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ABSTRACT

The krill and oceanographic surveys were conducted in the Antarctic Area III-E and IV during the 2018/19 austral summer season as part of fourth dedicated sighting survey of the New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A). These surveys, which were conducted by two research vessels *Yushin Maru No. 2 (YS2)* and *Kaiyo Maru No. 7 (KY7)*, are associated with the main objective II of NEWREP-A. The krill survey was conducted along the zig-zag tracklines designed for the whale sighting survey. Acoustic data using quantitative echosounders EK80 (YS2) and EK60 (KY7) were recorded continuously for total 72 days and 7,195n.miles. Net sampling using a small ring net (YS2 and KY7) and an Issak-Kid Midwater Trawl (IKMT) (KY7) was carried out to identify species and size composition of plankton echo signs at 54 stations and 22 stations respectively. Oceanographic observations were also conducted at 144 stations using a Conductivity-Temperature-Depth profiler (CTD) and seawater sampling occurred at 16 stations. Calibration among EK80 and EK60 quantitative echosounders, and simultaneous samplings between small ring net and IKMT were also conducted. Krill and oceanographic data are currently being examined, and results will be reported in related CCAMLR working groups.

KEYWORDS: EUPHAUSIIDS; ACOUSTICS; NET SAMPLING; OCEANOGRAPHY; ANTARCTIC

INTRODUCTION

Krill is a dominant prey for many predators in the Antarctic Ocean, and the Antarctic krill (*Euphausia superba*), ice krill (*E. crystallorophias*) and bigeye krill (*Thysanoessa macrura*) are all important prey species for the Antarctic minke whale (*Balaenoptera bonaerensis*) and other baleen whale species. Since the Antarctic minke whales highly dependent on those krill species, quantitative information of krill on a long-term basis is important for understanding ecological changes involving the Antarctic minke whales.

NEWREP-A includes surveys for krill abundance and distribution, which is relevant to the main objective II. Annual krill and oceanographic surveys were planned along with dedicated whale sighting surveys, aimed to obtain a relative index of abundance among years (GOJ, 2015). Last three surveys have been reported the 2016, 2017, 2018 meeting of the International Whaling Commission Scientific Committee (IWC SC, 2016, 2017, 2018).

The krill and oceanographic survey plan for 2018/19 was presented and discussed at the 2018 meeting of IWC SC (Hakamada *et al.*, 2018, IWC, 2018). The plan was also discussed separately with some the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) scientists with experience in krill surveys. The research plan considered suggestions from those meetings and specialists to the extent possible. This paper reports the results of the 2018/19 krill and oceanographic survey conducted under the NEWREP-A.

RESEARCH METHODS

Research vessel

Two research vessels, *Yushin-Maru No.2 (YS2; 747GT; Figure 1)* owned by Kyodo Senpaku Co. Ltd., and the trawler type vessel *Kaiyo-Maru No.7 (KY7; 649GT; Figure 2)* owned by Kaiyo Engineering Co. Ltd., were used for the krill and oceanographic survey.

Research area and period

The research area was comprised between 35°E and 115°E, south of 60°S that eastern part of Area III (III-E, 35°E-70°E), western part of Area IV (IV-W, 70°E-110°E) and eastern small part of Area IV (IV-E, 110°E-115°E). There were fell in CCAMLR Division 58.4.1 (80-150°E) and 58.4.2 (30-80°E) where overlying the eastern Enderby

Abyssal Plain, Kerguelen Plateau and western Wilkes Abyssal Plain in south east of Indian ocean. All area was divided into northern and southern strata by a line 45n.miles from the ice edge. The ice edge line was defined by observation of ice edge by the vessels as well satellite information base on National Snow and Ice Data Center (USA, Colorado). Although, The Prydz Bay was divided into south side by a line 66°S in Areas IV-W.

The YS2 surveyed the Area III-E for 65 days (from 13 December 2018 to 19 February 2019), KY7 surveyed the Area IV for 49 days (from 7 December 2018 to 28 January 2019), excluding transit and logistical reasons respectively. YS2 started the survey in Area III-SE eastward from 35°E at 13 December. Otherwise, KY7 started the survey in Area IV-SE westward from 115°E at 7 December. In early December, the ice edge existed approximately 64°S in the both areas. The polynya occurred south of 66°S in the Prydz Bay in late December, then it widened to 70°S for an ice free region in early January, therefore KY7 was able to enter the Prydz Bay on 11 January 2019.

Trackline design

The trackline was designed for the main purpose of this survey that the cetacean sighting survey for abundance estimates of large whales based on the DISTANCE sampling (Buckland *et al.*, 2015). It followed the accepted guidelines by the IWC SC for the International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (IDCR/SOWER) cruises (Matsuoka *et al.*, 2003, Mogue *et al.*, 2019; Figure 3). The trackline consisted of north and south zigzag course changing direction at each 5°00' longitudinal degree intervals in the northern stratum and at 2°30' in the southern stratum. The trackline of the Prydz Bay was set zigzag changed direction at 2°30' longitudinal degree intervals too. A randomized start point was determined based on the IWC SC guidelines (IWC, 2012).

Echosounder

The YS2 was equipped with a Quantitative Echosounder (EK80; Simrad, Norway) that was operated with frequencies at 38 kHz, 120 kHz and 200 kHz. The transducers were hull-mounted at a depth of 4.3 m below the sea surface. The KY7 was equipped with a Quantitative Echosounder (EK60; Simrad, Norway) that was operated with frequencies at 38 kHz, 120 kHz and 200 kHz. The transducers were hull-mounted at a depth of 4.7m below the sea surface. Acoustic data were recorded continuously while vessel steamed on the predetermined tracklines at a speed of 11.5knots and 11.0knots by YS2 and KY7 respectively. Maximum data recording depth was set at 500m by both vessels. Standard calibration (Demer *et al.*, 2015) of the EK80 and the EK60 was conducted respectively in the vicinity of Japan and also in the research area to determine the likely effective acoustic sampling range and a potential for detecting krill for multiple frequencies over the required survey depth. Following recommendations from specialists, calibration between EK80 and EK60 was conducted when YS2 and KY7 passed nearby around the crossing tracklines. The results of calibration between EK80 and EK60 will be reported in related CCAMLR working group and/or the mid-term review of NEWREP-A.

Small ring net sampling

The YS2 was equipped with a 1.5mm mesh size small ring net designed by Rigo-Sha Co., Ltd. Japan for vertical plankton hauls. The main purpose of this net sampling was not collecting quantitative information (*e.g.* number of individuals and length frequency distribution) but rather collecting qualitative information (*e.g.* species occurred in the echo signs) because the net is too small for collection representative sample of krill. The net had a 1.0m mouth diameter and a 2.4m length. During the sampling, the vessel stopped the engine so that the net could be hauled vertically. In principle net sampling conducted at the area, where is confirmed the echo sign.

The KY7 was also equipped with small ring net almost same as used in YS2, slightly different with a 0.5mm mesh size. The purpose of using this net in KY7 was to check efficiency of the small ring net by comparing the samples with those obtained by IKMT. If the small ring net samples collected a sufficient amount of samples to examine length frequency of krill and if the composition of plankton samples was similar to those collected by IKMT, the result of the small ring net sampling are somewhat useful. The small ring net hauls were carried out the same as in YS2, but were only conducted where IKMT collected swarms of krill.

Data Storage Tag Centi-ex (DST, Star Oddi CO., LTD, Iceland) were put on the mouth of the small ring net for recording temperature and depth at one second interval. The data of DST were moved to pc after salvaged. The lighting system LED used for attracting krill in the 2015/16NEWREP-A was not used following recommendation by CCAMLR WG-SAM (CCAMLR, 2016, Table 1).

The target depth of net sampling was set based on depth of the echo sign but the maximum depth was 200m. The depth of the net mouth was checked by angle of wire during hauling, then the accurate depth of the mouth was confirmed by DST record in the laboratory. Hauling speed of the net was about 1.0m/s that depended on sea state. All sampling was conducted during day time.

Issak-Kid Midwater Trawl (IKMT) sampling

The *KY7* was equipped with an IKMT designed by Nippon-Kaiyo Co., Ltd. Japan. The purpose of IKMT sampling was collecting quantitative information for krill (*e.g.* determination of the species occurring in echo signs and representative krill length frequencies). The IKMT was 3.66m in mouth diameter and 18.43m (ten feet) length. During the sampling, the *KY7* steamed about 2.0knots. Similar to small ring net, DST was installed at the mouth of the IKMT to record actual depth of the net.

The target depth of IKMT sampling was set based on depth of echo sign but the maximum depth was 200m. The depth of the mouth was monitored at the bridge of the *KY7* by PI sensor. Towing speed of IKMT was 1.0m/s. The 0.5mm mesh size was used for both small ring net and IKMT for the comparison of the two types of nets. All sampling is conducted during day time.

Krill sample treatment

Simple krill length measurements (AT) were conducted on board of the vessels. Measurement point of AT is from the front of the eye to the tip of the telson, the thin, tapered triangular plate at the end of the abdomen to the nearest 1mm (CCAMLR, 2011). Plankton samples were kept in bottles with 10% formalin and/or frozen at -20°C for further analysis in the laboratory.

Oceanographic observation

Hydraulic pressure, temperature, salinity, chlorophyll-*a* and dissolved oxygen were recorded from sea surface to 500m depth using Conductivity-Temperature-Depth profiler (CTD) SBE 19 plus V2 SeaCAT (Sea-Bird Electronics, USA) by *YS2*, SBE 19 plus SeaCAT (Sea-Bird Electronics, USA) by *KY7*. In principle, CTD casting was planned to be conducted at each latitude on tracklines with intervals of approximately 70n.miles. When seawater sampling was carried out, targeting depth of CTD was set to 1,000m. Seawater sampling was planned to be distributed evenly across survey area. The data of CTD was transferred to a pc at the laboratory.

Seawater sampling was carried out for the calibration of CTD sensors. Niskin water sampling bottle Model-1010 1.2L (General Oceanics, Inc., USA) by *YS2* and Model-1010 1.7L (General Oceanics, Inc., USA) by *KY7* were dropped to take seawater at depth from 0m to 200m every 20m. Depth information of sampling bottle was based on the angle of the wire during operating, accurate depth of sampling bottle was confirmed by using DST. The water for salinity calibration of CTD was kept in a 250ml clarity seawater bottle (WOCE type 5419-C, Rigo-Sha, Japan) and was stored at about 4°C. The sampled water for chlorophyll-*a* calibration of CTD, was filtrated with 100ml bottles by filter paper (233303 GF/F 2.5cm, Whatman, UK). The filter papers were kept in 8 ml centrifugal tubes (60.452, Sarstedt, Germany) filled with dimethyl formamide and were stored in a freezer at about -20°C (Saito, 2007).

Principle of priorities and time allocation for each survey components

The dedicated sighting survey was the highest priority component of this cruise, however we tried to conduct krill and oceanographic surveys to the maximum extent. In principle, almost all time was allocated to the sighting survey to secure sufficient sighting survey effort in the survey area. About an or two hour per day was allocated for net sampling and CTD.

RESULTS AND DISCUSSION

Echosounder

Calibrations of echosounders were made on 30 October 2018 by *YS2* and on 7 November 2018 by *KY7* before departure for the Antarctic. During the calibrations, the vessel's engines were stopped. The depth of anchoring for calibration was 37m at *YS2* and 48m at *KY7* in the vicinity of Japan. Calibrations in the survey area were made on 13 December 2018 by *YS2* and on 6 December 2018 by *KY7*. In the Antarctic, anchoring at that depth could not be achieved. During the calibrations, the vessel's engines were stopped and the drifting speed was approximate 0.7knots.

The comparison quantitative echosounders between EK80 of *YS2* and EK60 of *KY7* was conducted on 12 November 2018 in Japanese coastal sea. This comparison wasn't got sufficient echogram from Antarctic krill in Antarctic at 2016/17 and 2017/18 NEWREP-A (Wada *et al.*, 2017, 2018). Then it was recorded reflect of shallow sea bottom on this survey. We followed basic concept of the methodology by Simmonds *et al.*, (2005) and used reference as Watkins (2016), Choi (2017) and Wang (2018). The echosounder data were recorded by *YS2* and *KY7* moving in formation with one in the lead and the another about 400m astern, far enough to the side to be clear of the leader vessel's wake. The two vessels took the lead in turns and exchanged positions at the end of each transect. Both research vessels recorded quantitative echosounders data while shifting the leaders every 30 minutes four

times for two hours. As a result, calibrations between EK60 and EK80 were recorded shallow over 200m seabed backscattering constantly by all three frequency, 38kHz, 120kHz and 200kHz. The data will be analysis, then be offset echosounder data of past NEWREP-A.

Table 2 shows a summary of the total effort spent on the quantitative echosounder. The quantitative echosounder survey was conducted for a total of 7,195n.miles along the tracklines (3,365n.miles in Area III-E, 2,267n.miles in Area IV-W, 509n.miles in Prydz Bay and 1,055n.miles in Area IV-E). This distance of effort was almost predetermined trackline without confirming whales for sighting survey.

Net sampling

Number and horizontal location of net sampling stations

The small ring net sampling was conducted at a total of 54 stations by YS2 and KY7 (45 stations in Area III-E, six stations in Area IV-W, one station in the Prydz Bay and two stations in Area IV-E, Table 3 and Figure 4). Because of weather, sea ice conditions or survey priority reasons, some net sampling stations were skipped. The calibration of flow meter was conducted 10 December 2018 by YS2 and 6 December 2018 and 30 January 2019 by KY7 respectively.

IKMT sampling was conducted at a total of 22 stations by KY7 (ten stations in Area IV-W, seven stations in the Prydz Bay and five stations in Area IV-E, Table 3 and Figure 4). Logistical considerations were also taken into account to decide whether to proceed with net sampling such as the sea state, sea ice as well as other survey priorities.

Sampling contents

A total of eleven species, including three euphausiid species (Antarctic krill, ice krill and bigeye krill) and fish, were identified in the 73 net sampling contents. Several other species, *Hydromedusae*, *Siphonophorae*, *Polychaeta*, *Pteropoda*, *Copepoda*, *Amphipoda* and *Chaetognatha* were confirmed. There were no contents at three net sampling station.

Horizontal and vertical distribution of krill species

Tables 4 and 5 show the summary of frequencies of occurrence of krill species and *Copepoda* sampled at the small ring net sampling and IKMT sampling stations, and figures 5 and 6 show the horizontal distribution by krill species. Antarctic krill was sampled at 48 stations in entire of survey area (Figure 5). Distribution of surface water temperature was in the range of -1.7°C to 1.9°C. They were sampled at depths of 19m to 146m, depth of sea bottom range was 178m to 5,240m. The range of body length was from 12mm to 59mm. The incubating Antarctic krill was sampled at two stations in Area III-SE. Ice krill was sampled at a station in the Prydz Bay (Figure 6). Distribution of surface water temperature was 1.6°C. They were sampled at depths of 130m, depth of sea bottom was 670m. Bigeye krill were sampled at ten stations (Figure 6). Distribution of surface water temperature was in the range of -1.4°C to 1.0°C. They were sampled at depths of 35m to 146m, depth of sea bottom range was 2,100 to 5,240m. The range of body length was from 10mm to 25mm.

Comparison of small ring net and IKMT sampling

The small ring net and IKMT sampling was conducted with careful confirmation of krill swarm location by the monitor of quantitative echosounder and pilot vessel sensitively. Simultaneous samplings with the small ring net and IKMT were conducted at seven stations by KY7. At three cases, Antarctic krill was sampled by both small ring net and IKMT. However, for other four cases, the results from both nets were not consistent. In the 2016/17 and 2017/18 NEWREP-A survey totally seven cases were occurred, two cases were consistent for both net (Wada *et al.*, 2017, 2018). These results indicate that it is difficult to collect representative krill samples by the small ring net. However, the small ring net can contribute to obtaining qualitative information on the distribution of krill species and it require less time for conducting.

Oceanographic observation

The Oceanographic observation by CTD was conducted at 144 stations by YS2 and KY7 (69 stations in Area III-E, 42 stations in Area IV-W, eleven station in the Prydz Bay and 22 stations in Area IV-E, Table 6 and Figure 7). Before the cruise departure, the both CTD had been calibrated by Sea-Bird Electronics in the USA. The stations were separated approximate 70n.miles for covering each latitude on the trackline. In the previous JARPAII surveys, the average distance between stations was about 60n.miles (Watanabe *et al.*, 2014). The seawater sampling was conducted at 16 stations at the same locality where CTD observations were taken. These stations were distributed evenly across the survey area at a distance of approximately 120n.miles in radius. Seven stations in Area III-E and five stations in Area IV-W, two stations in the Prydz Bay and two stations in Area IV-E were sampled (Table 6 and Figure 7). A total of 176 seawater samples were taken then kept in clear bottles for salinity calibration and

filter paper filled with dimethyl formamide for chlorophyll-*a* calibration. Examination of seawater samples will allow calibrating CTD data, then identifying structure of ocean current likely Upper Circumpolar Deep Water, Lower Circumpolar Deep Water, and Shelf Water based on CTD data.

Response to recommendations of CCAMLR specialists and future works

Response to recommendations of CCAMLR specialists is summarised in Table 1. These issues included in Table 1 will be reported to the CCAMLR workshop. The preliminary results of abundance estimation of krill using the data sets of NEWREP-A and based on the standard abundance estimation method established by CCAMLR will be reported in the related working group of CCAMLR in the future.

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Table 1. Response to some recommendations of 2016 CCAMLR WG-SAM

Item	Paragraph	Note	Current state
Note for the survey	2.8	The sampling for krill was not likely to be adequate for validating the composition of acoustic marks obtained by the echosounders.	The first priority is sighting for whale in NEWREP-A. Accordingly, the trackline was considered for sighting for whale.
	2.8	The size of the net is likely to be too small and is not being used to target the acoustic marks, but to identify species existing at the stations.	At 2016/17, 2017/18 and 2018/19 NEWREP-A, IKMT was used by KY7. The IKMT has 3.66 m mouth and 18.43 m lengths. Unfortunately, it is difficult to using bigger net in YS2 by logistic reason.
	2.8	The use of a light on the net may lead to biased estimation of what organisms are being detected by the acoustics.	Since 2016/17 NEWREP-A, we didn't use LED light on the net.
	2.8	Insufficient krill are being obtained to estimate the length frequency of krill detected by the acoustics.	The first priority is sighting for whale in NEWREP-A. However, sampling effort has been increased as much as possible (2016/17 NEWREP-A: 13 IKMT stations and 32 small ring net stations, 2017/18 NEWREP-A: 11 IKMT stations and 47 small ring net stations, 2018/19 NEWREP-A: 22 IKMT stations and 54 small ring net stations).
	2.9	No clear description of the questions being addressed. (The sampling is insufficient to determine the relationship of whales to the densities of krill or physical ocean habitat.)	The trackline was considered for sighting for whale however net sampling and oceanographic were evenly distributed in the research area.
	2.9	Need random sampling across the area to better estimate and increase in the number of sampling locations.	At 2016/17 NEWREP-A, net samplings were conducted at least once a day. At 2017/18 and 2018/19 NEWREP-A, net samplings were conducted after confirming echo sign.
	2.9	Power analyses is important to determine an appropriate number of stations.	The analyses are preliminary stages. The result will be reported at related CAMMLR working group.

Table 2. Summary of quantitative echosounder survey in 2018/19 NEWREP-A.

Area	YS2		KY7		Total	
	days	n.miles	days	n.miles	days	n.miles
III-NE	39	2,175	-	-	39	2,175
III-SE	32	1,191	-	-	32	1,191
IV-NW	-	-	15	1,254	15	1,254
IV-SW	-	-	17	1,013	17	1,013
Prydz Bay	-	-	6	509	6	509
IV-NE	-	-	8	587	8	587
IV-SE	-	-	9	468	9	468
Total	65*	3,365	49*	3,830	72*	7,195

(*The survey was conducted some areas at same day)

Table 3. Number of net sampling stations in 2018/19 NEWREP-A.

Area	Small ring net			IKMT
	YS2	KY7	Total	KY7
III-NE	17	-	17	-
III-SE	28	-	28	-
IV-NW	-	0	0	3
IV-SW	-	6	6	7
Prydz Bay	-	1	1	7
IV-NE	-	2	2	1
IV-SE	-	0	0	4
Total	45	9	54	22

Table 4. Summary of contents of small ring net sampling stations and percent in 2018/19 NEWREP-A.

Area	<i>Euphausia superba</i>		<i>Euphausia crystallophias</i>		<i>Thysanoessa macrura</i>		<i>Euphausiids</i>		<i>Copepoda</i>		Other Zooplankton	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
III-NE	7	41%	0	0%	6	35%	0	0%	12	71%	16	94%
III-SE	21	75%	0	0%	3	11%	0	0%	18	64%	20	71%
IV-NW	-	-	-	-	-	-	-	-	-	-	-	-
IV-SW	4	67%	0	0%	0	0%	0	0%	0	0%	1	17%
Prydz Bay	0	0%	0	0%	0	0%	0	0%	0	0%	1	100%
IV-NE	0	0%	0	0%	0	0%	0	0%	1	50%	1	50%
IV-SE	-	-	-	-	-	-	-	-	-	-	-	-
Total	32	59%	0	0%	9	17%	0	0%	31	57%	39	72%

Table 5. Summary of contents of IKMT stations and percent in 2018/19 NEWREP-A.

(The IKMT was not conducted in Area III-E)

Area	<i>Euphausia superba</i>		<i>Euphausia crystallophias</i>		<i>Thysanoessa macrura</i>		<i>Euphausiids</i>		<i>Copepoda</i>		Other Zooplankton		Fish	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
IV-NW	2	67%	0	0%	1	33%	0	0%	0	0%	0	0%	1	33%
IV-SW	6	86%	0	0%	0	0%	1	14%	0	0%	2	29%	0	0%
Prydz Bay	6	86%	1	14%	0	0%	0	0%	1	14%	1	14%	1	14%
IV-NE	0	0%	0	0%	0	0%	0	0%	1	100%	1	100%	0	0%
IV-SE	2	50%	0	0%	0	0%	3	75%	1	25%	2	50%	0	0%
Total	16	73%	1	5%	1	5%	4	18%	3	14%	6	27%	2	9%

Table 6. Number of oceanographic stations in 2018/19 NEWREP-A.

Area	CTD			Seawater Sampling			DST		
	YS2	KY7	Total	YS2	KY7	Total	YS2	KY7	Total
III-NE	42	-	42	5	-	5	17	-	17
III-SE	27	-	27	2	-	2	28	-	28
IV-NW	-	19	19	-	3	3	-	3	3
IV-SW	-	23	23	-	2	2	-	14	14
Prydz Bay	-	11	11	-	2	2	-	7	7
IV-NE	-	8	8	-	1	1	-	3	3
IV-SE	-	14	14	-	1	1	-	4	4
Total	69	75	144	7	9	16	45	31	76



Figure 1. Research vessel *Yushin-maru No.2 (YS2)*.



Figure 2. Research vessel *Kaiyo-maru No.7 (KY7)*.

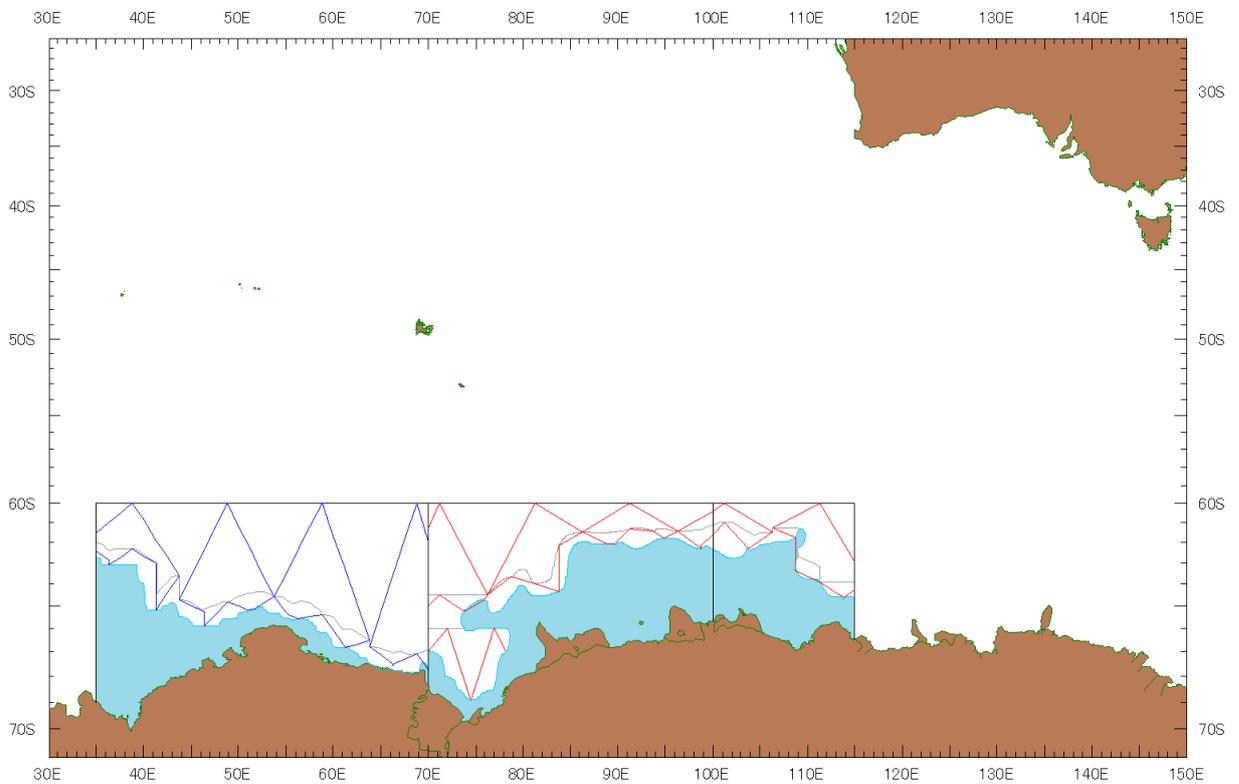


Figure 3. The tracklines made by two research vessels in 2018/19 NEWREP-A.
 (Blue line: surveyed trackline of YS2, Red line: surveyed trackline of KY7, Gray line: boundary between northern and southern research area, Turquoise line: ice edge, Brown: Antarctic continent)

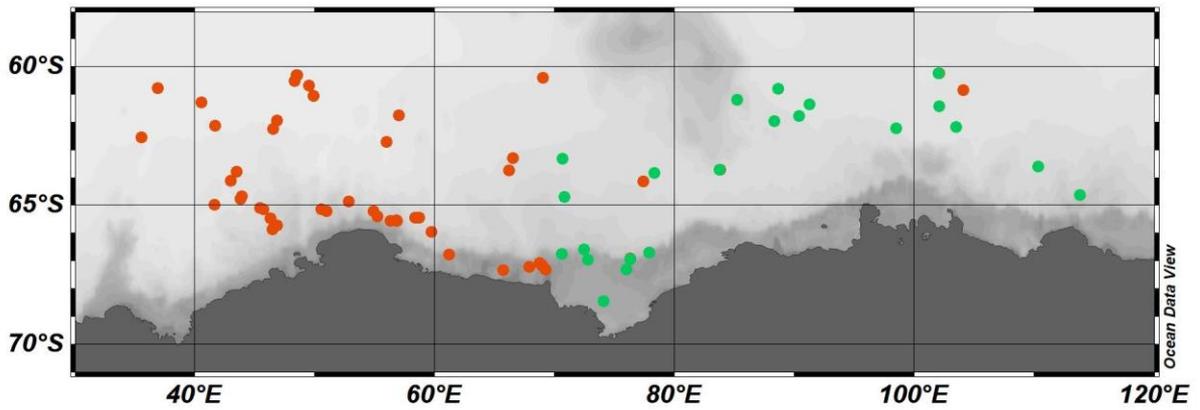


Figure 4. Sampling stations of small ring net and IKMT in 2018/19 NEWREP-A.
 (Red circle: small ring net, Green circle: IKMT)

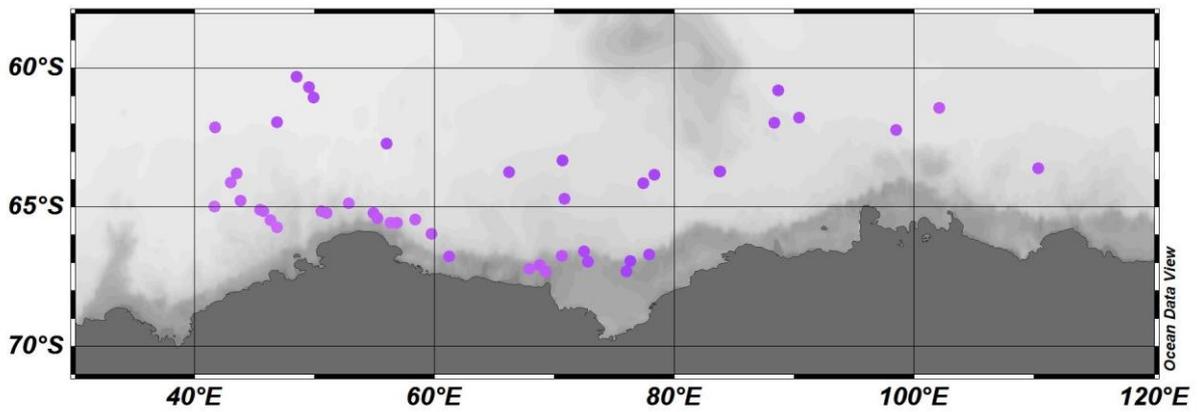


Figure 5. Sampling positions of Antarctic krill in 2018/19 NEWREP-A.

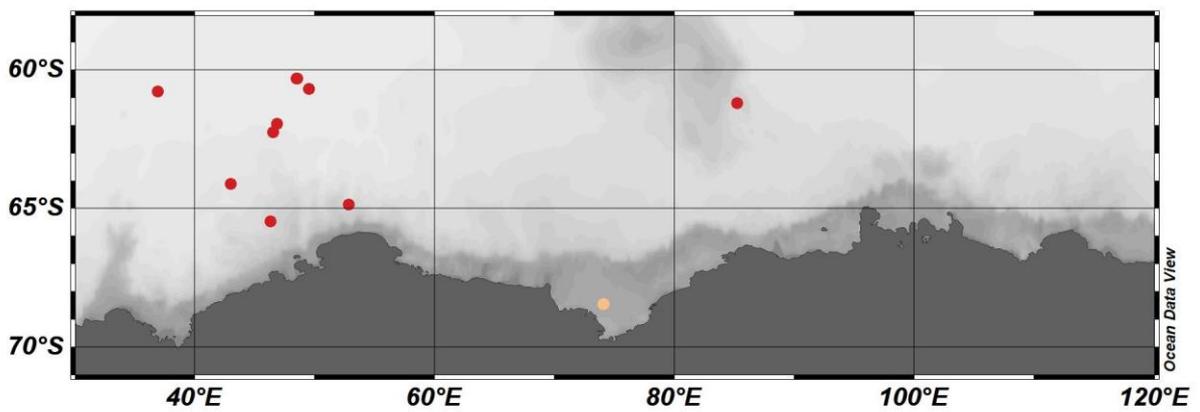


Figure 6. Sampling positions of ice krill and bigeye krill in 2018/19 NEWREP-A.
 (Orange circle: ice krill, Red circle: bigeye krill)

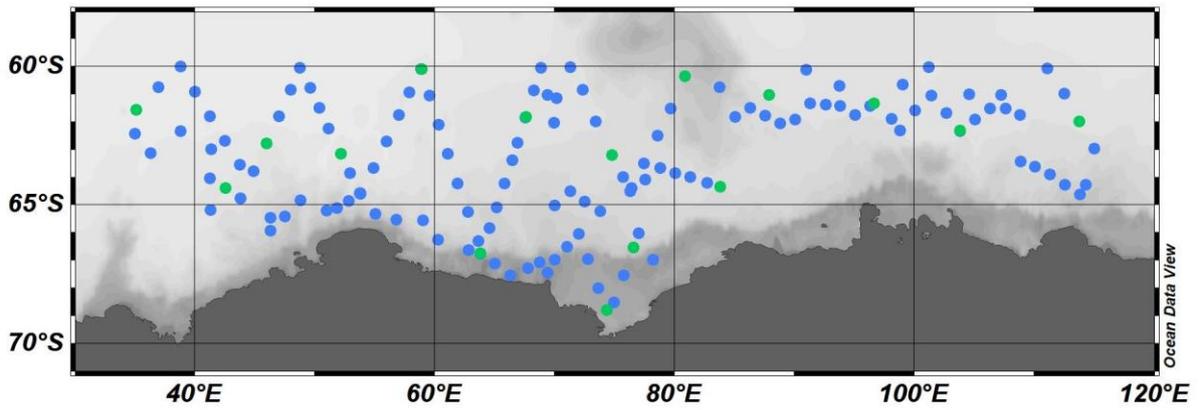


Figure 7. The stations of CTD and seawater sampling in 2018/19 NEWREP-A.
(Blue circle: CTD, Green circle: seawater sampling)

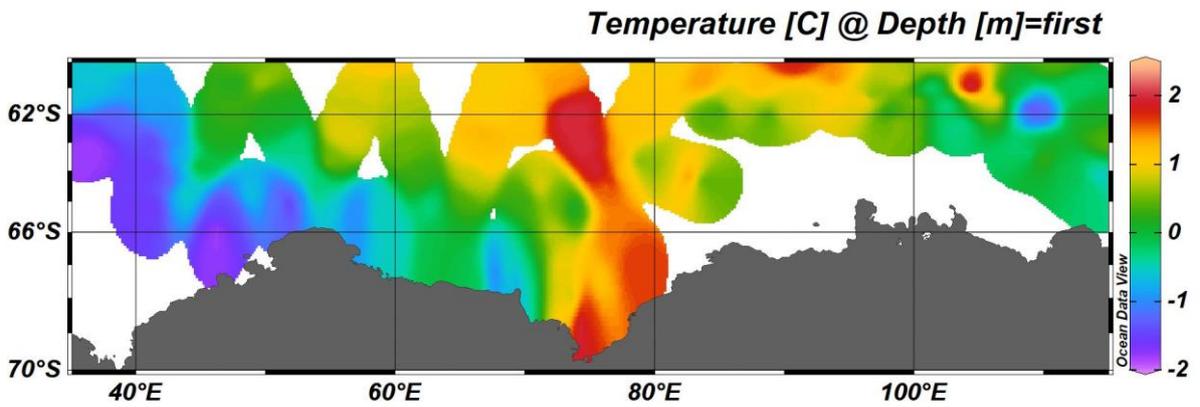


Figure 8. The surface temperature of research area in 2018/19 NEWREP-A.