number of taxonomists and the amount of resources dedicated to taxonomic studies is declining. This affects the ability for SO-CPR to resolve the identification issues for some zooplankton which are often collated as a single group. Pelagic foraminifera are a prime example. Due to insufficient taxonomic studies of pelagic foraminifera in the South Ocean it had been assumed that there was just one species south of the SAF, *Neogloboquadrina pachyderma* (Scott and Marchant, 2005), perhaps two species with *Globigerina bullodites*, a sub-Antarctic species, possibly intruding south of the SAF. More species were known to occur north of the SAF (Darling and Wade, 2008) with *G. bulloides* the dominant species (Moy et al., 2009). Due to the uncertainty of identifications foraminifera in the SO-CPR samples were collated as a single group, with the notation that is most likely just *N. pachyderma* south of the SAF. Julie Meilland (Meilland, 2015; Meilland et al., 2016) analysed CPR samples from the *Marion Dufresne* survey around Kerguelen and samples from the SO-CPR survey collected between Hobart and Casey station in the PFZ, POOZ and SIZ. She showed that there were in fact more species north and south of the SAF than expected, and specifically seven species were identified in the Southern Ocean south of the SAF, these being: *N. pachyderma*, *N. incompta*, *Turborotalia quinqueloba*, *G. bulloides*, *Orcadia riedieli*, *Globorotalia inflatae* and *Globigerinita uvula*. The first four species comprise most of the abundance of foraminifera, with *T. quinqueloba* more abundant than *N. pachyderma* over the studied region. Meilland provided training for SO-CPR analysts and foraminifera can now be identified to species. This is rather important because foraminifera with their carbonate shells, and being a very abundant group in SO-CPR samples, are a target group within the SO-CPR Survey for monitoring in relation to potential ocean acidification impacts. Meilland et al. (2016) also concluded that foraminifera, being very abundant in CPR samples, are very important in accurately describing spatial variation in pelagic ecosystems. Among the results of this collaboration is the integration of CPR foraminifera counts in to a large database gathering planktonic information from the water column since 1940 (<https://forcis.cerege.fr>).

Enhanced genetic methodology, and reduction in cost, may improve our ability to identify soft bodied zooplankton that cannot be accurately identified due to physical damage or preservation.

**Recommendation:** Application of genetic methodologies on CPR samples needs to be explored and applied.

## Zooplankton Assemblages and Oceanographic Fronts

Shipping logistics and schedules in the Antarctic are challenging, often with competition between research and resupply, overshadowed by meteorological, ocean, sea-ice conditions. Consequently, few intra-annual or within season studies have been undertaken in the Southern Ocean especially in relation to the various fronts and frontal zones in the Southern Ocean. There have only been three multi-ship surveys in which CPR tows have been used to try and identify spatial variations in zooplankton seasonal succession (Hunt and Hosie, 2006b, c; Takahashi et al., 2011b, 2017b).

One of the most important early biogeographic analyses by SO-CPR was to define the relationship between the physical environment of the Southern Ocean, specifically the ACC, and zooplankton communities south of Australia in the context of the current knowledge of the physical oceanography and zonation. Are there different communities in each frontal zone? Are they consistent in space and time, i.e. seasonal succession? The work conducted by Brian Hunt in the 2001/02 season involved detailed multivariate analyses of species composition using Cluster and Correspondence analyses, diversity and species indicator against environmental data collected simultaneously from a series of six transects south of Tasmania along 140°E. The transects were surveyed at approximately monthly intervals. The main environmental parameters were temperature, salinity and chlorophyll *a* (Hunt and Hosie, 2005, 2006b, c). Hunt demonstrated very clearly that the Sub-Antarctic Front, specifically the north branch of the SAF (SAF-N) is a major biogeographic barrier with a different zooplankton community north of the SAF. South of the SAF they reported the same typical summer composition described above, *Oithona similis*, numerous small calanoid copepods species, foraminifera and larvaceans. Within the ACC, there were very clear and distinctly different zooplankton assemblages found relative to each frontal zones and sub-zone. This clear north-south zonation was significantly correlated with temperature and salinity (Hunt and Hosie, 2005). Rather than being completely different communities with different or unique species composition, these assemblages were effectively one community, easily distinguishable between fronts due to subtle variation in the proportion of the species (Hunt and Hosie, 2005) (Fig. 6). The zooplankton assemblages exhibited distinctly sharp boundaries relative to fronts and sub-branches. The zooplankton assemblages were better at showing separation of frontal zones than direct measurement of the oceanography by shipboard underway recording systems. In other words, the plankton were very good at amplifying and expressing their responses to oceanic conditions.

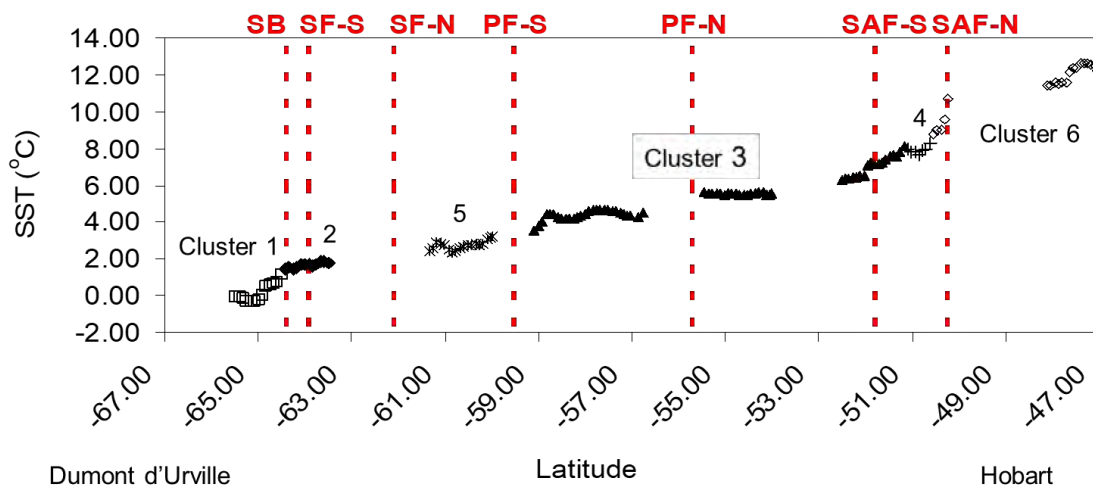


Figure 6. Location of zooplankton assemblages identified by cluster relative to the Southern Ocean fronts and overlain on top of the sea-surface temperature (SST) profile, recorded along the 140°E transect from 25 February to 3 March. The cluster groups are based on night time samples (higher zooplankton abundance), hence the gaps. Cluster 1 in the SIZ had 1 unique species, Cluster 3 in the open ocean area had 3 unique species, and Cluster 6 north of the SAF had 9 unique species. Frontal

positions are indicated for the SB (Southern Boundary); SF-S and SF-N (Southern Antarctic Circumpolar Current Front—South and North); PF-S and PF-N (Polar Front—South and North); SAF-S and SAF-N (Sub-Antarctic Front—South and North). Redrawn from Figures 2a and 5 from (Hunt and Hosie, 2005).

Hunt repeated this work over the same transects several times within the same season and was able to show that the assemblages maintain their compositional integrity within each zone, and the assemblage maintain their associations with the fronts despite the fronts moving latitudinally through the year (Hunt and Hosie, 2006b, c). Subsequent research by Sokolov and Rintoul (2009a) has shown that the frontal zones have moved south and we expect the zooplankton distribution ranges will move with the fronts, as modelled later by Mackey et al. (2012). There was virtual no seasonal variation in zooplankton composition relative to the SAF and PFZ. Seasonal succession was dominated by changes in the densities of the common taxa, with no evidence for a seasonal shift in the species composition (Hunt and Hosie, 2006c). Seasonal variation in the zooplankton, in the SIZ was more marked with both changes in the abundance of zooplankton and species composition. The seasonal community succession appeared to be influenced by the low sea ice extent and the southward projection of the ACC in this region with the relatively warm ACC waters, together with low krill biomass, favouring higher densities of small grazers such as copepods during the January/February bloom conditions. The persistence of relatively warm surface waters in March and the seasonal decrease in chlorophyll *a* biomass provided favourable conditions for salps, which were able to penetrate south of the Southern Boundary of the ACC. By comparison seasonal succession in the SAZ and PFZ was governed more by change in zooplankton densities through the summer, notably for *Oithona similis*, foraminifera and larvaceans, with an abrupt peak occurring in February and March. Hunt and Hosie (2005, 2006b, c) demonstrated that the CPR was an ideal instrument for regularly, repeatedly and quickly monitoring and surveying plankton over large and finer scales across the Southern Ocean. This laid the foundation for future biogeographic, bioregionalisation and trend studies.

Takahashi et al. (2011b) were able to repeat four transects in the same region 2007/08 as part of the CAML and IPY, and used the same multivariate analysis methods. While they didn't observe the abrupt peak in abundance in the PFZ as observed by Hunt and Hosie (2006c), they did observe the same pattern of total zooplankton abundance being comparatively consistent along the four transects, especially in the PFZ and POOZ, as seen before. This was followed by another seasonal study along 110°E in the 2011/12 season by Takahashi et al. (2017b) who again recorded consistently high abundances through the PFZ and POOZ, commensurate with the previous studies with much the same species composition across the zones (Fig. 7). They concluded that detecting the different seasonal patterns of micro- and meso-zooplankton species/taxa shows that the accumulation of intra-annual seasonal CPR data in various areas is still necessary to understand seasonal and annual micro- and meso-zooplankton abundance, and fundamental long-term changes.

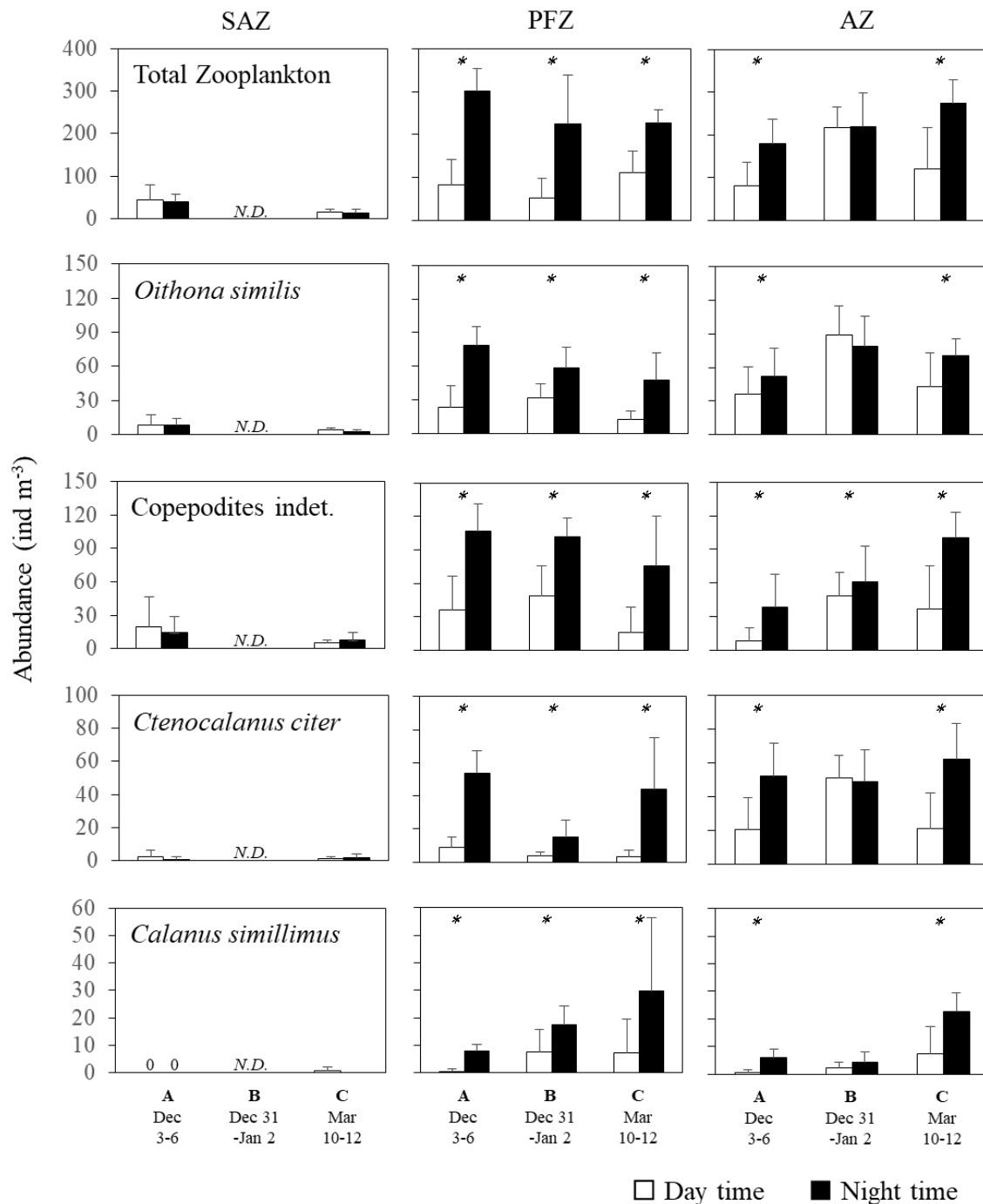


Figure 7. Abundance levels of major zooplankton groups in day (white bar) and night samples (black bar) from three frontal zones. Results of Tukey's test of abundance levels at each transect are shown. \*Significant differences ( $P < 0.05$ ). (from Takahashi et al., 2017b)

A related experiment was conducted and involved three widely separated transects being sampled near simultaneously southwards across the ACC by *Kaiyo Maru*, *Aurora Australis* and *Shirase* (Takahashi et al., 2002; Umeda et al., 2002; Hosie et al., 2003). This provided the opportunity to see if species composition contained with the AAC remained consistent as the ACC flowed through the region. Was the species composition the same upstream and downstream? The three ships



commenced sampling within 14 days of each other, which is as close to simultaneous sampling as possible given the different scientific and logistic priorities of the vessels; *Kaiyo Maru* along 25° 30'E starting on 24 November 1999, *Aurora Australis* along 158° 57'E on 1 December 1999, and by *Shirase* along 110°E on 8 December (Fig. 8, Hosie et al., 2003). The ships also started at different positions relative to the SAF and PF. *Kaiyo Maru* started sampling north of the SAF, *Shirase* between the SAF and PF and *Aurora Australis* just on the PF. Consequently, different distribution patterns of abundance were seen across the fronts, but they tended to be low north of the SAF and higher south of it as previously noted. Within the same zone, PFZ or POOZ, the abundances were the same on the three transects and there were no statistically significant differences in abundance. Species composition and the relative proportions were similar between transects for the ten most abundant taxa (Fig. 9) (Hosie et al., 2003). The composition and proportions were similar to those obtained from other CPR tows through the PFZ and POOZ; the only exception was the higher proportion of foraminifera caught by *Kaiyo Maru*. The similarity in abundance and composition between the transects, as well as consistency with previous tows, strongly indicates minimal spatial variation along the ACC. This allows more confidence in properly identifying and assessing temporal variation.

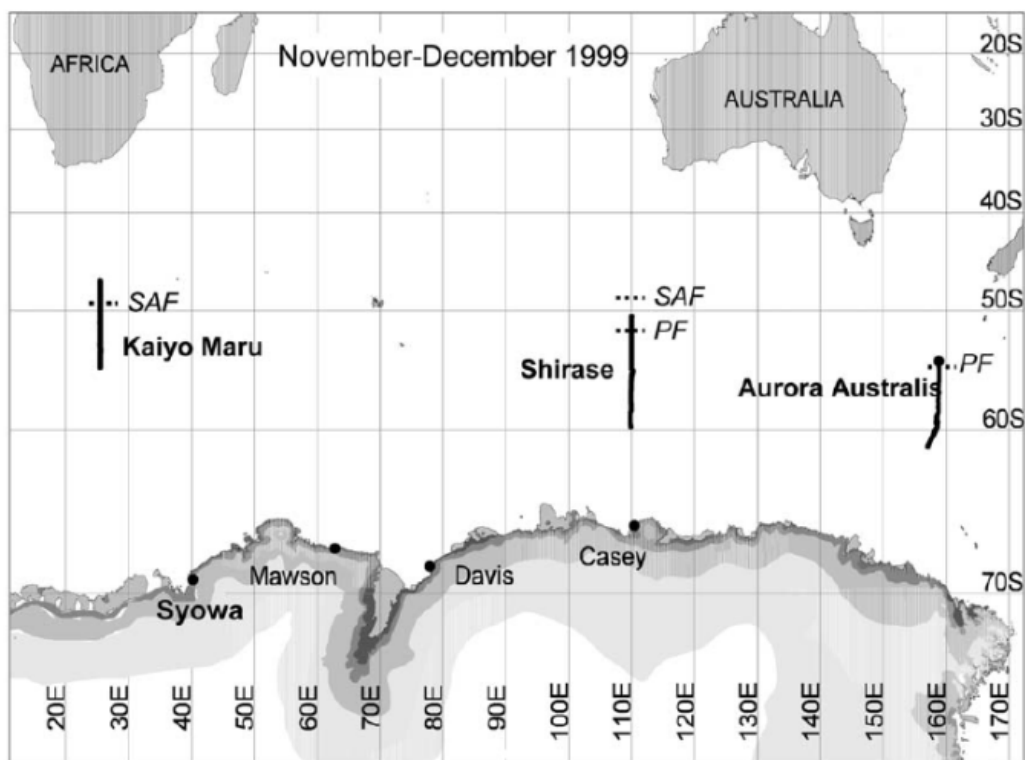


Figure 8. CPR tracks of the three-ship survey from the 1999–2000 season. *Kaiyo Maru* 24–25 November 1999, *Aurora Australis* 1–2 December 1999 and *Shirase* 7–9 December 1999. Approximate position of SAF and PF relative to each transect derived from ships' environmental monitoring system or Orsi et al. (1995). (from Hosie et al., 2003).

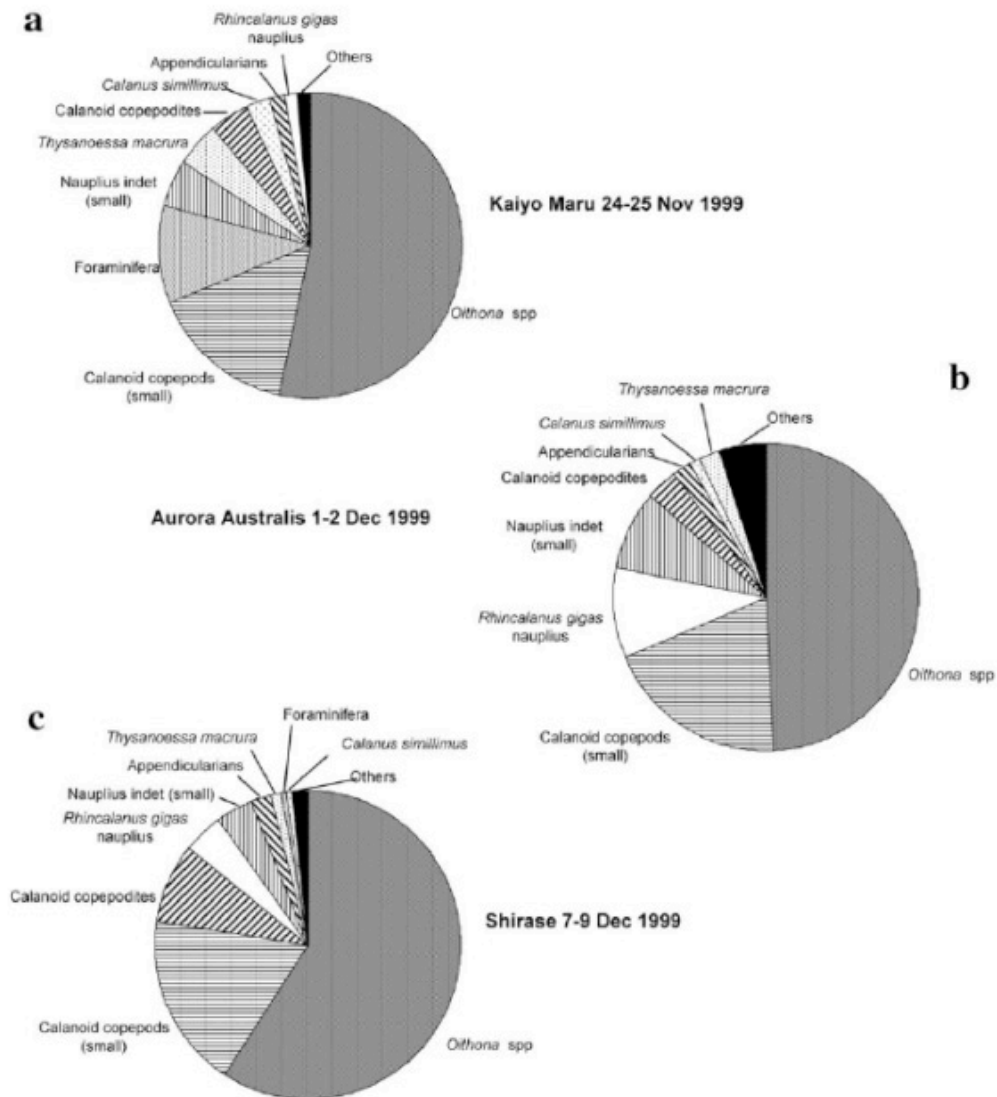


Figure 9. Major taxa recorded on each transect of the three-ship survey, November–December 1999: (a) *Kaiyo Maru*, (b) *Aurora Australis* and (c) *Shirase*. (from Hosie et al., 2003)

In relation to inter-annual variation along the same transects, the TV *Umitaka-maru* traversed the same transects southeast from Cape Town and north-east to Fremantle over consecutive austral summers; 2005, 2006, 2008, and 2009 (Takahashi et al., 2010a). This provided an opportunity to conduct CPR tows for four seasons along almost the same cruise track at the same time of year. Zooplankton abundance and community structure were comparatively consistent among transects with the usual dominance by *Oithona* and other calanoid copepod species. However, they detected the two notably different assemblages. The first on the Cape Town to Syowa transect in 2008 was dominated by larvaceans of the genus *Fritillaria* spp.. Which comprised 37% of total zooplankton abundance. This coincided with the lowest chlorophyll *a* levels recorded on all transects. Takahashi et al. (2010a) noted that larvaceans are suited to oceanic oligotrophic waters and therefore suited to low phytoplankton density. The second different assemblage, more an episodic event, was dominated by very abundances of foraminifera in 2005 (Fig. 10).

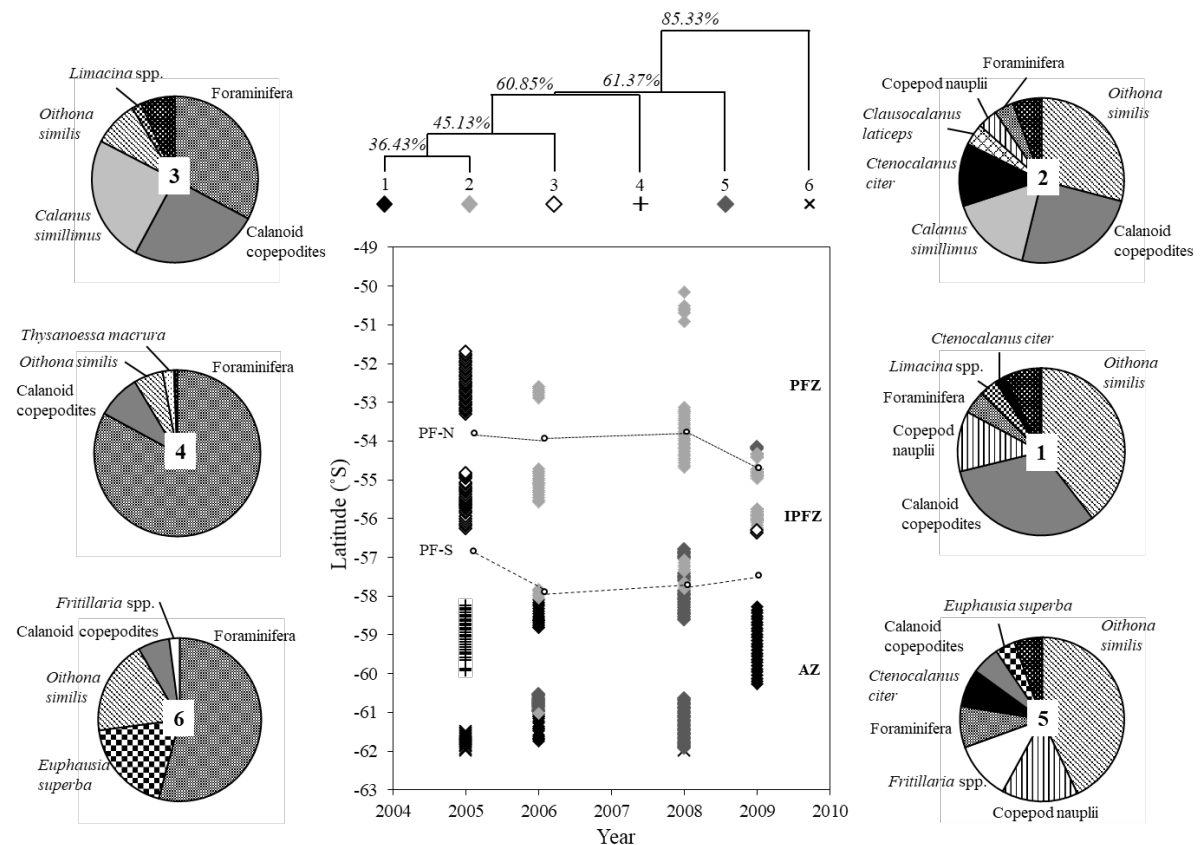


Figure 10. Results of the cluster analysis. The upper graph indicates the clusters identified and their level of separation, and the lower panel indicates the spatial and temporal distribution of samples in each cluster. The percentage contribution of major zooplankton groups in each cluster identified from cluster analysis is also shown. (from Takahashi et al., 2010a)

### *Foraminifera* bloom

Unlike *Fritillaria* spp., foraminifera prefer high phytoplankton density. Elevated average chlorophyll *a* biomass in 2005 ( $0.64 \text{ mg m}^{-3}$ ) most likely provided favourable conditions for the foraminifera to bloom in high numbers over a very large area, at times ranging from 33 to 83 % of total zooplankton abundance, representing a near ten-fold increase (Takahashi et al., 2010a). Foraminifera in previous seasons were usually less than 9 % of the total zooplankton abundance. Analysis of the dataset showed foraminifera in 2005 dominated the zooplankton across an extensive area of the eastern Antarctic region, from below South Africa to south Tasmania. This resulted in the decrease in the abundance of other dominant species such as *Oithona similis*. The abundance of foraminifera returned to low numbers in subsequent years but occasional elevated abundances of foraminifera are still recorded, e.g. south of Tasmania in spring 2009 and later south of New Zealand in summer 2010. Hunt and Hosie (2006c) reported such elevated abundances in February to March in 2002 during their multi-transect study. The consequences of these large blooms remains unknown but similar increases in foraminifera numbers have been reported in The North Atlantic by The CPR Survey. It is yet to be established if these foraminifera blooms are natural occurrences or a response to changing oceanic conditions. This is somewhat important as the foraminifera are a group likely to be affected by ocean acidification. Note, planktonic foraminifera distribution is highly seasonal and also known to be patchy. Such patchiness in foraminifera distribution at a localised level could sometimes be misinterpreted as a large scale bloom. The same can be said for other taxa.

Foraminifera are a large group of protists; they produce a shell made of calcium carbonate. Because calcium carbonate is susceptible to dissolution in acidic conditions, foraminifera may be strongly affected by a changing climate and ocean acidification. Moy et al. (2009) compared the shell weights of the modern foraminifera *Globigerina bulloides* collected from sediment traps in the Southern Ocean with the weights of foraminifera shells preserved in the underlying Holocene-aged sediments. They found that the modern shell weights are 30–35% lower than those from the sediments, consistent with reduced calcification induced by ocean acidification. They noted that “it was unclear whether reduced calcification will affect the survival of this and other species, but a decline in the abundance of foraminifera caused by acidification could affect both marine ecosystems and the oceanic uptake of atmospheric carbon dioxide.” As noted previously, foraminifera are specifically targeted for monitoring by SO-CPR.

**Recommendation:** Foraminifera remain a target group for monitoring change in abundances and effects of ocean acidification, along with other calcareous zooplankton.

## General Biogeography

### *CPR Atlas*

At a wider regional level than single transects we published an atlas of the distribution of the 50 most abundant species along with developmental stages of krill which comprised more than 90% of the total abundance (McLeod et al., 2010). Clear zonal distribution can be seen readily, especially between warmer water species like the sub-Antarctic *Neocalanus tonsus* (Fig. 11) and cold water species like *Euphausia superba* which typically is found mainly in the SIZ (Fig. 12). Fig. 13 shows the ubiquitous distribution of *Oithona similis*. Other species also have wide distributions the ACC but can show preferences to the north, e.g. *Calanus simillimus* or to the south, e.g. *Calanoides acutus*, *Fritillaria* sp.. The atlas shows that there is often a higher abundance of some zooplankton species eastward (downstream) of the Kerguelen Plateau between 80 and 120°E, distinct for *O. similis*, *T. macrura* larvae, *Rhincalanus gigas* nauplii (Fig. 13). Satellite imagery regularly shows downstream blooms of phytoplankton linked to iron enrichment (McLeod et al., 2010), which then diminishes further east below Australia as does the zooplankton abundance. The focus of the atlas was south and west of Australia as that was the region with the highest level of sampling at the time with approximately 22,500 sample records. In future, with the inclusion of a growing network of research partners in the CPR survey, we will be able to provide a circum-Antarctic atlas. The atlas will assist researchers studying plankton, relationships between predators and prey, productivity and future changes in response to climate change by providing baseline information of known distributions of the common zooplankton. This atlas was a major contribution to the Census of Antarctic Marine Life (CAML).

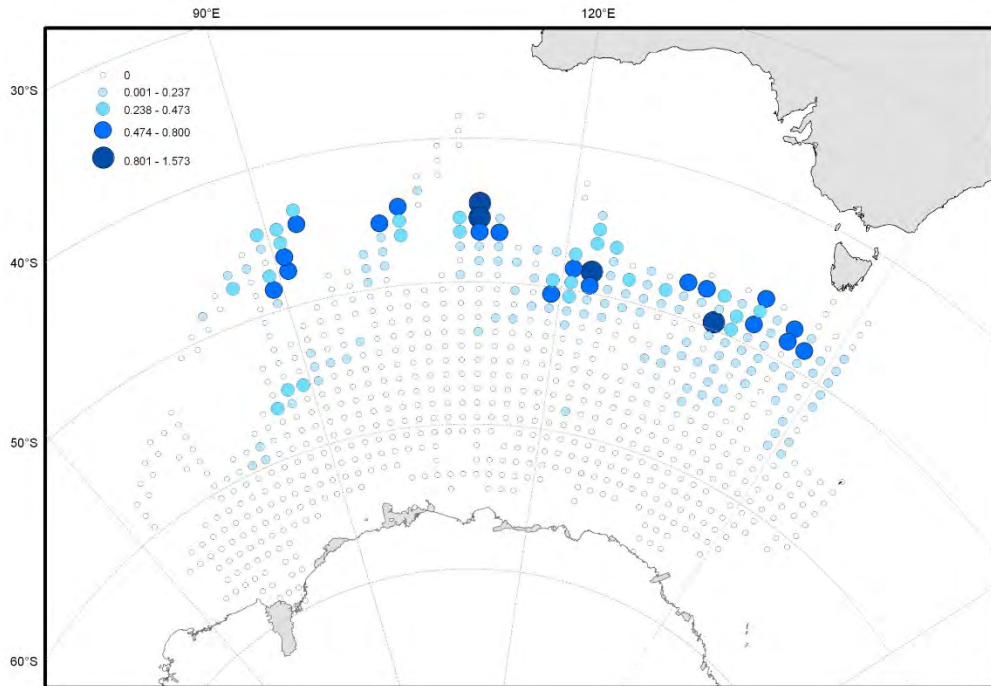


Figure 11. Near-surface abundance of calanoid copepod *Neocalanus tonsus* collected by the SO-CPR survey from 1991 to 2008. The  $\log_{10}(x+1)$  transformed data are displayed in  $1^\circ$  latitude by  $2^\circ$  longitude bins. Abundance is relative to the size of the shaded circle. Note: the focus of the SO-CPR Atlas is the region between  $60^\circ\text{E}$  and  $160^\circ\text{E}$ . (from McLeod et al., 2010)

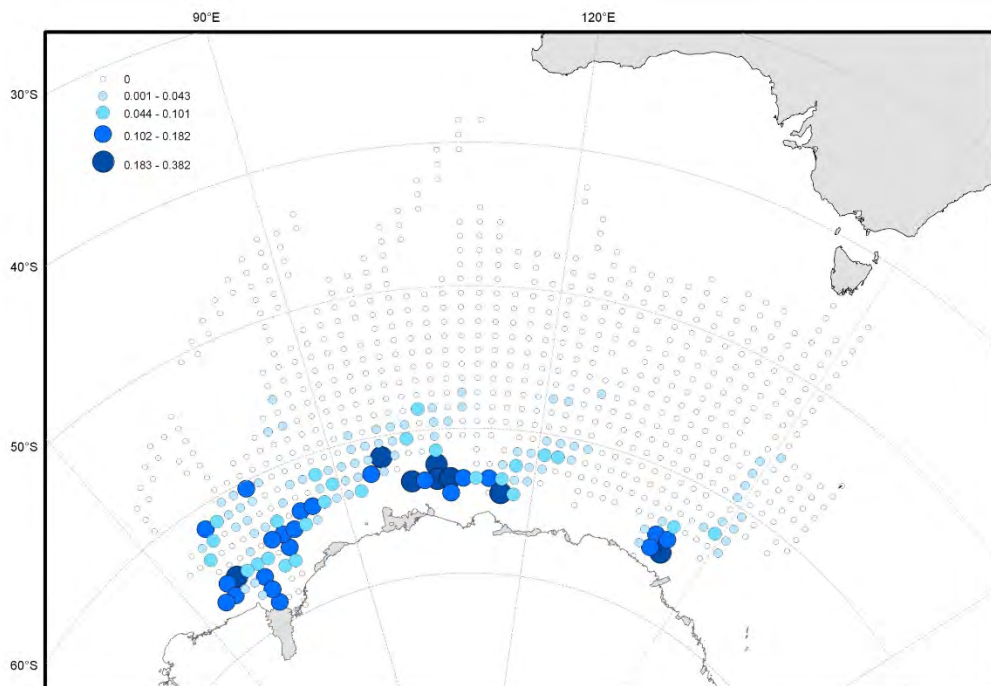


Figure 12. Near-surface abundance of euphausiid *Euphausia superba* adults. (from McLeod et al., 2010)

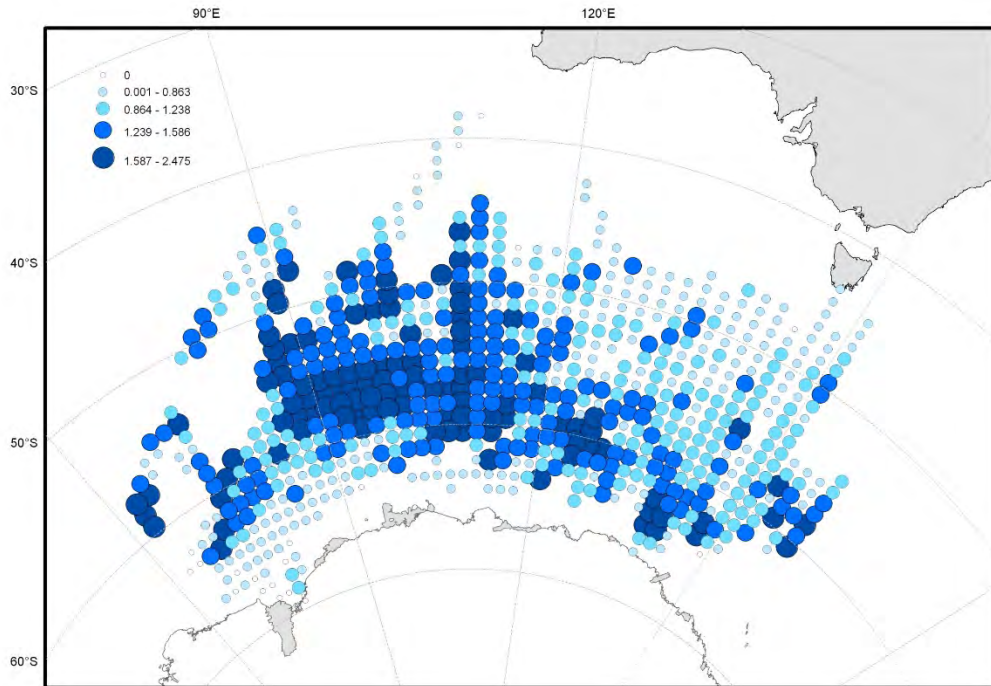


Figure 13. Near-surface abundance of cyclopoid copepod *Oithona similis*. (from McLeod et al., 2010)

#### *Circum-Antarctic Biogeographic patterns - SCAR Biogeographic Atlas*

One of the principal products of CAML and SCAR-MarBIN was the SCAR Biogeographic Atlas of the Southern Ocean. Seven chapters used CPR data when describing the distributions of specific groups, e.g. copepods, euphausiids, hyperiid amphipods, pteropods, for determining the bioregionalisation of the Southern Ocean (Cuzin-Roudy et al., 2014; Griffiths et al., 2014; Hosie et al., 2014; Koubbi et al., 2014; Kouwenberg et al., 2014; Roberts et al., 2014; Zeidler and De Broyer, 2014). The main CPR chapter was an extension of the CPR Atlas with the production of predictive biogeographic maps of whole zooplankton assemblages, zoogeographic divisions of plankton, using the same 50 taxa used by McLeod et al. (2010). This provided patterns by month or season which can assist other scientists studying climate change, plankton, predator-prey relationships by providing information on the degree of variation or in fact consistency in zooplankton biogeographic regions. Such information is also useful for fisheries and conservation management. Effectively, we produced maps of what assemblages you would expect to find relative to time and location.

All available CPR data up to March 2008, the end of CAML field work, were used for the Generalised Dissimilarity Modelling (GDM) of communities. Data were analysed by month. July, August, September, were grouped, as were April and May, due to the low number of samples in each of those months. A total of 25,026 five nautical mile equivalent samples were used in the analyses. GDM is a technique that models rates of change in species community composition between sites as a function of changing environmental characteristics (Ferrier et al., 2007). A number of global environmental variables were tested and five were selected for the final models, these being:

- Long-term mean summer sea surface temperature (satellite data)
- Long-term mean summer sea surface chlorophyll-*a* (satellite data)
- Long-term mean sea-ice extent (satellite data)

- Bathymetric depth
- Distance from the Antarctic continental slope

The zooplankton species composition was determined for each group for each month, as well as for the entire dataset (all months combined). A total of 18 taxa were then identified as the principal taxa that characterised each group, although not all taxa were considered important in every month, nor particularly abundant. These taxa were, cyclopoid copepod *Oithina similis*, calanoid copepods *Calanoides acutus*, *Calanus propinquus*, *C. simillimus*, *Clausocalanus brevipes*, *Ctenocalanus* sp., *Neocalanus tonsus*, *Rhincalanus gigas* nauplii, euphausiids *Euphausia frigida*, *E. superba*, *Thysanoessa macrura*, larvaceans *Fritillaria* spp., *Oikopleura* spp., pteropod *Limacina* spp., chaetognath *Eukrohnia hamata*, salp *Salpa thompsoni*, foraminifera and ostracods.

The GDM defined four distinct biogeographic zones that were commensurate with the known physical zones of the SAZ, PFZ, POOZ and SIZ. These are evident in the all-months prediction, and also evident in each month from December to May, these being the months with most data (Fig. 14). The modelling was less clear for July to November when there was less data and the southern region was covered by ice preventing CPR tows. The four biogeographic zones are more-or-less communities and will comprise several of the finer scale assemblages identified by Hunt and Hosie (2005, 2006b, c). Hosie et al. (2014) described these biogeographic zones as:

- the Inner Seasonal Ice Zone (ISIZ), a region covering much of the continental shelf and slope area,
- the Outer Seasonal Ice Zone (OSIZ), the rest of the SIZ south of the maximum sea ice extent,
- the Open Ocean Zone (OOZ), in between the OSIZ and the Sub-Antarctic Front (SAF), which comprises the PFZ and POOZ, along with numerous finer scale zooplankton assemblages, and at times includes part of the higher latitudes covered in winter by sea-ice,
- the Sub-Antarctic Zone (SAZ), the region north of the SAF and in some places including the Sub-Tropical Zone (STZ).



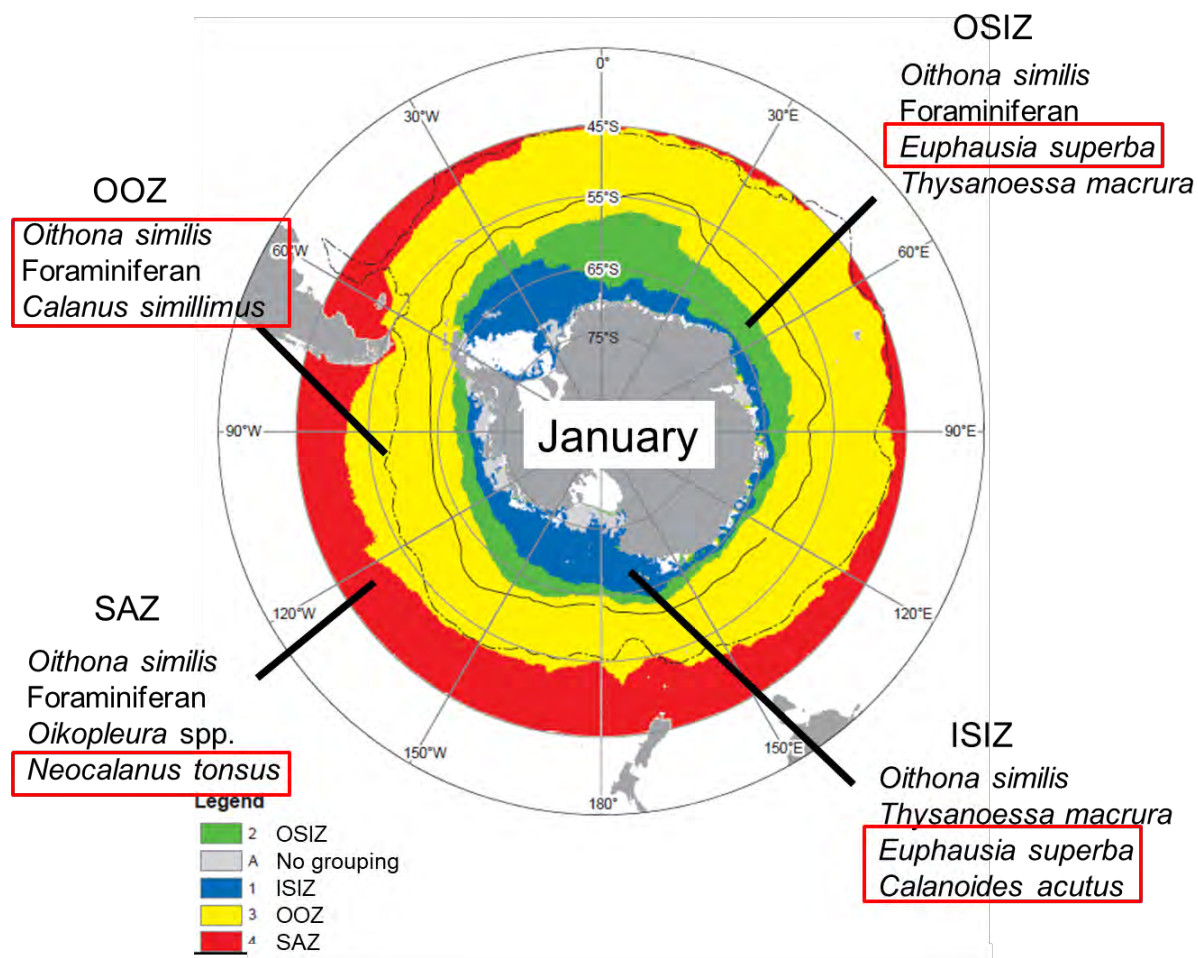


Figure 14. Generalised Dissimilarity Modelling (GDM) prediction for January for each of the major Zones; Sub-Antarctic Zone (SAZ), Open Ocean Zone (OOZ), Outer Sea-Ice Zone (OSIZ), Inner Sea-Ice Zone (ISIZ). Typical species characterising each zone are shown with the statistically dominant species highlighted inside a red box. (from Hosie et al., 2014).

The two most important variables affecting the prediction for the entire data set were (in order) sea surface temperature (SST) and sea-ice. Chlorophyll *a* (Chl *a*) and slope were interchangeably the third most important variable. However there is considerable variation between months.

All the GDM predictions identified the Sub-Antarctic Front (SAF) as a distinct biogeographic boundary as noted by Hosie and Hunt (2005, 2006a, b). The SAZ was typically characterised by the sub-Antarctic copepod *Neocalanus tonsus*. The zooplankton communities associated with the region of the SIZ and the continental shelf within were typically identified by Antarctic krill *Euphausia superba*, and copepods *Calanus propinquus*, *Calanoides acutus*. The ISIZ and OSIZ were predicted in months from December onwards when the sea-ice had retreated sufficiently to permit CPR sampling in the high latitudes. The Southern Ocean south of the SAF was more-or-less one zooplankton community in the near-surface. There was no real biogeographic separation between the POOZ and PFZ and was treated as one biogeographic zone (community), the Open Ocean Zone. However, within this zone there are still distinct finer scale zooplankton assemblages associated with the various fronts and sub-branches as identified by Hunt and Hosie (2005, 2006b, c). There were notable latitudinal variations in the boundaries of the biogeographic zones between months which will reflect the expected latitudinal variation in the oceanographic fronts (Sokolov and Rintoul, 2009a, b).



The Ooz notably comprised species with wide circumpolar distributions. It was also the region that consistently had the highest total zooplankton abundance and numbers of species. Some species tended to be more abundant in the south and decreasing towards the north (e.g. *Fritillaria* spp., *Thysanoessa macrura*), or more abundant towards the north (e.g. *Oikopleura* spp., *Ctenocalanus* spp., *Clausocalanus brevipes*) or more abundant in the mid-latitudes (e.g. *Oithona similis*, *Calanus simillimus*, *Euphausia frigida*). This is similar to the maps in the CPR atlas (McLeod et al., 2010). *O. similis* was in fact abundant and numerically dominant in all zones of the Southern Ocean, which is to be expected for this cosmopolitan species, but it was most abundant in the Ooz. This follows the boosted regression tree (BRT) distribution modelling of Pinkerton et al. (2010) who predicted this species as being consistently most abundant in the mid ocean band ice free region of the Southern Ocean (see section on Species Level Biogeographic Modelling).

Overall, the GDM models predicted the same biogeographic zones, in terms of species composition and distribution, as being consistent uniform bands around Antarctica, and that these are also quite consistent for most of the year. Nonetheless, there can still be distinct latitudinal and longitudinal variation and possibly consistent localised hotspots of abundance for individual species as demonstrated with the BRT spatial-temporal models for *Oithona similis* (see Fig. 15) (Pinkerton et al., 2010).

In relation to seasonal patterns, total zooplankton abundance in the Ooz increase rapidly in spring from very low winter abundances to reach a peak in December, remaining high in January and February before declining in March, April, May. Zooplankton abundances in the ISIZ and OSIZ peaked two months later, which coincides with the approximate one to two month period of being ice free. Zooplankton abundances also peaked in February in the SAZ. The numbers of species (of the original selected 50) increased over summer and declined in autumn. The number of species peaked in January-February in the ISIZ, March in the OSIZ, remained high from December to March in the Ooz and increased steadily in the SAZ, peaking in March before declining rapidly in April-May.

### **Species Level Biogeographic Modelling**

The application of species distribution modelling techniques to the SO-CPR data set has proved useful for bioregionalisation studies and for predicting the spatial and seasonal distribution and abundance of major groups of zooplankton in the Southern Ocean (Pinkerton et al., 2010; Pinkerton et al., 2020). The success of the prediction lies in sensitivity of zooplankton to closely align with and reflect the oceanographic conditions of their environment.

Pinkerton et al. (2010) applied the Boosted Regression Trees (BRT) multivariate statistical modelling technique to the CPR dataset up to 2006 to derive relationships between environmental conditions and the distribution of the adult stage of the cyclopoid copepod *Oithona similis* in the Southern Ocean. *O. similis* was initially chosen as it is numerically the most abundant species in the SO-CPR data set and seemed the best primary candidate to test the method. Analysis from Pinkerton et al. (2010) supported the hypothesis that abundance is related to environmental conditions in a broadly similar way throughout the Southern Ocean. Pinkerton et al. (2020) followed a similar approach but extended it to cover six key taxonomic groups of zooplankton: Copepoda (Calanoida), Euphausiidae, Foraminifera, *Fritillaria* spp., *Oithona similis*, and pteropods. In both studies, rather than using environmental data collected simultaneously with the CPR as per Hunt and Hosie (2005, 2006b, c), global data sets of environmental properties were used which would allow prediction of likely zooplankton communities into areas not sampled by CPR. Environmental parameters included values of sea surface temperature, chlorophyll, ice conditions and bathymetry.

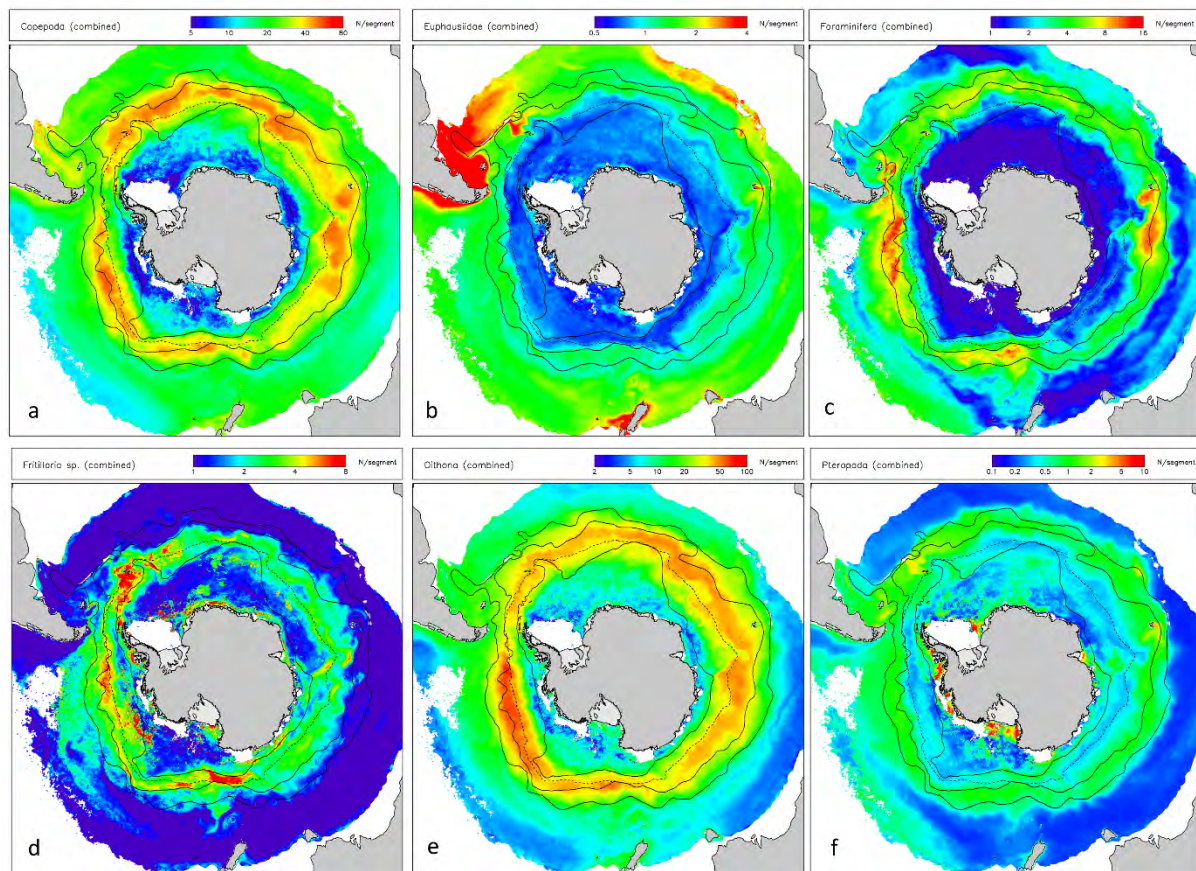


Figure 15. Predictions from Boosted Regression Tree (BRT) models for spatial patterns in the relative abundance (October–March) of: (a) Copepoda (Calanoida); (b) Euphausiidae; (c) Foraminifera; (d) *Fritillaria* spp.; (e) *Oithona similis*; (f) pteropods. Low values of coverage of environmental space were excluded from the prediction and shown in white (from Pinkerton et al., 2020).

It was possible to obtain reasonable models for the environmental suitability of these six zooplankton groups between October and March for most parts of the Southern Ocean (Fig. 15). No investigation was undertaken where the environmental characteristics were not well represented or where CPR sampling could not be undertaken. For example, the CPR cannot be towed under sea-ice, notably ice shelves, fast ice, or persistently heavy pack, so predictions were not made into these areas. For most groups of zooplankton (except Euphausiidae), there were higher environmental suitabilities (indicative of higher abundances) in a broad circumpolar band between the Polar Frontal Zone and the southern boundary of the Antarctic Circumpolar Current. The modelling of Pinkerton et al. (2020) predicted a hotspot in pteropod numbers in the South Georgia area consistent with a global analysis of net samples (Hunt et al., 2008) despite no SO-CPR data from this area being included in the CPR analysis. Pinkerton et al. (2010) identified five potential and relatively persistent "hotspots" of abundance of adult *Oithona similis* were identified: in the southern Scotia Sea around 59–64°S 40–55°W, two areas off east Antarctica near 110°E and 65°S 120–150°E, in the frontal zone north of the Amundsen Sea 59 and 62°S 140°W, and a small area in the outer Bellingshausen Sea around 65°S 80°W. Some of these hotspots appear to match predator patterns, and these hotspots were also present in the re-analysis of the larger dataset (Pinkerton et al., 2020).

The BRT modelling addressed two important questions. First, it is possible to use BRT to define the relationship between environmental conditions observable at large scales and the occurrence of this key zooplankton species across the Southern Ocean, i.e. circumpolar extrapolation of patterns based on data from a limited region is feasible. Secondly, that the relationships are relatively geographically consistent. Effectively, the BRT modelling of observed zooplankton abundance data from the SO-CPR coupled with global environmental data predicted the circumpolar distribution of probability of occurrence and actual abundance in areas where CPR sampling had occurred and also had not occurred. When sampling did happen in areas not previously sampled, notably the new CPR surveys conducted between New Zealand and the Ross Sea, the observed occurrence and abundance patterns were reasonable close to the predictive models. Those new CPR tows did show that the area of high abundance for total zooplankton were broader than the predictive models in the Ross Sea area (Robinson et al., 2014). The BRT modelling also predicted very low abundances in the southern Amundsen and Bellingshausen Seas. These low abundances have since been confirmed by CPR tows by Russia in 2008 during CAML.

Pinkerton et al. (2010, 2020) showed that the BRT method can be used for modelling large scale open water zooplankton patterns, and specifically the SO-CPR dataset has proved to be appropriate and valuable such mapping despite the low and variable efficiency of capture of many zooplankton species. In particular, Pinkerton et al. (2020) reproduced the currently-understood qualitative large-scale patterns of occurrence and abundance of six key taxonomic groups of zooplankton: Copepoda (Calanoida), Euphausiidae, Foraminifera, *Fritillaria* spp., *Oithona similis*, and pteropods. The comparison with net-haul samples reported in scientific literature have shown that the BRT abundance model explained 32–54% of the variance of *in situ* measurements including in areas geographically removed from SO-CPR sampling. The approach also allowed seasonal variation in these six groups of zooplankton to be investigated, and provided information on long-term trends in these species over the period of satellite observation of the ocean (1997–present) (Fig.16). Based on the BRT models, Pinkerton et al. (2020) concluded that between 1997 and 2018 the environmental suitability for copepods (both cyclopoid and calanoid), Foraminifera, and *Fritillaria* spp. had increased by 0.72% per year average, and at higher rates in frontal regions especially in the Indian sector of the Southern Ocean. In contrast, for pteropods in some areas (particularly over the Ross Sea shelf) the environmental suitability has significantly worsened over the last 20 years.



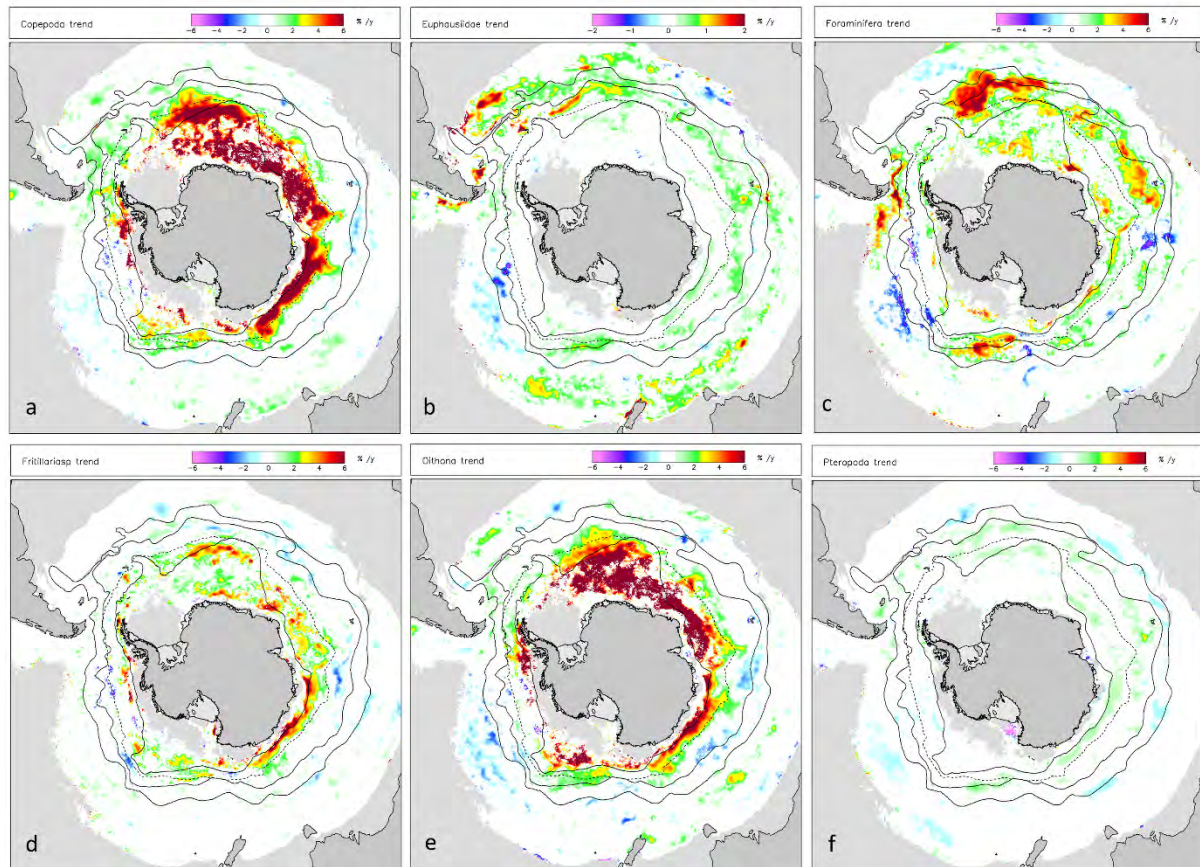


Figure 16. Trends in environmental suitability from Boosted Regression Tree (BRT) models for: (a) Copepoda (Calanoida); (b) Euphausiidae; (c) Foraminifera; (d) *Fritillaria* spp.; (e) *Oithona similis*; (f) pteropods. Low values of coverage of environmental space were excluded from the prediction and shown in white (from Pinkerton et al., 2020).

**Recommendation:** Reassessment and refinement of the zooplankton bio-regionalisation using the BRT method for the whole dataset should be conducted regularly as a means of monitoring and predicting possible biogeographic changes.

### Distributional changes and invasive species

Mackey et al., (2012) hypothesised the potential changes in macro-zooplankton distributions in the southern Atlantic in response to increase ocean temperatures. Their modelling indicated that most species would have a southward movement in response to a 1°C rise in temperature. For some warmer water species such as *Themisto gaudichaudii* and *Euphausia triacantha* the movement relative to their historical range could be of up to 12° latitude south. Cold water species would experience contraction in their ranges, and these species in particular will be vulnerable to temperature rises. *Euphausia crystallorophias* already has a very restricted range in the neritic waters of the Antarctic continent, with a preference for sub-zero water temperatures (Hosie refs, e.g. 1994), and cannot move further south.

It has been noted previously that there has already been a southward movement in the fronts of the ACC by about 0.6° of latitude (Sokolov and Rintoul, 2009b). Apart from the expected movements of species assemblages associated with the fronts as observed by Hunt and Hosie (2006b, c), no distinct

southward movement of any zooplankton species has been clearly detected so far. The CPR database is large and extensive, and would be ideal for detailed annual assessment of change in southern range boundaries of key abundant species. Contraction in the ranges of colder species is also not clear, partly because zooplankton in the SIZ tend to be less abundant in the very upper surface waters. *Euphausia crystallorophias* is poorly represented in the CPR database primarily because of logistical limitations of operating a CPR in neritic waters where ice usually prevails. The annual warming of surface layers in the neritic zone is also likely to prevent upward migration of this species, reducing the likelihood of being caught.

There is no evidence to date of any non-indigenous species being recorded by CPR south of the SAF. The SAF is probably a sufficient major biogeographic barrier to prevent incursions so far, or the waters of the ACC are too cold for northern species. It does need noting that there is substantial eddy activity in the region south of Australia, stronger than south of New Zealand and the Drake Passage (Phillips and Rintoul, 2000). Such activity may be the vector that carries sub-Antarctic and Sub-tropical species south of the SAZ into Antarctic waters.

In December 2010, the SO-CPR Survey found the heterotrophic dinoflagellate HAB *Noctiluca scintillans* in the sub-Antarctic Zone up to 240 km south of Tasmania (Fig. 17; McLeod et al., 2012). This is probably the most southerly oceanic record of *Noctiluca* and the furthest extension into the oceanic realm globally. This species is considered to be a coastal/neritic species and often causes problems in coastal systems. Within Australia, it has slowly been extending its range north from its first record of Sydney in 1860 into Queensland waters, south into Victorian and Tasmanian coastal waters and westward to South Australia and Western Australia (Hallegraeff et al., 2008). The *Noctiluca* south of Tasmania was constrained within an eddy that appeared to have detached from the East Australian Current (EAC). The species was in good condition and had been feeding heavily on phytoplankton. There was a significant decline in both phytoplankton and copepod abundances in association with the high abundances of *N. scintillans* indicating competition between the flagellate and copepods. McLeod et al. (2012) linked the progress of *Noctiluca* and eddy into the Southern Ocean to the continuing strengthening and southward extension of the East Australia Current, which appears to be caused by altered circulation patterns associated with global warming (Cai et al., 2005). The prediction is the EAC is likely to continue to strengthen and transport more warm water and eddies further south (Fig. 18). Thus may result in viable populations of *Noctiluca* become established in the Southern Ocean in the future with unknown effects for the food web.

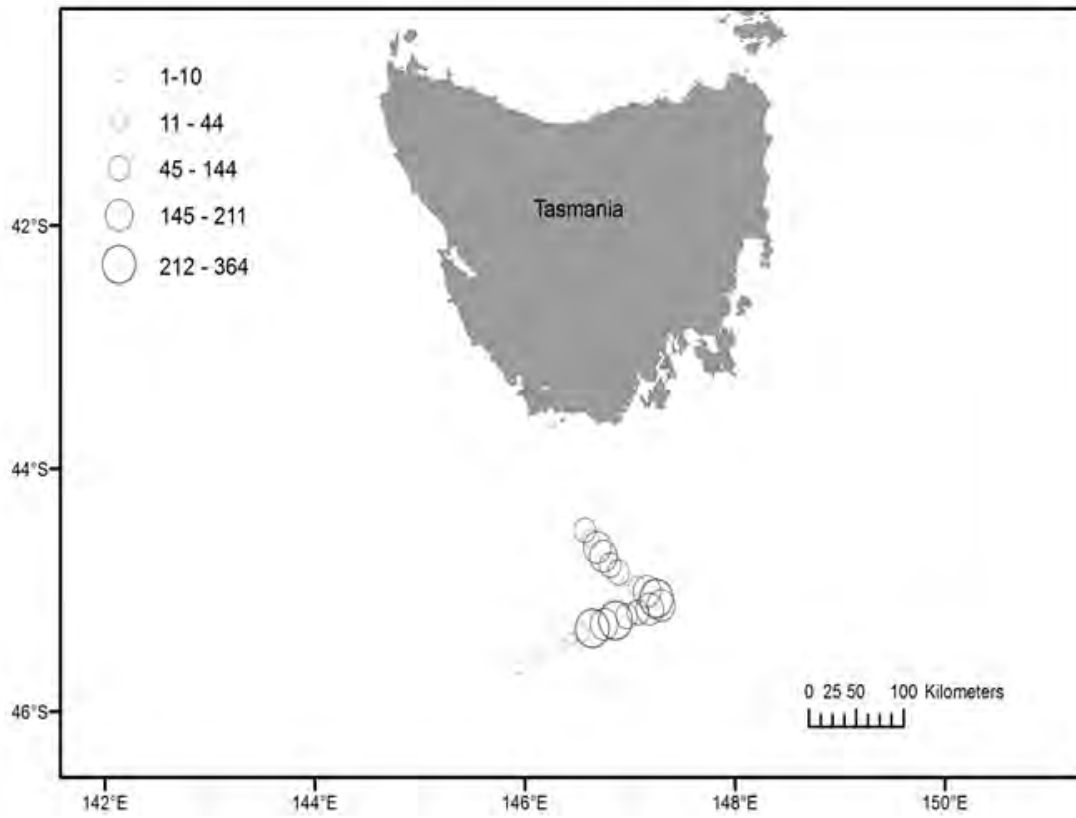


Figure 17. Southern most extension of *Noctiluca scintillans* captured by CPR on 10-12 December 2010 south of Tasmania. The legend shows number of cells per 5 nautical mile segment. (from McLeod et al. 2012).

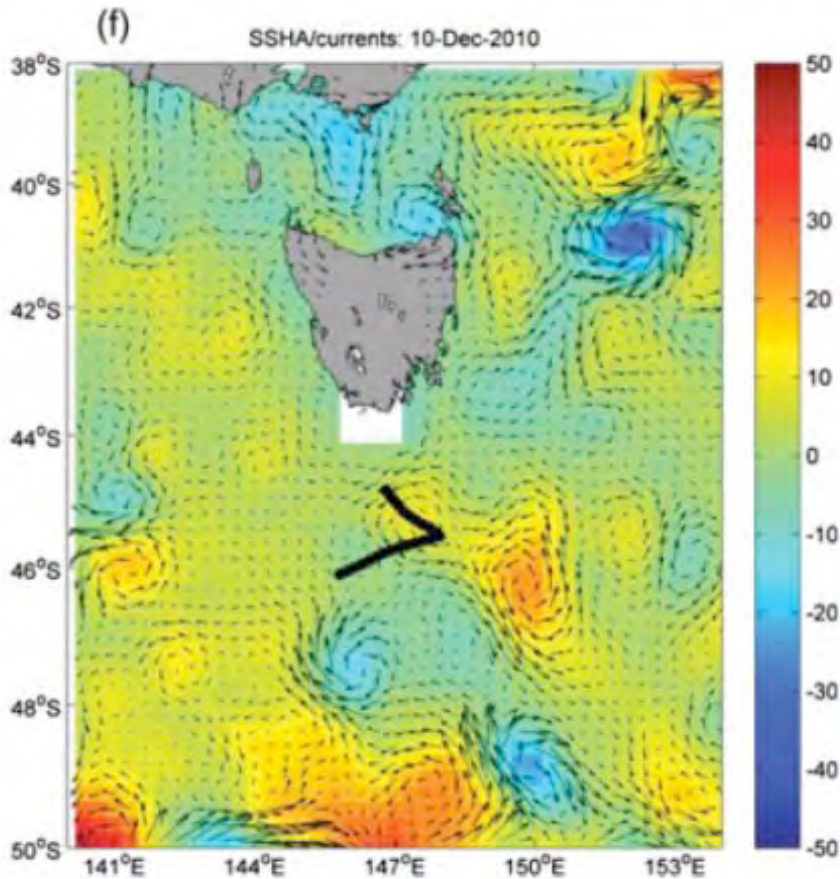


Figure 18. Sea surface height anomaly (SSHA) for 10 December 2010 showing the warm-core eddy (red) south of Tasmania sampled by the CPR on 10-12 December 2010 (Fig. 16). The black line indicates the extent of *Noctiluca scintillans* from the CPR transecting through the western edge of the eddy.

### Trends in zooplankton patterns

Having established the zooplankton biogeographic patterns of the region, with some indication of the degree of intra-annual or seasonal variation, the next step in the analyses of CPR data was to determine the existence and extent of long term trends. The following involves the assessment of long term changes in total zooplankton abundance, and average copepod community size, an index of the relative dominance of smaller or larger copepod species, between the eastern Antarctic region and the Ross Sea region. These analyses contributed to a report on the comparison zooplankton patterns in the Ross Sea region, in comparison with the eastern Antarctic, for the New Zealand Ministry for Primary Industries (Robinson et al., 2014). The work subsequently contributed the GACS Global Marine Ecological Status Reports 10 and 11 (Edwards et al., 2014, 2016), and to the Transboundary Water Assessment Programme, specifically for Chapter 5.3 The Status of Zooplankton Populations of the UNESCO and UNEP “The Open Ocean: Status and Trends” (Batten et al., 2016).

#### *Long-term trends in mean Total Zooplankton Abundance (TZA).*

Two regions of the Southern Ocean were compared, the East Antarctic region from 60 to 160°E where there has been the highest density of CPR tows to date, and the Ross Sea region between New

Zealand and the Ross Sea, 160°E to 150°W where CPR tows have been conducted regularly since 2006 (Fig. 19). The East Antarctic region includes FAO/CCAMLR Area 58 south of 55°S and the Ross Sea region includes Area 88 south of 60°S. The TZA, as the name implies, is the sum of abundance of all zooplankton collected in a section (sample) of CPR silk expressed as numbers per cubic metre, each sample normally representing 5 nautical miles of tow which equates to ~1.5 m<sup>3</sup> of water filtered. The four predominant latitudinal zones SAZ, PFZ, POOZ, and SIZ were compared within the East Antarctic and Ross Sea regions (Fig. 20).

Figures 21 to 24 show the annual mean TZA for each zone in the East Antarctic (black line) and Ross Sea (red line) regions. Each year shown represents the austral sampling season which starts about September and finishes about March/April, hence year 2000 represents the 1999/2000 season, and 2004 is 2003/04. The value shown are means for each sampling season. The figure show a steady increase in abundance in all four zones of the East Antarctic region over about a 20 year period, with perhaps a plateauing after 2005 in the PFZ. All increases were however statistically significant. The specific causes of these increases still needs to be identified. By contrast the Ross Sea region showed no trend in any of the four zones over a 12 year period of CPR tows in that region. However, the total abundances of zooplankton in the Ross Sea region were much higher than the abundances in the East Antarctic region. The TZA in the Ross Sea varied markedly between years with large peaks in abundance occurring in 2008-09 in the SAZ, 2009-10 in the SIZ and in 2010-11 in the other oceanographic zones. The cause of these peaks has yet to be determined. The large inter-annual variation in zooplankton abundance in the RS region contrasts with the observed patterns in the East Antarctic region where there is less inter-annual variation in total zooplankton abundance. The overall higher abundances in the Ross Sea region coincided with the Phytoplankton Colour Index (PCI) scores on the CPR mesh being consistently higher in the Ross Sea region, as were chlorophyll *a* concentrations from ocean colour satellites, indicating a higher standing stock or productivity in the RS region.

Sampling in the Ross Sea region is perhaps still relatively too short to make clear comment on trends, other than the Ross Sea region does not show any similarity to the patterns of the Eastern Antarctic region despite the PFZ and POOZ of the Ross Sea region being “downstream” of the Eastern Antarctic region in relation to the ACC. The Ross Sea region also has consistently higher productivity.



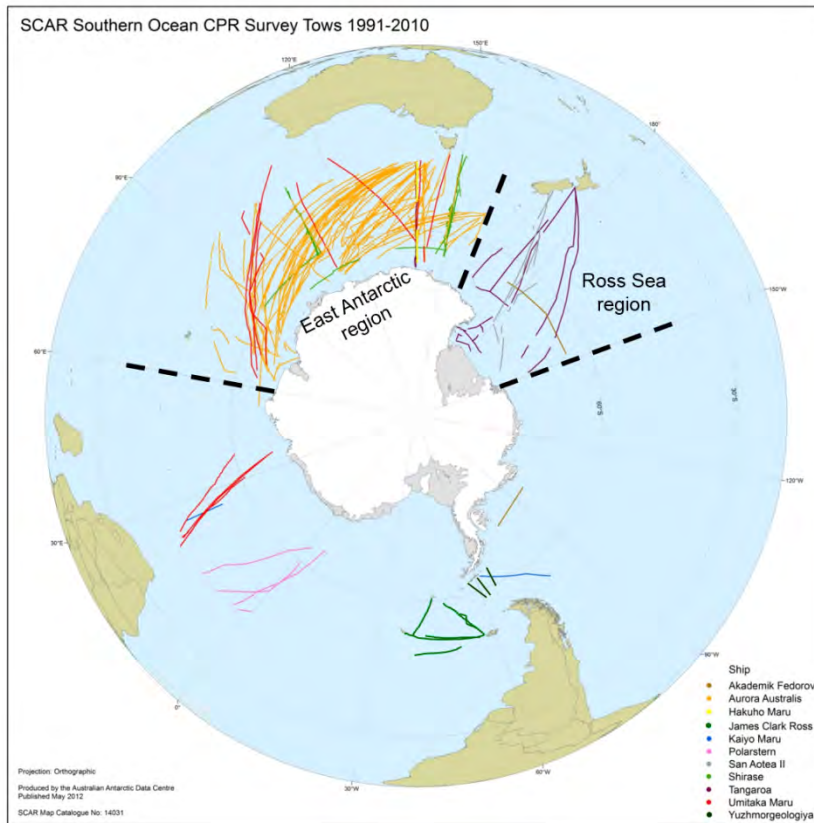


Figure 19. Map of tows of the SCAR Southern Ocean CPR Survey showing the two main regions of analysis in this report, the East Antarctic region and Ross Sea region.

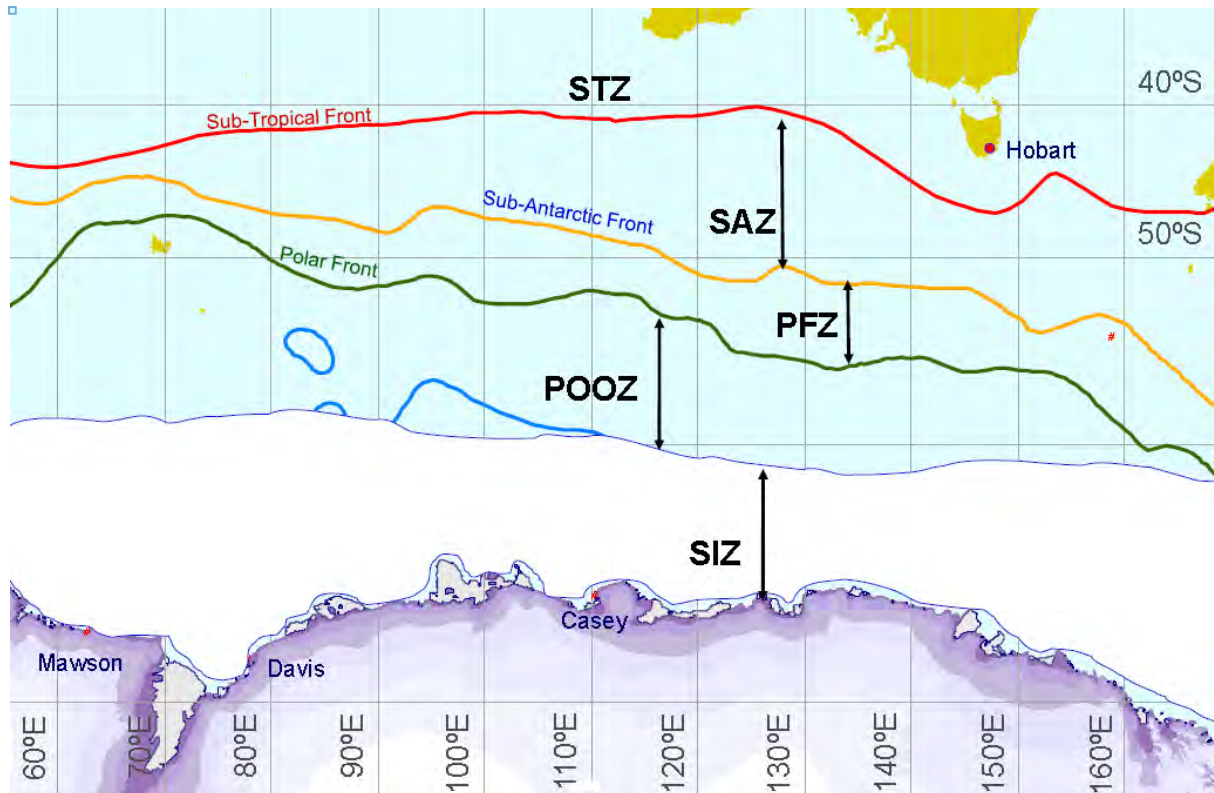


Figure 20. Description of the latitudinal zones; Sea-Ice Zone (SIZ), Permanent Open Ocean Zone (POOZ), Polar Frontal Zone (PFZ) and Sub-Antarctic Zone (SAZ). The positions of the fronts and ice edge is approximate.

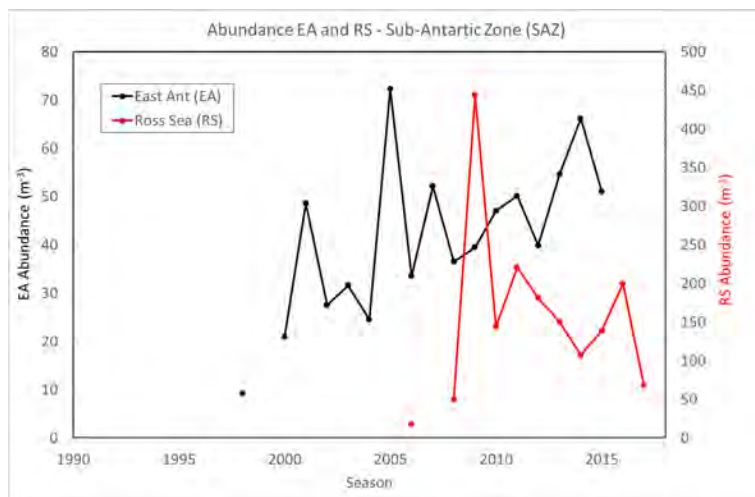


Figure 21. Mean Total Zooplankton Abundance for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Sub-Antarctic Zone. Season refers the austral sampling season, hence year 2000 is the 1999/2000 austral sampling season.

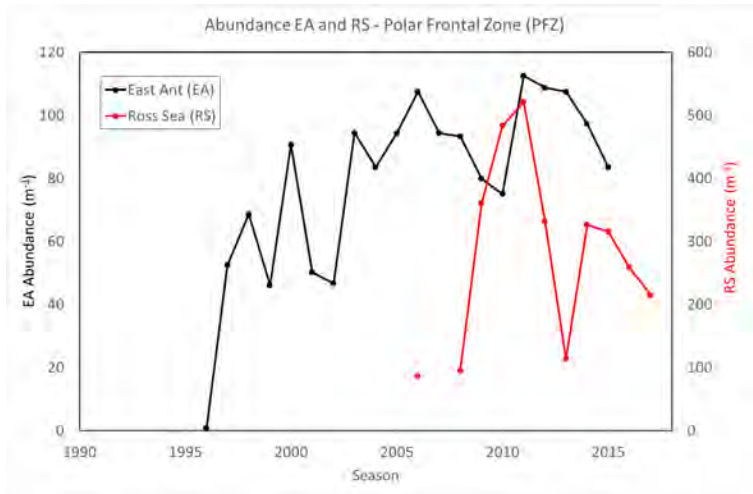


Figure 22. Mean Total Zooplankton Abundance for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Polar Frontal Zone.

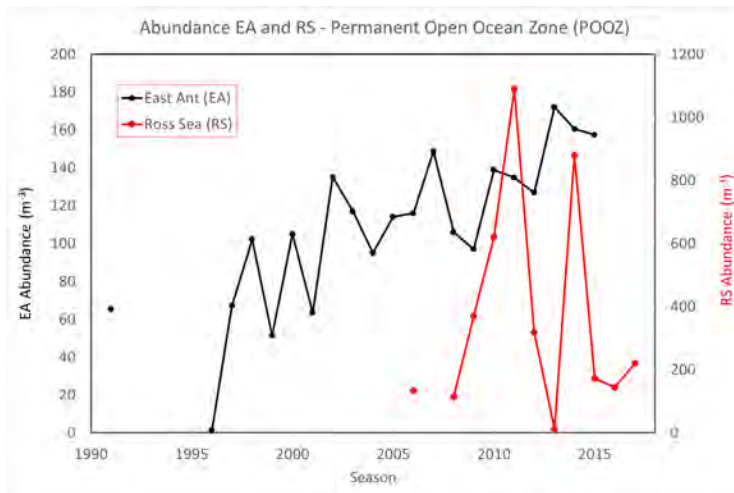


Figure 23. Mean Total Zooplankton Abundance for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Permanent Open Ocean Zone.

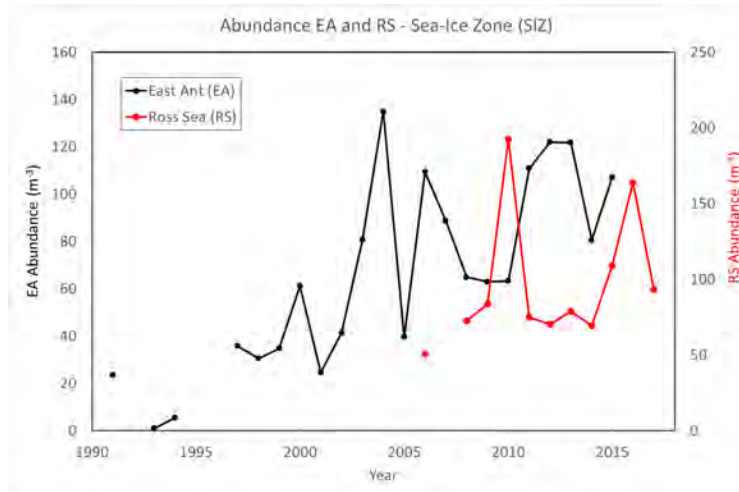


Figure 24. Mean Total Zooplankton Abundance for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Sea-Ice Zone.

Since 1991, the EA region has shown an increasing trend in total zooplankton abundance in the SAZ, PFZ, POOZ and SIZ. The five years of FV *San Aotea II* data, supplemented with the three RV *Tangaroa* voyages providing data since 2006, show neither increasing nor decreasing trends in any part of the RS region in terms of total zooplankton abundance. Total zooplankton abundance in the RS varied markedly between years with large peaks in abundance occurring in 2008-09 in the SAZ, 2009-10 in the SIZ and in 2010-11 in the other oceanographic zones. The cause of these peaks has yet to be determined. The large inter-annual variation in zooplankton abundance in the RS region contrasts with the observed patterns in the EA region where there is less inter-annual variation in total zooplankton abundance.

#### *Long-term trends in Average Copepod Community Size (ACCS)*

The ACCS is a weighted mean calculation using the mid-range length of an adult female copepod (see Appendix 4) as an index of the relative size of a species, multiplied by the number of individuals of that species. This is calculated for all copepod species in the sample, summed for all species and then divided by total number of all copepods to produce a single ACCS value (Beaugrand et al., 2003).

$$\bar{S} = \frac{\sum_{i=1}^N (L_i \times X_i)}{\sum_{i=1}^N X_i}$$

For each sample the length  $L$  (mm) of each copepod species  $i$  (adult female length) is multiplied by its abundance  $X$ , summed for all species  $N$  and divided by the total copepod abundance. Copepods which could not be identified to at least order are excluded from the ACCS analysis.

The ACCS does not indicate changes in the size of a species. The ACCS provides an index to compare changes in the dominance of species composition. The averaged index will increase if the

relative number of individuals, and hence dominance, of a larger species increases. Conversely the ACCS index will decrease if there is a shift in dominance to smaller copepod species.

The premise for using the ACCS is that warming oceans would see a shift to warm waters species that tend to be smaller. Certainly, tropic species are typically very small, temperate species tend to be bigger, and the largest species are typically found in sub-polar/polar waters or in deeper cooler waters. The near surface Antarctic waters, notably in higher latitude are typically characterised by large very abundant species such as *Calanoides acutus*, *Calanus propinquus*, *Rhincalanus gigas* and several species of *Paraeuchaeta*. The Southern Ocean also has quite a broad size range of numerous abundant small species of 1-2 mm common in the CPR samples, e.g. species of *Clausocalanus*, *Ctenocalanus*, and then there is the numerically most abundant species the cosmopolitan *Oithona similis* at < 1mm.

As noted in the Climate and Oceanography section, there has been a systematic and substantial warming of the Southern Ocean since 1930 with much of this concentrated in the Antarctic Circumpolar Current (ACC) (Aoki et al., 2003, Böning et al., 2008, Gille, 2002, 2008). Both surface and deeper waters have warmed (Aoki et al., 2003, Gille, 2008). This warming can be attributed to the southward movement of the ACC (Sokolov and Rintoul, 2009b) and possibly also increased heat flux and subduction of warmed surface waters (Gille, 2008, Rintoul et al., 2012). Has this affected the copepod species composition?

The ACCS was used to compare dominance in copepod species between the four zones, SAZ, PFZ, POOZ, SIZ, and between the East Antarctic and Ross Sea regions. Figures 25 to 28 show the corresponding annual variations in ACCS scores. As with the abundances the ACCS scores increased significantly in all four zones of the East Antarctic region, at least up to about 2008 or perhaps 2010 in the SIZ. From then there is the indication that the ACCS has plateaued or perhaps starting to decrease. The earlier increase in ACCS values indicates a shift in the abundance and dominance to larger species. This is contrary to the hypothesis that warming waters, as has been occurring in the region, would favour smaller warm water copepods. Other factors could be driving the shift in species dominance and need to be determined. Rather than increase in the abundance larger species, could it be a decline in smaller species' abundance, e.g. reducing dominance of *Oithona similis*? The CPR operations in the Ross Sea region again showed no trend or similarity to the East Antarctic patterns upstream in the ACC other than the rapid increase in the ACCS score for the POOZ. This zone is relatively narrow south of New Zealand and the values could be a reflection of the low number of samples across the zone. The increase is not copied in the ACCS scores for the PFZ to the north or SIZ to the south. Continued tows in the future will reveal if there is any correspondence in ACCS patterns, within the same zones, between the East Antarctic and the Ross Sea regions. Additional data will also show if the ACCS in East Antarctic has plateaued, perhaps starting to decrease or is a hiatus in a continuing upward trend.

As with the study of long term changes in abundance above, sampling in the Ross Sea region between 160°E and 150°W is still relatively too short to make clear comment on trends, other than the Ross Sea region does not show the same patterns as the Eastern Antarctic region despite the PFZ and POOZ of the Ross Sea region being “downstream” of the Eastern Antarctic region in relation to the ACC.

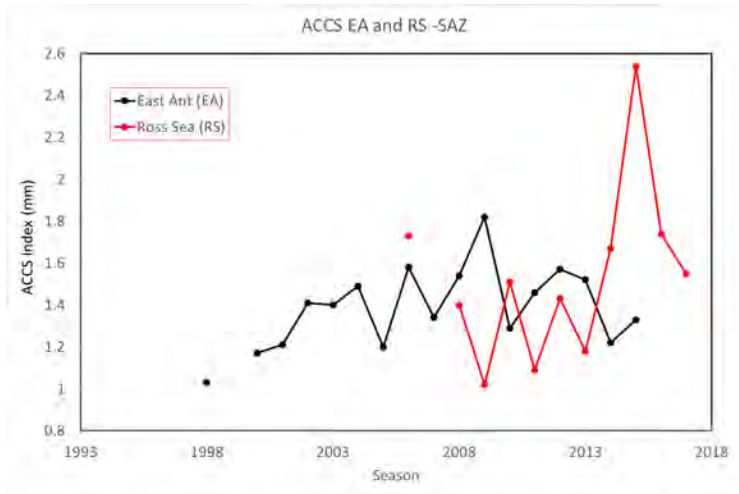


Figure 25. Average Copepod Community Size for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Sub-Antarctic Zone. Season refers the austral sampling season, hence year 2000 is the 1999/2000 austral sampling season.

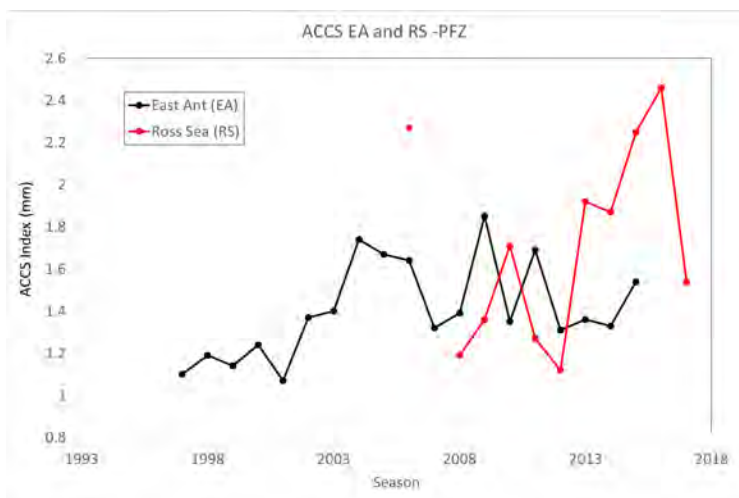


Figure 26. Average Copepod Community Size for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Polar Frontal Zone.

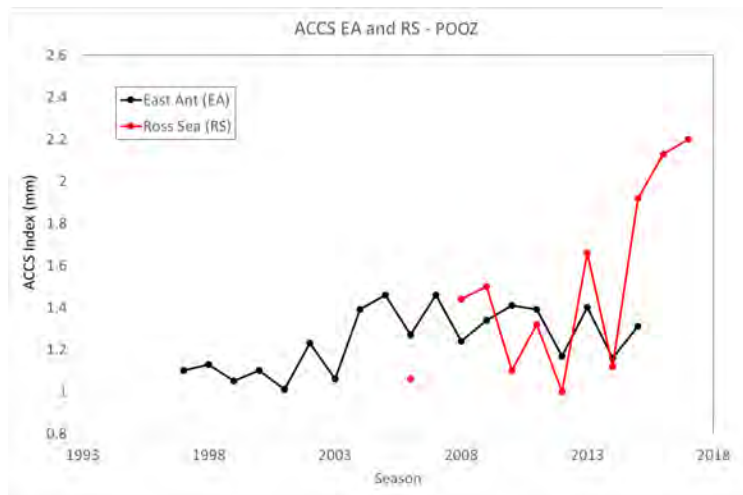


Figure 27. Average Copepod Community Size for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Permanent Open Ocean Zone.

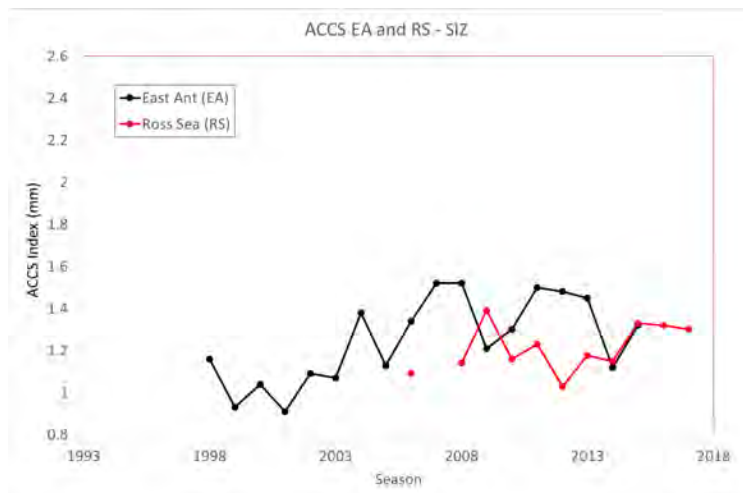


Figure 28. Average Copepod Community Size for the East Antarctic (EA) black, and Ross Sea region (RS) red for the Sea-Ice Zone.

### Longer term changes

Takahashi et al. (2010b) reported the opportunity to replicate one of Sir Alister Hardy's early CPR transects across Drake Passage and to compare species composition between 1927 and 2000. Comparison of the 2000 data set with figures from Hardy (1936) suggests the two sets of tows recorded markedly different species composition. Unfortunately, the original 1927 data cannot be located for direct comparison. However, Hardy's figure shows his tow recorded much higher abundances of larger copepods and chaetognaths (Fig. 29), whereas the tow in 2000 recorded a dominance of smaller copepods, notably *Oithona similis*, plus other species. Natural spatial and seasonal variation cannot be discounted between the 1927 and 2000 tows, nor can the fact that Hardy's original (Type I) CPR had a larger aperture than the Type II in use since 1931. The differences do highlight the need for the establishment of routine CPR tows in this sector of the ACC.



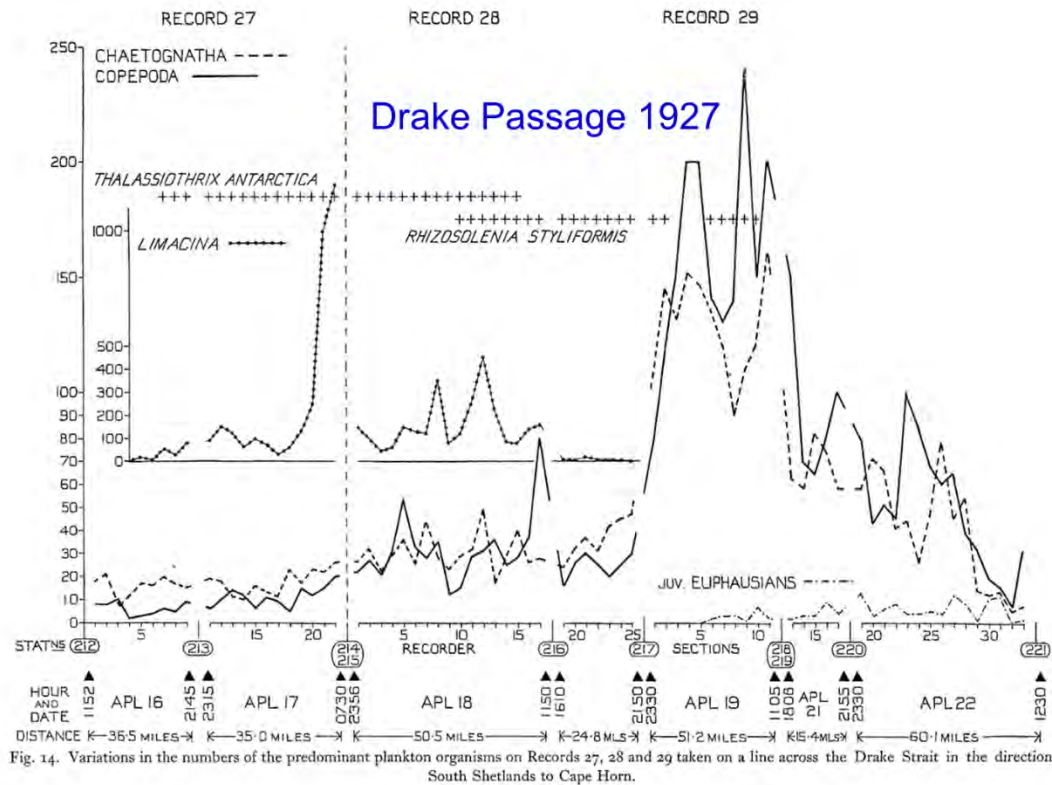


Figure 29. Sir Alister Hardy’s successful tow across Drake Passage in April 1927 showing high abundances of both copepods and chaetognaths. (from Hardy, 1936)

### Effects of (or relationship with) large scale climatic events

One question that is routinely asked in scientific meetings, and was one of the 80 questions identified in the SCAR Horizon Scan (No. 74) (Kennicutt et al., 2014a, b), is “How can natural and human-induced environmental changes be distinguished ...?” Or in other words, having found probable evidence of climate change impact on Antarctic biota, what portion if any is attributable to “natural effects”? Those who deny the possible impact and consequences of human-induced effects are quick to claim it is all natural cycles. In leaping into studies on climate change effects, we have failed to first understand the scope or boundaries of natural cycles.

From a plankton perspective, as the base of the food web, two other Horizon Scan questions are intertwined:

Q50 What are the synergistic effects of multiple stressors and environmental change drivers on Antarctic and Southern Ocean biota?

Q60 What are the impacts of changing seasonality and transitional events on Antarctic and Southern Ocean marine ecology, biogeochemistry and energy flow?

Notable seasonal and inter-annual events likely to have effects are the Southern Annular Mode (SAM), and the El Niño Southern Oscillation (ENSO).

Cowen (2016) in her study of SAM, ENSO and their combined effects on Southern Ocean plankton, noted that before understanding the ecological responses of primary and secondary production to changing climates, it is essential to ascertain the influence of natural climate variability on plankton.



Cowen further noted that evidence already existed to support the effects of multiple modes of climate variability such as SAM and ENSO on Southern Ocean circulation. Subsequently, changes in circulation would be expected to affect plankton.

Cowen (2016) first examined 16 years of satellite observations in the Southern Ocean to provide additional evidence of the effects of SAM and ENSO before comparing with the zooplankton. Positive SAM and La Niña events were found to produce similar patterns in sea surface temperature, and chlorophyll *a* concentration anomalies in the Southern Ocean. The stronger patterns associated with La Niña events were hypothesised as a coupling between SAM and ENSO, where weak positive SAM events and strong La Niñas show strong signals in SST, and thus primary productivity. The primary productivity responses to this coupling were increases close to the Antarctic continent and through to the southerly Sub-Antarctic areas, and decreases in the northerly Sub-Antarctic and Sub-Tropical Zone. El Niño events were found to produce a clear increase in primary productivity though the Antarctic Circumpolar eddy field.

14 years of CPR zooplankton observations, presence and abundance, from across the Southern Ocean region 60°E – 180°E were then analysed for 12 abundant zooplankton species, plus total zooplankton abundances, in relation to various environmental observations and the forcing of the climate modes, SAM and ENSO expressed as a derived Multiple ENSO Index (MEI) (Tables 6 and 7). Species selected were the copepods *Calanoides acutus*, *Calanus simillimus*, *Clausocalanus brevipes*, *Clausocalanus laticeps*, *Ctenocalanus citer*, *Metridia lucens*, *Neocalanus tonsus*, *Oithona similis*, *Rhincalanus gigas* adults and nauplii separately, the larvacean *Fritillaria* spp. and the euphausiid *Thysanoessa macrura*. All 12 species showed a response to the climate modes, although mechanisms for each species were often difficult to attribute. Results for *O. similis* and *M. lucens* were inconclusive. For *C. simillimus*, *Fritillaria* spp., *N. tonsus*, *R. gigas* and total Southern Ocean zooplankton abundances, increases in either presence or abundance were attributed to increases in primary productivity as a result of the phases of the climate modes. Increases in *T. macrura* presence was linked to warming SSTs during El Niño events, with this species expected to have accelerated growth rates with increasing water temperature. Increases in the frequency of presence and abundance of *C. citer* and *C. acutus* were attributed to strengthening wind speeds during negative SAM and El Niño/positive SAM events. Stronger wind speeds most likely result in increased mixing, subsequently increasing macronutrient supply, which promoted phytoplankton growth. However, while *C. citer* is considered to be predominantly herbivorous there was no strong relationship between this species and chlorophyll. It was most abundant in low chlorophyll areas – perhaps a function of the time-lag/grazing down effect.

Cowen (2016) identified some interesting and conflicting results, notably in relation to *Calanus simillimus*. Negative SAM events increased the presence of *C. simillimus*. Conversely, abundances increased during La Niña and positive SAM events. This is interesting as both La Niña and positive SAM events provide similar anomalies in the Southern Ocean (L'Heureux and Thompson, 2006; Ciasto and Thompson, 2008; Schneider et al., 2012). The three relationships with the climate modes were linked to warmer SST anomalies and increased chlorophyll *a* anomalies. As *C. simillimus* is an omnivorous species (Ward et al., 1996), it can be concluded that increased chlorophyll *a* anomalies could facilitate increased abundances. Cowen noted that these relationships were both complex and somewhat contradictory, as warming SSTs are generally associated with decreased chlorophyll and increased chlorophyll is related to cooler SSTs. This raises question of how the SST and chlorophyll relationships are responding independently? She also noted similar contradictory results for *Clausocalanus brevipes* and *C. laticeps* with increased abundances during La Niña events, linked to increases in SST and increases in chlorophyll *a* anomalies. These seemingly contradictory relationships serve to highlight that we simply lack sufficient information on the ecology of most zooplankton species necessary to understand how the species respond to their environment.

*Where to next?* Cowen's (2016) research has provided the first step in understanding the effects of the various modes of climate variability on zooplankton over a wide area of the Southern Ocean. This

work needs to extend to other species, and then to the next level to the various assemblages or communities previously identified. Specifically, we need substantially more information on the ecology of the individual species in order to properly interpret and understand the responses of zooplankton to changes in the environment and then to be able to model the flow on effects.

**Recommendation:** Substantially more information is required on the ecology of individual species in order to properly interpret and understand the responses of zooplankton to changes in the environment and then to be able to model the flow on effects.

Table 6. Shows the results for presence of the 12 species plus total abundance of zooplankton<sup>1,2</sup>.

| Species Model                 | Int. | Month | SST<br>an. | WS<br>an. | Chl<br>an. | SST  | WS   | Chl  | MEI  | SAM  | Lat  | Lon  |
|-------------------------------|------|-------|------------|-----------|------------|------|------|------|------|------|------|------|
| <i>Calanoides acutus</i>      | ***↑ | ***↓  | -          | ***↑      |            | ***↓ |      | ***↑ |      | ***↓ | ***↑ | -    |
| <i>Calanus simillimus</i>     | ***↑ | ***↓  | ***↑       | -         | ***↑       | ***↓ | ***↑ | ***↓ | -    | ***↓ | ***↑ | ***↑ |
| <i>Clausocalanus brevipes</i> | ***↓ | ***↑  | ***↓       | ***↓      | ***↑       | ***↑ | ***↑ | **↓  | -    |      | ***↓ | ***↓ |
| <i>Clausocalanus laticeps</i> | -    | -     | **↓        | ***↓      | ***↑       | -    | ***↑ | ***↓ | ***↑ | **↓  | -    | -    |
| <i>Ctenocalanus citer</i>     | *↓   | -     | -          | -         | -          | -    | ***↑ | -    | ***↑ | ***↑ | -    | -    |
| <i>Fritillaria spp.</i>       | **↓  | ***↑  | ***↓       | -         | -          | -    | -    | -    | -    | ***↑ | ***↓ | -    |
| <i>Metridia lucens</i>        | **↓  | -     | -          | ***↓      | ***↓       |      | ***↑ | ***↓ | ***↑ |      | ***↑ | ***↑ |
| <i>Neocalanus tonsus</i>      | ***↓ | ***↑  | ***↓       | *↓        | ***↑       | ***↑ | ***↑ | ***↓ | ***↑ | -    |      | -    |
| <i>Oithona similis</i>        | ***↑ | ***↓  |            | -         |            | ***↓ | -    | ***↑ | **↑  | -    | ***↑ | ***↑ |
| <i>Rhincalanus gigas</i>      | -    | ***↑  | -          | ***↑      | ***↑       | ***↓ |      | ***↓ | **↓  | *↓   | ***↑ | -    |

|                      |      |      |      |   |      |      |   |      |      |     |
|----------------------|------|------|------|---|------|------|---|------|------|-----|
| <i>Rhincalanus</i>   | -    | ***↑ | ***↓ | - | ***↑ | ***↓ | - | -    | -    | **↓ |
| <i>gigas nauplii</i> |      |      |      |   |      |      |   |      |      |     |
| <i>Thysanoessa</i>   | ***↑ | -    | ***↑ | - | -    | ***↓ | - | ***↓ | ***↑ | -   |
| <i>macrura</i>       |      |      |      |   |      |      |   |      |      |     |
| <i>Total</i>         | -    |      |      | - | -    | -    | - | -    | -    | -   |
| <i>abundances</i>    |      |      |      |   |      |      |   |      |      |     |

<sup>1</sup>The most parsimonious models for each species based on Akaike Information Criteria (AIC) are presented. Variables read from left to right, Int. = Intercept, Month, SST an. = Sea Surface Temperature Anomalies, WS an. = Wind Speed Anomalies, Chl an. = Chlorophyll Concentration Anomalies, SST an. = Sea Surface Temperature, WS = Wind Speed, Chl = Chlorophyll, Lat = Latitude, and Lon = Longitude.

<sup>2</sup>Variables which are included in the most parsimonious model are indicated with a symbol and an arrow. Each symbol indicates the significance level of the variable in the model. \* indicates the variable is significant at the 0.01 level, \*\* significant at the 0.001 level, \*\*\* significant greater than 0.001 level. ↑ indicates increased presence is associated with higher estimates of the variable, ↓ indicates increased presence is associated with lower estimates of the variable.

Table 7. Shows the results for abundance of the 12 species plus total zooplankton abundance<sup>1,2</sup>.

| Species Model                 | Int. | Month | SST<br>an. | WS<br>an. | Chl<br>an. | SST  | WS   | Chl  | MEI  | SAM  | Lat  | Lon  |
|-------------------------------|------|-------|------------|-----------|------------|------|------|------|------|------|------|------|
| <i>Calanoides acutus</i>      | -    | ***↓  | -          | -         | -          | ***↓ | -    | -    | -    | -    | -    | ***↓ |
| <i>Calanus similimus</i>      | ***↑ | ***↓  | **↑        | ***↓      | **↑        | -    | ***↑ | ***↓ | **↓  | ***↑ | ***↑ | ***↓ |
| <i>Clausocalanus brevipes</i> | -    | ***↑  | ***↑       | -         | ***↑       | **↑  | -    | *↓   | **↓  | -    | -    | **↓  |
| <i>Clausocalanus laticeps</i> | -    | ***↓  | -          | ***↑      | -          | ***↓ | ***↓ | -    | -    | -    | ***↑ | ***↑ |
| <i>Ctenocalanus Citer</i>     | -    | -     | -          | ***↑      | -          | ***↓ | ***↓ | ***↓ | ***↑ | *↑   | ***↑ | ***↑ |
| <i>Fritillaria spp.</i>       | -    | ***↑  | ***↓       | ***↓      | **↑        | ***↑ | -    | **↑  | ***↑ | ***↑ | ***↓ | ***↑ |
| <i>Metridia lucens</i>        | -    | -     | -          | -         | ***↓       | -    | -    | **↑  | -    | -    | -    | -    |
| <i>Neocalanus tonsus</i>      | -    | -     | -          | **↓       | -          | ***↑ | ***↑ | ***↑ | ***↓ | -    | -    | **↓  |
| <i>Oithona similis</i>        | ***↑ | **↑   | -          | -         | -          | ***↓ | ***↓ | ***↑ | ***↑ | ***↑ | ***↑ | ***↑ |
| <i>Rhincalanus gigas</i>      | -    | -     | ***↑       | *↑        | -          | ***↓ | -    | -    | -    | -    | ***↑ | **↑  |

|                      |      |      |   |      |      |      |      |      |      |      |
|----------------------|------|------|---|------|------|------|------|------|------|------|
| <i>Rhincalanus</i>   | -    | ***↑ | - | **↓  | -    | **↓  | ***↑ | ***↑ | **↑  | ***↑ |
| <i>gigas nauplii</i> |      |      |   |      |      |      |      |      |      |      |
| <i>Thysanoessa</i>   | -    | **↓  | - | ***↓ | -    | -    | **↓  | -    | -    | -    |
| <i>macrura</i>       |      |      |   |      |      |      |      |      |      |      |
| Total abundances     | ***↑ |      | - | ***↑ | ***↓ | ***↓ | ***↑ | ***↑ | ***↑ | ***↑ |

<sup>1</sup>The most parsimonious models for each species based on Akaike Information Criteria (AIC) are presented. Variables read from left to right, Int. = Intercept, Month, SST an. = Sea Surface Temperature Anomalies, WS an. = Wind Speed Anomalies, Chl an. = Chlorophyll Concentration Anomalies, SST an. = Sea Surface Temperature, WS = Wind Speed, Chl = Chlorophyll, Lat = Latitude, and Lon = Longitude.

<sup>2</sup>Variables which are included in the most parsimonious model are indicated with a symbol and an arrow. Each symbol indicates the significance level of the variable in the model. \* indicates the variable is significant at the 0.01 level, \*\* significant at the 0.001 level, \*\*\* significant greater than 0.001 level. ↑ indicates increased abundance is associated with higher estimates of the variable, ↓ indicates increased abundance is associated with lower estimates of the variable.

## Molecular genetic methods in collaboration with CPR

The coarseness of morphology-based taxonomy is one of the limitation of CPR methodology. Zooplankton samples from CPR are often physically damaged when spooled onto the mesh so that specific morphological features are lost, e.g. the delicate shells of thecosome pteropods. Soft bodied zooplankton such as larvae, hydromusae, ctenophores can damaged or distorted by the preservation process. Recent rapid growth of molecular genetic methods could make a large advance in plankton taxonomy so far restricted by morphology-based identification. DNA can be used to identify small species/taxa, gelatinous plankton and undeveloped larval zooplankton. Kirby and Reid (2001) successfully extracted and amplified a mitochondrial DNA sequence for the first time from preserved samples in the CPR archive. It effectively involved, unfixing formalin samples, and disentangling the DNA to obtain sufficient base pairs for identification. That technique is still limited in time and cost.

Metabarcoding (DNA-based species identification of complex samples) is revolutionizing analysis of biodiversity of marine zooplankton (Bucklin et al., 2016), and it has a potential to allow fast, accurate and less expensive collection of data for long-term monitoring. However, CPR samples preserved in formalin has limited genetic analysis of silks to DNA barcoding of individual specimens taken from the mesh (Kirby and Lindley, 2005, Licandro et al., 2015) or detection of single species (Vezzulli et al., 2012). Deagle et al. (2018) investigated the potential to use DNA metabarcoding as a tool for rapid collection of taxonomic data from CPR samples free of fixatives, i.e. formalin was not added to the CPR unit. As the results of the comparison between DNA-based biodiversity data and the conventional morphology-based sample processing, DNA increased the number of metazoan species identified and provided high taxonomic resolution of some groups that are traditionally hard to identify in the SO-CPR survey (e.g. zooplankton larvae, notably echinoderms, and hydrozoans). On the other hand, this high detectability of DNA analysis may be affected by cross-contamination during

sampling. DNA from larger euphausiids masked the presence of smaller zooplankton species. Also, Deagle et al. (2018) pointed out that differences by the two methods, morphology and DNA, can occur. These will be due to some specimens being unidentifiable based on morphology, lack of existing DNA reference sequences and potential incorrect taxonomic assignments with either method. Some challenges remain yet in order to fully utilize the molecular genetic methods with the long-term CPR monitoring. However, it is a promising technique that could play an important role in future plankton monitoring.

## Network Analysis

Networks are structured graphs consisting of sets of nodes connected by edges (lines) which provides a rich framework for visualising data and exploring connections inherent within the data. Each node in a network represents an entity or concept of interest—for ecological data, the entities of interest are commonly individual species or sample sites, although any choice of entity could potentially be used. Relationships between the entities of interest are indicated by edges between nodes in the network. A network is most commonly diagrammatically represented using circles or other shapes for the nodes, with lines between nodes showing the edges. The edges can be weighted to indicate the strengths of the relationships, and can also be directed, indicating that the relationships have an inherent direction (e.g. predation or temporal succession). Network analysis has been used rarely for the visualisation of ecological data, but are actually well suited to this purpose, including data that one might not normally think of as a network. For example, network analysis is ideal for examining trophic linkages with nodes being species and edges being the perceived interactions between species. Raymond and Hosie (2009) presented a simple method for transforming the CPR data, a basic data matrix, into network format, and show how this can be used as the basis for interactive exploratory analyses of ecological data. The subsequent network analyses revealed zooplankton community structures that are in good agreement with previously published results, e.g. known relationships between taxonomic groups, or associations through common biogeography (Hunt and Hosie 2005, 2006b, c).

The type of nodes and edges used on the CPR data included:

- Tow segments (samples) as nodes and edges indicating sites with common species. The temporal changes in environmental data could be observed. Specific taxa could be highlighted in this type of network to explore spatial linkages (Fig. 30).
- Nodes representing taxa and edges indicating taxa with similar spatio-temporal distributions (Fig. 31).

Variations in community structure were observed to be related to the temporal and spatial pattern of sampling, as well as to physical environmental factors such as the number of days since sea ice melt, which revealed subtle variation in species composition with variation in ice cover. The analyses also revealed a number of errors in the data, including taxon identification errors and instrument failures – an important issue in data quality control. The method allows an analyst to generate networks, in two or three dimensions, from different combinations of variables in the data set, and to examine the effects of varying parameters such as the scales of spatial, temporal, and taxonomic aggregation. Not only can the nodes be examined in detail, but edges can effectively be “plucked” to see what happens through the network. This flexibility allows the analyst to rapidly gain a number of new perspectives on ecological data such as CPR data, and provides a powerful mechanism for exploration. It enables investigators to form hypotheses and conceptual models for further investigation and experimentation. However, to date, there is still insufficient application of the network method on the growing CPR dataset. There is also the opportunity to directly link the CPR data with allied environmental and higher predator data.



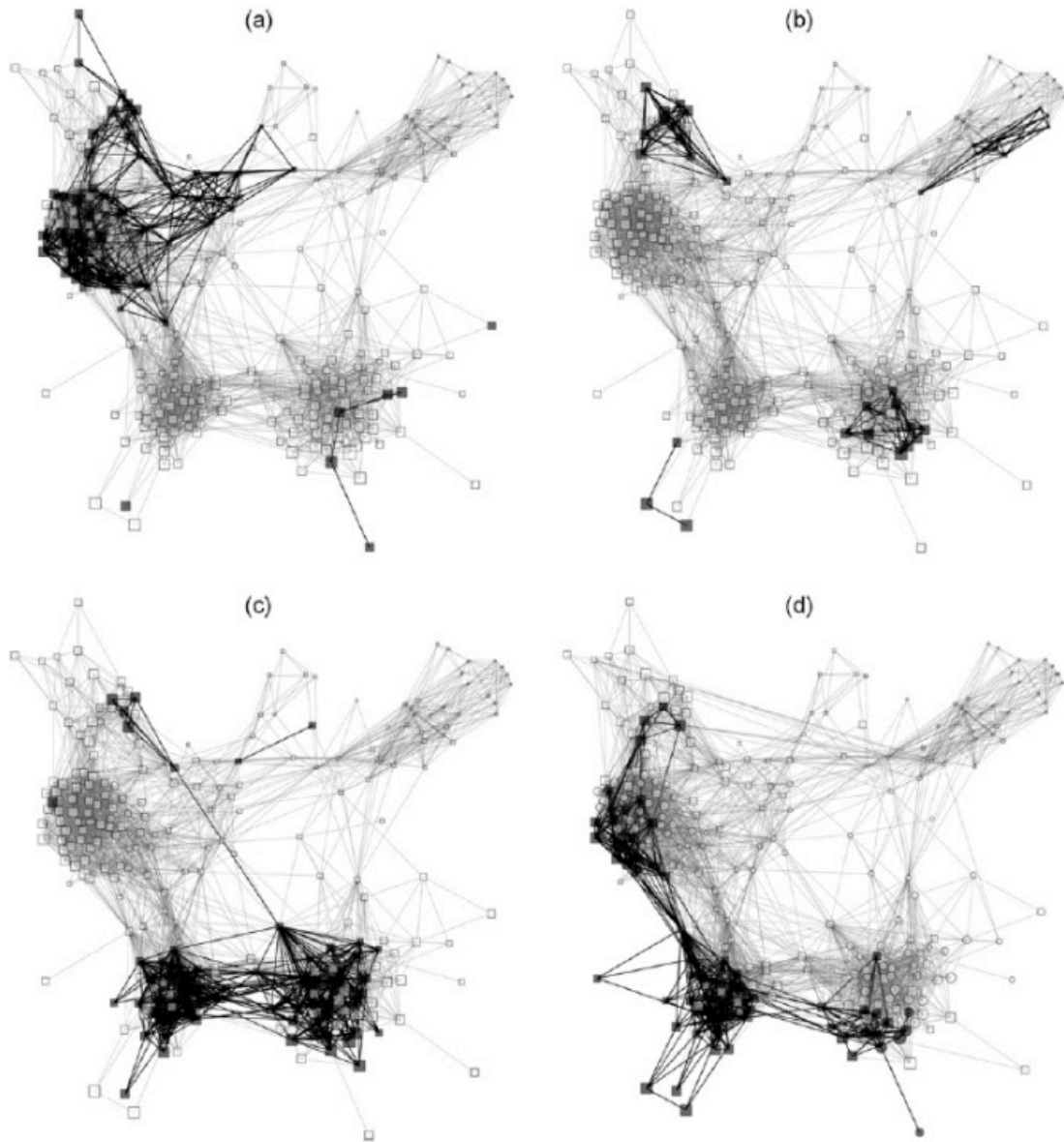


Figure 30. A network of CPR data from a series of tows conducted along the 140°E meridian south of Tasmania, 7 February and 3 March 2002. Nodes represent tow segments (sample sites) and the edges indicate sites with common species. Dark grey nodes and edges are specifically associated with the taxa (a) *Salpa thompsoni*, (b) *Pelagobia longicirrata*, (c) *Themisto gaudichaudii*, and (d) *Metridia lucens*. (from Raymond and Hosie, 2009).

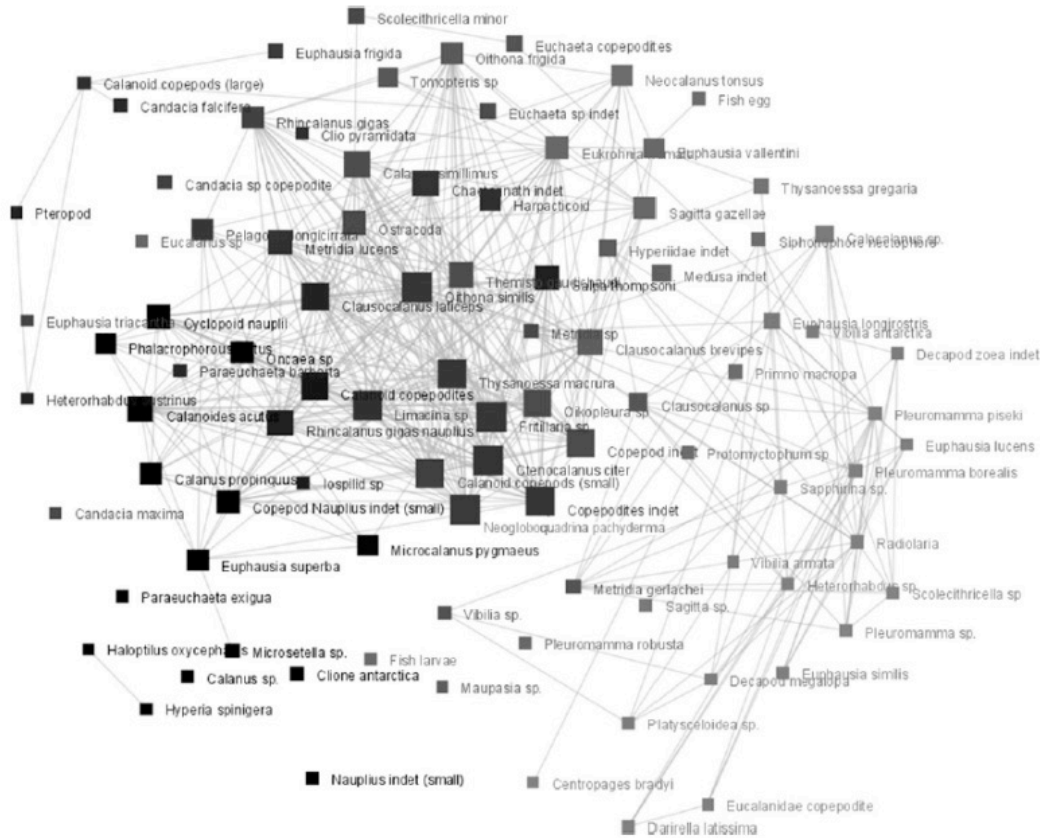


Figure 31. Plankton community network, in which nodes represent taxa and edges indicate taxa with similar spatio-temporal distributions. The network has been constructed from the full set of CPR tow segments. (from Raymond and Hosie, 2009)

**Recommendation:** A more extensive application of network analysis to the CPR data, together with environmental and higher predator data, may well suit a postgraduate or postdoctoral study under the sponsorship of the SCAR groups on CPR, Biodiversity Informatics, and Birds & Marine Mammal.

## GAPS IN OUR KNOWLEDGE

The gaps in our knowledge of Antarctic plankton in general are extensive and warrants a separate report. We will only highlight the key issues pertaining to the CPR, which are common to other areas of biology and often discussed Antarctic meetings.

*Winter:* Sampling in winter is a chronic issue for nearly all biological studies, in particular for the CPR survey. Very few ships venture into the Antarctic region in winter, mainly because there are a few vessels capable of doing so, which also have the necessary icebreaking capability. While a ship may not specifically plan to enter the winter sea-ice, the presence of ice is still a navigational issue. Navigation in winter in the Southern Ocean has its hazards and ships need to be well suited. It is also a costly and difficult logistic exercise especially if national Antarctic agencies are still planning full summertime operations. A few winter samples have been taken by the RSV *Aurora Australis* and the S.A. *Agulhas II*. Detailed analyses of the *Aurora Australis* samples showed very low numbers of



number in winter in the Permanent Open Ocean, Polar Frontal and Sub-Antarctic zones, which is perhaps unsurprising. However, it does set a reference point for understanding seasonal change.

*Coastal/neritic zone:* The coastal areas are not well sampled. CPR tows often cease on approach to coastal areas as towing of CPR become somewhat impractical when ships are busy navigating through the inshore waters with the usual hazards of sea-ice, icebergs and rapidly changing bottom topography. Unfortunately neritic species are not well monitored, notably *Euphausia crystallorophias*, which could disappear with warming waters as it cannot move further south. Monitoring of plankton in inshore waters may require smaller, lighter CPR units designed for use over shorter distances and be deployable from small craft. There could be issues of compatibility with offshore CPR data.

*Regional gaps:* As noted previously, most of the CPR sampling has been concentrated in the area from south-east of Africa eastwards to the Ross Sea. Only three tows have been conducted in the Pacific area east of the Ross Sea, only a few more across Drake Passage and to date none in the Weddell Sea. There have been tows conducted in the South Atlantic between the Falklands and South Georgia, the SF route, by the CPR Survey MBA UK, in conjunction with the British Antarctic Survey and the South Georgia Government, but this route has been suspended since April 2017. These tows are part of the MBA CPR Survey database and not included in the SO-CPR data.

*Taxonomic resolution:* The ability to identify soft-bodied zooplankton which are easily damaged, notably medusa, larvaceans, meroplankton larvae, is an issue for all CPR surveys around the world. Genetics or some other bioassay might provide the solution for gaining both qualitative and quantitative estimates of the abundance of gelatinous zooplankton.

*Microplastics:* While specifically designed for sampling plankton, the CPR has proved itself as a means of quickly monitoring large areas for plastics as it does for plankton. The application of FTIR or Raman methods should produce the required quantitative assessment of origin and abundance of plastics without burdening plankton analysts. This is not so much a gap in the CPR studies, but a valuable augmentation.

*Ecology of zooplankton:* Apart from the spatial and temporal gaps, our biggest gap is basic understanding of the ecology of zooplankton. The SO-CPR has already produced valuable information on biogeography of zooplankton species and communities, as well as changes in those systems, e.g. changing abundances and average copepod community size. However, interpreting these results, and understanding the mechanisms of interactions and any changes with the environment is hampered by our poor understanding of the ecology of zooplankton. We have spent millions of dollars and the same in hours trying to understand the ecology of Antarctic krill *Euphausia superba*, a major key species in the Antarctic food web, and there is still much we don't know. But, it is not the sole key species, with other euphausiids and copepods being important alternative pathways in the Antarctic food web. Our knowledge of the ecology of the major copepods species and other euphausiids is very poor, before we consider other groups such as salps, larvaceans, chaetognaths, pteropods etc. There is speculation that ocean acidification may cause the demise of the very abundant thecosome pteropods such as *Limacina* sometime later this century (Royal Society, 2005). Is this true and how will this affect the zooplankton community structure and food web? The reality is, there are too few zooplankton specialists working in the Antarctic area, with insufficient research funds flowing in their direction.

**Recommendation:** All attempts should be made to include CPR tows on any vessels operating in the Antarctic region in winter, or through the Pacific and Atlantic sectors at any time.

**Recommendation:** All efforts should be made to reinstate and maintain CPR tows in the South Georgia/Scotia Arc area as this is an important krill fishing area.

**Recommendation:** More research effort is required to understand the ecology of zooplankton, even for just the major abundant copepods, other euphausiid species and salps, in order to understand the effects and consequences of change in that community and through the rest of the Antarctic food web.

## FINAL COMMENTS

There are numerous and various threats potentially affecting Antarctic plankton and krill with undoubted cascading effects through the rest of the ecosystem. Any one of these alone can have serious effects, without acting in concert with other threats.

There are definite warm-water species in the north (SAZ), e.g. *Neocalanus tonsus*, and cold-water species in the south, *Euphausia superba*, *Calanus propinquus*, *Calanoides acutus*, and in particular *E. crystallorophias*, but there are large number of species with broad circumpolar distributions which indicates reasonably wide temperature tolerances. Moderate increases in sea-water temperature are predicted to have a severe impact on the survival of sea-ice species like Antarctic krill *E. superba* (Hill et al., 2013), and the same is most likely to apply to the neritic ice krill *E. crystallorophias*. The ice krill already has a very restricted coastal range and has nowhere else to retreat with warming waters. For many species increases in temperature may not be as problematic, other than a predicted poleward shift in their distribution (Mackey et al., 2012). *Oithona similis*, for example, is a cosmopolitan species that seems to occur in most marine environments and therefore seems unlikely to be negatively affected by temperature increases. Potentially, there may be high tolerance to temperature increase in the near-surface zooplankton communities at least. Nonetheless, rising seawater temperature will diminish sea-ice habit. Warming waters will favour warm-water tolerant species over cold-water stenothermic species potentially leading to fundamental changes in species composition and community structure and function. Warming conditions are also likely to favour invasive species.

The effects of ocean acidification are potentially more disruptive (Kawaguchi et al., 2013, Bednaršek et al., 2012). It will affect calcareous phytoplankton and zooplankton but experiments with live krill have shown that ocean acidification can also affect physiology and development. This is probably true for much of the plankton. The combination of even small changes in temperature, pH, and UV could have a greater effect on krill, but we cannot comment on the same for zooplankton because we lack the detailed ecological information for individual species. That lack of autecological information for just some of the more abundant zooplankton species limits our ability to interpret and possibly predict the outcomes of large scale events on the Antarctic marine ecosystem, either in relation to the degree of impact or resilience. We need such research.

The ecological significance and subsequent economic consequences of the regime shift detected by CPR in the North Sea in the mid-1980s would not have been fully appreciated had it not been for the preceding 30+ years of continuous monitoring demonstrating the long-term stability of the marine ecosystem prior to the shift (Reid et al., 1998a; Beaugrand et al., 2003). The SO-CPR Survey has now been going for nearly 30 years with very good data coverage for about 25 years. While the SO-CPR is still in its infancy compared to the northern CPR Survey of nearly 90 years, each year of data enhances the value and power of the SO-CPR dataset considerably. Compared with the same early period of the northern CPR Survey, the SO-CPR has perhaps achieved much more in its first 30 years because of access to modern quantitative computer analytical methods and modelling to analyse the CPR data together with other environmental and biological data. This has allowed us, at a much earlier stage, to relate zooplankton patterns to local, oceanic and global events. The current strength of the SO-CPR Survey is the substantial biogeographic analyses already conducted in relation to both real patterns and modelled. We also have the advantage of being able to look to and follow in the footsteps of the northern CPR Survey and its achievements in detecting and monitoring significant plankton events.

There is still much more we need to do. Despite the best efforts of SO-CPR and SCAR's Expert Group supporting it, we still have significant temporal and spatial gaps in the survey, which probably can only really be rectified through substantial changes in when and where shipping operations are conducted in the Antarctic. Nonetheless, the continuation of the current SO-CPR programme, the monitoring and mapping of zooplankton, with the continued accumulation of data, will further improve our baseline information on zooplankton abundances and distributions allowing us to detect and hopefully help understand the effects climate change impacts on the ecosystem. The SO-CPR Survey is also well established to allow early detection of any invasive species. The CPR remains the most efficient and cost effective way of quickly, routinely, and consistently gathering real on site data on zooplankton over ocean basin scales.

### **Acknowledgements**

The success of the SO-CPR Survey would not have been possible without the contribution and support of researchers (including fellow collaborators, data analysts and modellers, end users), sample analysts past and present, workshop and support technicians, plus on board volunteers/expeditioners who helped deploy and maintain the CPR at sea. We are extremely grateful for all their efforts. The various home institutes of these individuals and the SO-CPR team are equally thanked for their support, funding and foresight in recognising the need for the SO-CPR Survey. We are very grateful to the crews of the various ships who have deployed the CPR over the years; *Aurora Australis* (Australia), old *Shirase* and new *Shirase* (Japan), *Kaiyo Maru* (Japan), *Hakuho Maru* (Japan), *Umitaka Maru* (Japan), *Polarstern* (Germany), *San Aotea II* (New Zealand), *Tangaroa* (New Zealand), *Yuzhmorgeologiya* (USA), *Akademik Federov* (Russia), *S.A. Agulhas II* (South Africa), *Marion Dufresne II* (France), *Ary Rongel* (Brazil), Chilean Research vessel (INACH Chile). We also acknowledge the valuable support, encouragement and endorsement of the Scientific Committee on Antarctic Research (SCAR) and its various Expert and Action Groups that have sought to use the CPR data. We offer a special thanks to the Sir Alister Hardy Foundation for Ocean Science (SAHFOS), now the Marine Biological Association of the UK based CPR Survey. Their invaluable support and advice was crucial in the early development of the SO-CPR Survey, and still very much welcomed today.

These data were sourced from the Scientific Committee on Antarctic Research (SCAR) sponsored Southern Ocean CPR (SO-CPR) Survey Database, hosted by the Australian Antarctic Data Centre (AADC). The AADC is part of the Australian Antarctic Division (AAD, a division of the Department of the Environment). The SO-CPR Survey and database are also funded, supported and populated by the Australian Government through the Department of the Environment-AAD approved AAS projects 472 and 4107, Australian Integrated Marine Observing System (IMOS), the Japanese National Institute of Polar Research (NIPR), the New Zealand National Institute of Water and Atmospheric Research (NIWA), the German Alfred Wegener Institute (AWI), the United States of America - Antarctic Marine Living Resources programme (NOAA US-AMLR), the Russian Arctic and Antarctic Research Institute (AARI), the Brazilian Programa Antartico Brasileiro (PROANTAR), the Chilean Instituto Antartico Chileno (INACH), the South African Departmental of Environmental Affairs (DEA) and the French Institut polaire francais - Paul-Emile Victor (IPEV) and Universite Pierre-et-Marie-Curie (UPMC).

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## **APPENDIX**

### **CPR workshop list**

- May 2000, NIPR  
First training workshop for NIPR personnel.
  
- March 2001, AAD  
Data analysis workshop with the AAD and NIPR personnel.
  
- November 2008, AAD  
CPR methodology and maintenance training workshop for NZ and Brazilian plankton analysts.
  
- March 2009, AAD  
Sample processing and data handling workshop for NZ plankton analyst.
  
- May 2009, SAHFOS  
Data analysis workshop (GH, KT, BH).
  
- November 2009, Rio de Janeiro  
CPR methodology and maintenance training workshop to train 14 people from Brazil, Argentina, Chile, Peru, Ecuador and Venezuela.
  
- November 2010, NIPR Tokyo  
Southern Ocean standards workshop attended by both SO-CPR and SAHFOS analysts.
  
- September 2011, SAHFOS  
Standards workshop with the SAHFOS and SO-CPR analysts.
  
- September to October 2012, AAD  
Training workshops for Brazilian, French, Korean scientists.
  
- May to June 2013, AAD  
SO-CPR and EG-CPR training and meeting.
  
- August 2013, NIWA Wellington  
Data analysis workshop
  
- March 2015, Cape Town  
Training workshop for South African personnel.
  
- December 2016, AAD  
Standards workshop
  
- November 2018, AAD  
Standards workshop