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ABSTRACT

Krill and oceanographic surveys were conducted in the Antarctic Area V-W during the 2016/17 austral summer season as part of second New Scientific Whale Research Program in the Antarctic Ocean (NEWREP-A) dedicated sighting survey. These surveys, which were conducted by two research vessels *Yushin Maru No. 3* (*YS3*) 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 procedure. Acoustic data using quantitative echosounders EK80 (*YS3*) and EK60 (*KY7*) were recorded continuously for 33 days. Net samplings using a small ring net (*YS3* and *KY7*) and an Issak-Kid Midwater Trawl (IKMT) (*KY7*) were carried out to identify species and size compositions of plankton echo signs at 32 stations and 13 stations, respectively. Oceanographic observations were also conducted at 53 stations using a Conductivity-Temperature-Depth profiler (CTD) and water sampling occurred at six stations. Calibration among EK80 and EK60 echosounders, and between small ring and IKMT were conducted. Krill and oceanographic data are currently being examined, and results will be considered for the planning of the next survey in the 2017/18 austral summer season.

KEYWORDS: KRILL; ACOUSTICS; NET SAMPLING; OCEANOGRAPHY; SCIENTIFIC PERMITS; ANTARCTIC;

INTRODUCTION

Krill is a dominant prey for many predators in the Antarctic, 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 *Balaenoptela bonaerensis*. Since the Antarctic minke whales highly depend on those krill species, quantitative information of krill in the long-term basis is important to understand ecological changes involving the Antarctic minke whales.

NEWREP-A includes surveys for krill abundance and distribution, which is relevant to the main objective II, and two kinds of krill surveys were planed (Government of Japan, 2015a): (i) annual krill and oceanographic surveys along with a dedicated whale sighting survey, aimed to obtain a relative index of abundance among years and (ii) a dedicated krill survey that follows Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) standard methodology with parallel tracklines to obtain more accurate index of abundance (CCAMLR-type dedicated krill survey).

The krill and oceanographic survey plan for 2016/17 was presented and discussed at the 2016 meeting of the International Whaling Commission Scientific Committee (IWC SC) (Hakamada *et al*., 2016, IWC, 2016), and to the 2016 meetings of the CCAMLR WG-EMM and CCAMLR WG-SAM (Matsuoka et al., 2016, CCAMLR, 2016). The plan was also discussed separately with some CCAMLR scientists with experience in krill surveys. The final plan considered suggestions from those meetings and specialists to the extent possible.

This paper reports the results of the 2016/17 krill and oceanographic survey conducted under the NEWREP-A.

RESEARCH METHODS

Research vessel

Two research vessels, *Yushin-Maru No.3* (*YS3*; 742GT) owned by Kyodo Senpaku Co. Ltd., and the trawler type vessel *Kaiyo-Maru No.7* (*KY7*; 649GT) owned by Kaiyo Engineering Co. Ltd., were used for the krill and oceanographic survey.

Research area and period

The research area was comprised between 130°E and 165°E, south of 60°S (part of Area V-W). The research area was divided into northern and southern strata by a line 45n.miles from the ice-edge (line defined by observation of ice edge from the vessels as well satellite information).

The *YS3* and *KY7* surveyed the research area for 33 days (13 December 2016 to 14 January 2017) and 27 days (from 13 December 2016 to 8 January 2017), respectively.

Trackline design and timing

The trackline was designed for the main purpose of this survey (cetacean sighting survey for abundance estimates of large whales) based on the DISTANCE sampling (Buckland *et al*., 2015), and 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, Isoda *et al*., 2016, Isoda *et al*., 2017; Figure 1). The trackline consisted of a zigzag course changing direction at each 5°00' longitudinal degree intervals in the northern stratum and at 2°30' in the southern stratum. A randomized start point was determined based on the IWC SC guidelines (IWC, 2012).

Echosounder

The *YS3* was equipped with a Quantitative Echosounder (EK80; Simrad, Norway). EK80 was operated with frequencies at 38kHz, 120kHz and 200kHz. The transducers were hull-mounted at a depth of 4.3m below the sea surface. Maximum data recording depth was set at 500m. Acoustic data were recorded continuously while *YS3* steamed on the predetermined tracklines at a speed of 11.5knots. Standard calibration of the EK80 was made using a standard methods (Demer *et al*., 2015) 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.

The *KY7* was equipped with a Quantitative Echosounder (EK60; Simrad, Norway). EK60 was operated with frequencies at 38kHz, 120kHz and 200kHz. The transducers were hull-mounted at a depth of 4.7m below the sea surface. Maximum data recording depth was set at 500m. Acoustic data were recorded continuously while *KY7* steamed on the predetermined tracklines at a speed of 11.0knots. Standard calibration of the EK60 was conducted in the vicinity of Japan and also in the research area.

Following recommendations from specialists, calibration between EK80 and EK60 echosounders was conducted when *YS3* and *KY7* passed nearby around the crossing tracklines of northern and southern strata.

Small ring net sampling

The *YS3* was equipped with 1.5mm mesh size small ring net designed by Nippon-Kaiyo 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 was 1.0m mouth diameter and 3.0m length. During the sampling, the vessel stopped the engine so that the net could be hauled vertically. In principle net sampling was planned to be conducted once a day according to the echo sign and progress of the sighting survey.

The *KY7* was also equipped with the same small ring net as used in *YS3*. 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 sufficient amount of samples to examine length frequency of krill and the composition of plankton samples similar to those collected by IKMT, the result of the small ring net are somewhat useful. The small ring net hauls were carried out as same as in *YS3*, but only conducted where IKMT collected swarm of krill.

Data Storage Tag Centi-ex (DST, Star Oddi CO., LTD, Iceland) was put the level of the mouth of the small ring nets 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 2016/17NEWREP-A (Wada, 2016) was not used following recommendation by CCAMLR WG-SAM (CCAMLR, 2016).

The target depth of net sampling was set based on depth of the echo sign but the maximum depth was limited to 200m. The depth of the net mouth was checked by wire during hauling, then the accurate depth of the mouth was confirmed by DST record in the laboratory. Hauling speed of the net was 1.0m/s.

Issak-Kid Midwater Trawl (IKMT) sampling

The *KY7* was also 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. In principle IKMT sampling was planned to be conducted once a day. In average it took approximately 11minutes per haul (without including time for setting). DST was installed at the mouth of the IKMT to record actual depth of the net.

The target depth of net 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. 0.5mm mesh size was used for both small ring net and IKMT for the comparison of the two types of nets.

Krill sample treatment
Simple krill length measurements (AT) were conducted on board of the research 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 (CCAMLR, 2011). Plankton samples were kept in bottles with 10% formalin or frozen at -20℃ for further analysis in the laboratory.

Oceanographic observation
Hydraulic pressure, temperature, salinity and dissolved oxygen were recorded from sea surface to 500m depth using Conductivity-Temperature-Depth profiler (CTD) SBE 19 plus SeaCAT (Sea-Bird Electronics, USA). In addition to the above data, chlorophyll was also recorded using SBE 19 plus V2 SeaCAT (Sea-Bird Electronics, USA) by *YS3*. In principle, CTD casting was planned to be conducted once a day. When seawater sampling was carried out, targeting depth of CTD was set to 1,000m depth. The data of CTD was transferred to a pc at 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 *YS3* and Model-1010 1.7L (General Oceanics, Inc., USA) by *KY7* were dropped to take seawater at depth from 0 to 200m by every 20m. Depth information of sampling bottle was based on the angle of wire while operation, accurate depth of sampling bottle inform from DST at laboratory. The water was kept in a 250mL clarity seawater bottle (WOCE type 5419-C, Rigosha, Japan) for salinity calibration of CTD by *YS3* and *KY7* and was stored at about 4°C. For chlorophyll calibration of CTD in *YS3*, sampled water was also stored in 100mL bottles after paper filtering (233303 GF/F 2.5cm, Whatman, UK). The filter papers were kept in 8mL centrifugal tubes (60.452, Sarstedt, Germany) filled with dimethylformamide and were stored in a freezer at about -20°C (Saito, 2007).

Principle of priorities and time allocation for each survey components
Dedicated sighting survey was the most prioritised component of this cruise for both vessels, however we tried to conduct krill and oceanographic surveys to the utmost extent. Other priorities of the survey components differed among the vessels. For *YS3*, the survey priorities were as follows (from high to low); photo-identification, biopsy sampling, echosounder, small ring net and oceanographic. For *KY7*, the survey priorities were IKMT sampling, echosounder, oceanographic, satellite tracking, photo-identification, biopsy sampling and small ring net. In principle, almost all time was allocated to the sighting survey to secure sufficient sighting survey effort in the survey area. Less than an hour per day at once was allocated for net sampling and CTD.

RESULTS

Echosounder
Calibrations of echosounders were made on 14 November 2016 (*YS3*) and on 11 November 2016 (*KY7*) before departure for the Antarctic. Calibrations in the research area were made on 24 December 2016 (*YS3*) and on 21 December 2016 (*KY7*). During the calibrations, the vessel's engines were stopped. The depth of anchoring for calibration was 35m (*YS3*) and 52m (*KY7*) in the vicinity of Japan. In the Antarctic that depth could not be anchored, the drifting speed was 0.75knots (*YS3*) and 0.90knots (*KY7*).

The comparison between vessel's echosounders was carried out on 20 December 2016 at night time. We followed basic concept of the methodology by Simmonds *et al*., (2005), however the vessels kept same direction during the experiment for safety reason. The echosounder data were recorded by *YS3* and *KY7* moving in formation with one in the lead and the other 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 echosounders data while shifting the leaders four times for 2 hours 30 minutes.

Table 1 shows a summary of the total effort spent on the quantitative echosounder. Figure 2 shows the actual track of the echosounder survey where data were recorded continuously. The parts off effort in Figure 2 were due to time spent out of track lines confirming whale species under the whale sighting survey. The echosounder survey was conducted for a total of 3,221.1n.miles along the tracklines (33 days and 2,196.5n.miles in the case of *YS3*; 20 days and 1,024.6n.miles in the case of *KY7*).

Net sampling survey

Number and geographical location of net sampling stations

The small ring net sampling was conducted at a total of 32 stations, i.e. 29 stations by *YS3* and three stations by *KY7* (Table 2, Figure 3). Because of weather, sea ice conditions or survey priorities reasons, some planned net sampling stations were skipped or shifted.

IKMT sampling was conducted at a total of 13 stations by *KY7* (Table 2). Figure 3 shows the 13 stations on the tracklines. Other 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 research priorities.

Distribution of krill species

Table 3 shows the frequencies of occurrence of krill and copepod species sampled at the small ring net sampling stations, and Figure 4, 5, 6 shows the geographical distribution by species on the tracklines. Antarctic krill was sampled at 11 stations, one in the stratum V-NW and ten in the stratum V-SW. Surface temperature in the stations where Antarctic krill was found ranged from -1.7 to 1.5°C, and the depth from 22 to 104m. The range of body length (measured on board) was 17-52mm. Bigeye krill was sampled at 13 stations, six in the stratum V-NW and seven in the stratum V-SW. Surface temperature in the stations where bigeye krill was found ranged from -1.7 to 1.8°C, and the depth from 22 to 109m. The range of body length was 12-28mm.

Table 4 shows the frequencies of occurrence of krill and copepod species sampled at the IKMT sampling stations, and Figure 4, 5, 6 show the geographical distribution by species on the tracklines. Antarctic krill was sampled at five stations, two in the stratum V-NW and three in the stratum V-SW. Surface temperature in the stations where Antarctic krill was found ranged from -0.6 to 3.1°C, and the depth ranged from 30 to 200m. Bigeye krill was sampled at three stations, two in the stratum V-NW and one in the stratum V-SW. Surface temperature in the stations where bigeye krill was found ranged from 1.3 to 2.5°C, and the depth ranged from 30 to 200m.

Evaluation of small ring net sampling

Comparison between the small ring and IKMT was carried out at three stations by the *KY7*. At two stations when the IKMT sampled 8.4kg and 4.9kg Antarctic krill, only amphipoda and polychaete were sampled by the small ring net. Overall no good match between both nets was found. IKMT sampling was conducted with careful confirmation of krill swarm location by the monitor of echosounder. The advantage of IKMT is adjustment of horizontal location of the krill swarm while it is difficult to stabilise the vessel just above the krill swarm when the vertical small ring net is hauling. These preliminary results suggest that it is difficult to collect representative krill samples by the small ring net sampling.

Specialists had suggested that a total of 100 tows will be required for the comparison between different types of nets to increase the power of the analysis. However the sampling efficiency of the two types of nets is obvious, therefore the use of the small net sampling in vessel where an IKMT is available should be limited in future. For vessels where IKMT is not available, small ring nets could be used for occasional target-hauling by echo signs or krill patches.

Oceanographic observation

The Oceanographic survey by CTD was conducted at a total of 53 stations (32 stations with *YS3* and 21 stations with *KY7*: Table 5). Before departure the cruise, CTD had been calibrated by Sea-Bird Electronics. Figure 7 shows the 53 stations on the echosounder tracklines. These stations were separated between 40 and 80n.miles. During the previous JARPAII surveys the average distance among stations was about 60n.miles (Watanabe *et al.*, 2014). In a total of 11 cases, CTD castings were conducted at the same locality of the small ring net sampling (ten stations by *YS3* and one station by *KY7*). In a total of two cases CTD castings were conducted at the same locality of the IKMT sampling by *KY7*. The CTD casting took in average 29 minutes per cast without including the time for set up. These data are under analysis.

The seawater sampling was conducted at six stations, three stations each by *YS3* and *KY7* (Table 5). Figure 8 also shows the six stations on the tracklines. Samples are being examined.

ACKNOWLEDGEMENT

We would like to thank the Government of Japan for providing support for this survey, and all the crew of *YS3* and *KY7* for their effort at the field. We thank colleagues from the Institute of Cetacean Research, National Research Institute of Fisheries Engineering, National Research Institute of Far Seas Fisheries, Tohoku National Fisheries Research Institute, CCAMLR specialists and Hiroto Murase for useful suggestions and logistical support.

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Table 1. Summary of echosounder survey in 2016/17 NEWREP-A.

Stratum	*YS3*		*KY7*		Total
	days	n.miles	days	n.miles	n.miles
V-NW	19	1,289.5	11	608.6	1,898.1
V-SW	18	907.0	11	416.0	1,323.0
Total	33*	2,196.5	20*	1,024.6	3,221.1

(*The survey was conducted V-NW and V-SW in same day)

Table 2. Number of net sampling stations in 2016/17 NEWREP-A.

Stratum	*YS3*	*KY7*		Total	
	Small ring net	Small ring net	IKMT	Small ring net	IKMT
V-NW	16	2	8	18	8
V-SW	13	1	5	14	5
Total	29	3	13	32	13

Table 3. Summary of small ring net sampling in 2016/17 NEWREP-A.

Stratum	Number of stations	*Euphausia superba*		*Thysanoessa macrura*		Copepod		Other	
V-NW	18	1	6%	6	33%	13	72%	17	94%
V-SW	14	10	71%	7	50%	4	29%	13	93%
Total	32	11	34%	13	41%	17	53%	30	94%

Table 4. Summary of IKMT in 2016/17 NEWREP-A.

Stratum	Number of stations	*Euphausia superba*		*Thysanoessa macrura*		Copepoda		Other	
V-NW	8	2	25%	2	25%	6	75%	7	88%
V-SW	5	3	60%	1	20%	0	0%	2	40%
Total	13	5	38%	3	23%	6	46%	9	69%

Table 5. Number of oceanographic stations in 2016/17 NEWREP-A.

Stratum	*YS3*			*KY7*			Total		
	CTD Casting	DST Casting	Seawater Sampling	CTD Casting	DST Casting	Seawater Sampling	CTD Casting	DST Casting	Seawater Sampling
V-NW	17	16	2	12	9	1	29	25	15
V-SW	15	13	1	9	6	2	24	19	16
Total	32	29	3	21	15	3	53	44	6

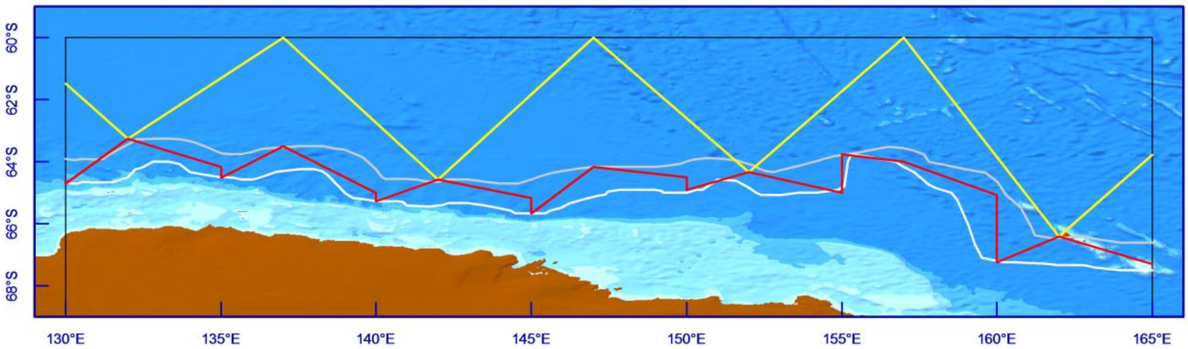

Figure 1. Research area and planned tracklines of the krill and oceanographic survey in 2016/17 NEWREP-A. (Black line: research area, Yellow line: planned trackline in V-NW, Red line: planned trackline in V-SW, White line: ice edge, Gray line: boundary of V-NW and V-SW, Aqua: Antarctic shelf, Brown: Antarctic continent)

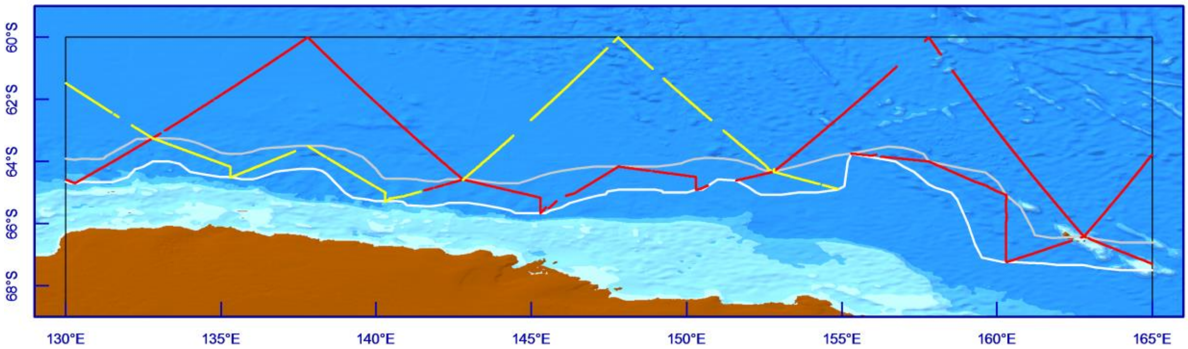

Figure 2. Result of the echosounder survey in 2016/17 NEWREP-A.
(Red line: EK80 actual trackline by *YS3*, Yellow line: EK60 actual trackline by *KY7*)

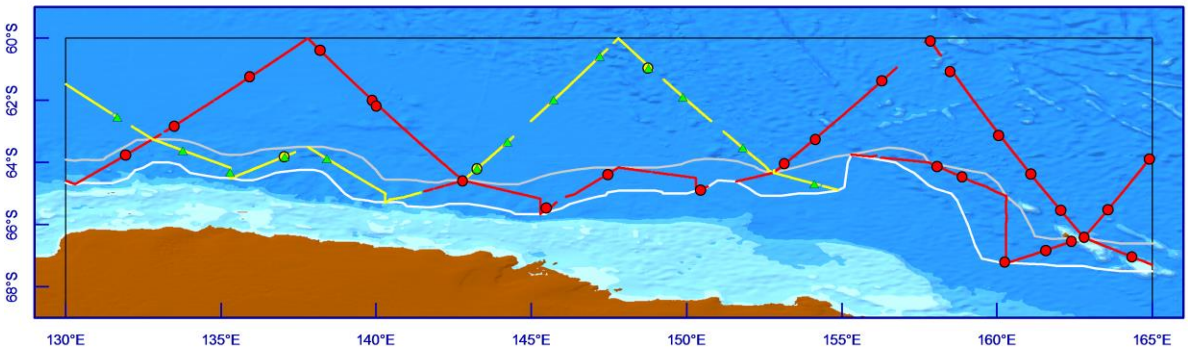

Figure 3. The small ring net sampling and IKMT station in 2016/17 NEWREP-A.
(Red circle: small ring net by *YS3*, Yellow circle: small ring net by *KY7*, Green triangle: IKMT by *KY7*)

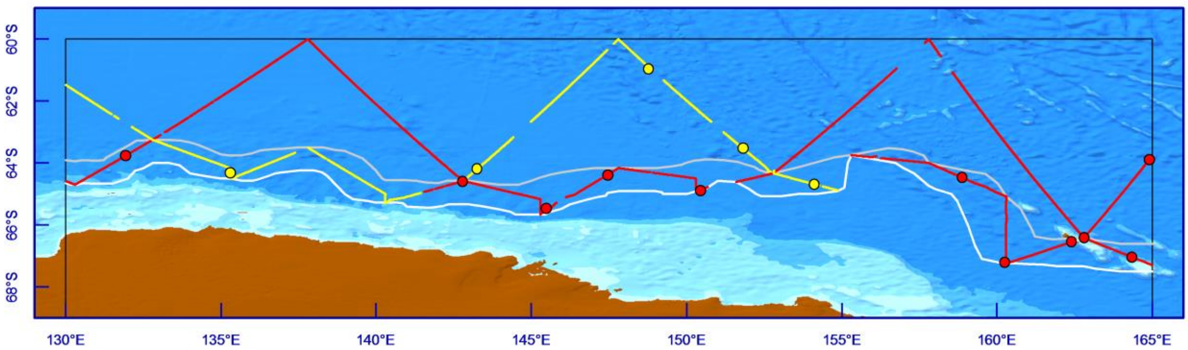

Figure 4. The sample of Antarctic krill in 2016/17 NEWREP-A.
(Red circle: small ring net by *YS3* and *KY7*, Yellow circle: IKMT by *KY7*)

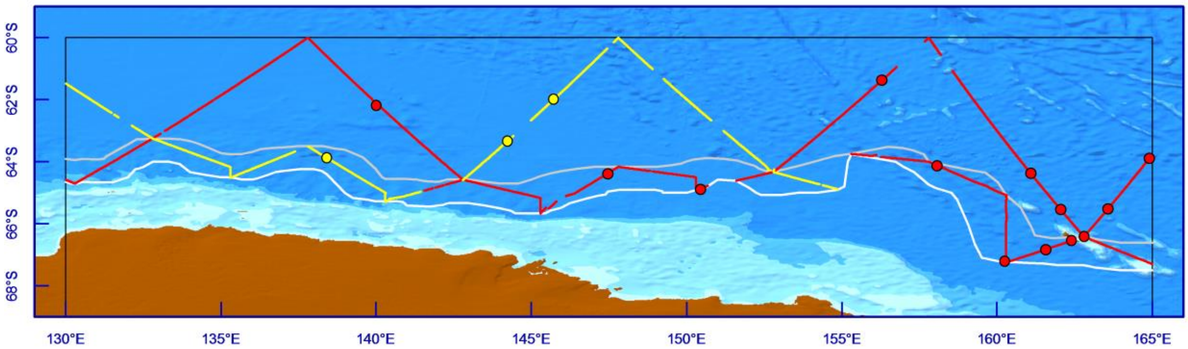

Figure 5. The sample of bigeye krill in 2016/17 NEWREP-A.
(Red circle: small ring net by *YS3* and *KY7*, Yellow circle: IKMT by *KY7*)

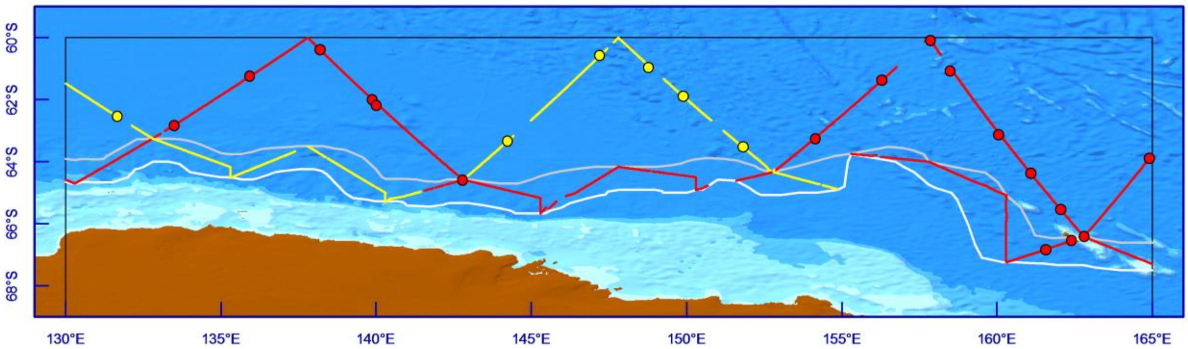

Figure 6. The sample of copepod in 2016/17 NEWREP-A.
(Red circle: small ring net by *YS3* and *KY7*, Yellow circle: IKMT by *KY7*)

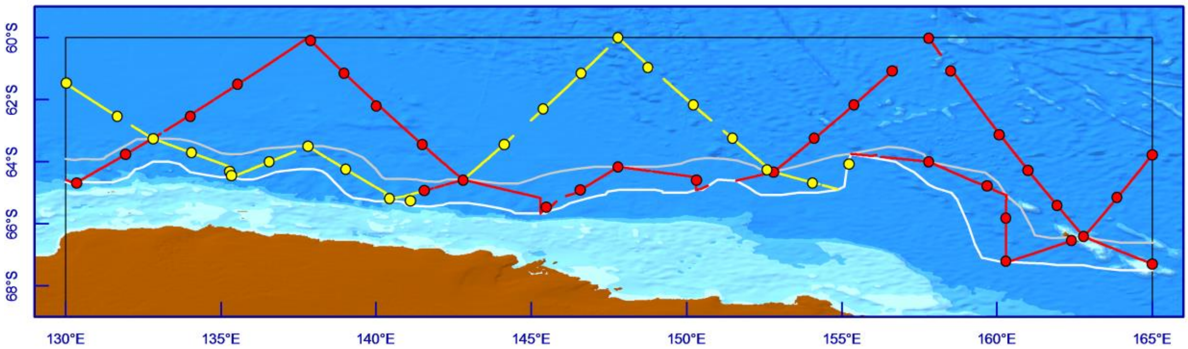

Figure 7. The CTD station in 2016/17 NEWREP-A.
(Red circle: *YS3*, Yellow circle: *KY7*)

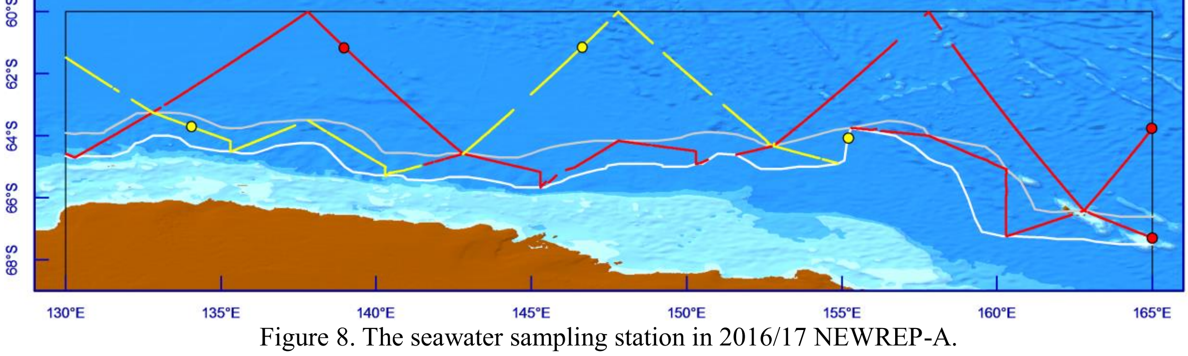

Figure 8. The seawater sampling station in 2016/17 NEWREP-A.
(Red circle: *YS3*, Yellow circle: *KY7*)

Figure 9. Research vessel *Yushin-maru No.3* (*YS3*).

Figure 10. Research vessel *Kaiyo-maru No.7* (*KY7*).

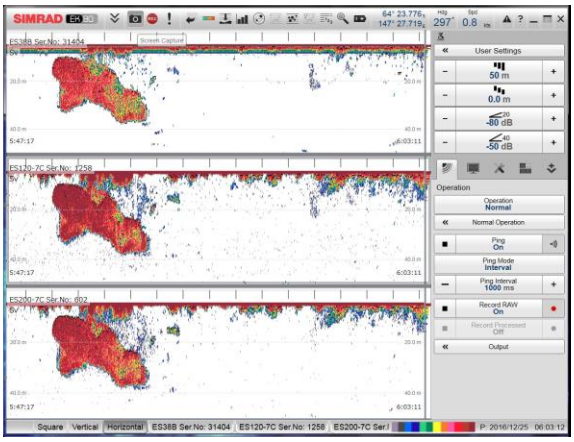

Figure 11.
Echo sign of Antarctic krill by EK80 at *YS3*.

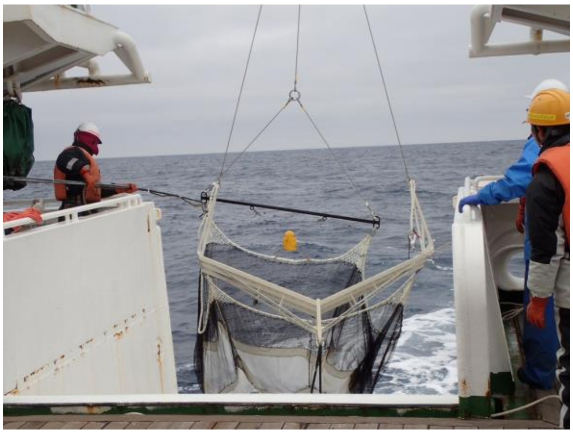

Figure 12. IKMT sampling at *KY7*.

Figure 13. Antarctic krill sample by IKMT at *KY7*.

Figure 14. Antarctic krill sample by IKMT at *KY7*.