Appendix E

Acoustic Survey Trawl Sampling Protocol

# Objectives of biological data collection

There are two primary objectives for the net sampling programme:

* To validate and identify acoustic targets, confirming which targets can be considered as krill and obtaining krill length frequency data for Target Strength estimation;
* To describe krill demography and large-scale distribution patterns of size groups and maturity stages as well as regional recruitment indices.

# Gear selection

Tow samples should be conducted with trawl nets with a minimum mouth opening of 8 m2 and a mesh size ≤4 mm knot-to-knot, or ≤7 mm (stretched) diamond-shaped, from mouth to rear. The most appropriate nets for collecting krill samples are considered to be either the macroplankton trawl, with a mouth opening of 36 m2 and 7 mm stretched diamond-shaped meshes from mouth to rear (Krafft et al. 2018), or the RTM8 (Rectangular Midwater Trawl; Baker et al. 1973), with a mouth opening of 8 m2 and ≤4 mm mesh size. Nets should be equipped with a TDR and flowmeter.

# Standard Gear – the Macroplankton trawl

The Macroplankton trawl (Melle et al, 2006; Wenneck et al., 2008; Krafft et al., 2010; Heino et al., 2011) has from 2010 been used on a regular basis to obtain quantitative samples of macro-zooplankton, particularly krill, during the Norwegian South-Orkney surveys conducted with the fishing vessels FV Saga Sea and FV Juvel (Skaret et al., 2023), and more recently the CV Antarctic Provider. This trawl will also give improved quantitative estimates of various types of jellyfish (schyphozoan medusae, siphonophores and salps). Trawl tows should also be used to ground-truth acoustic scattering layers for the type of organisms they contain, particularly when the scattering structures are potentially of zooplankton origin. During shooting/ deployment, it is recommended to reduce ship speed to a minimum to avoid the trawl net from sampling on its way to maximum depth. After reaching maximum depth, the winch should be stopped for about 3 minutes to allow the trawl to stabilize before starting to haul/ retrieve the net obliquely. During hauling, the ship speed should be increased to approximately 1.5-2 knots, and trawl vertical haul speed should be around 16-22 m/min. The total time of the net haul from surface to bottom to surface should be ~40 minutes.

# Standard Gear – the RMT8 trawl

The RMT8 (Rectangular Midwater Trawl; Baker et al. 1973) was used during the CCAMLR-2000 and Large-Scale 2019 Krill Surveys of Area 48. Below is a description for a typical RMT8 net. Alternatives net types should modify this protocol to achieve same results. At each station a quantitative standard double oblique tow will be conducted from the surface down to 200 m (250 m optimal), or within 10 m of the bottom at stations shallower than 250 m in depth. This depth range is considered the best compromise between the time available for sampling and the likely vertical depth range of krill. During the hauls, a constant ship's speed of 2.5 ± 0.5 knots is suggested. It is recommended to maintain a wire speed of 0.7 to 0.8 m/sec (42 to 48 m/min) during paying out and of 0.3 m/sec (18 m/min) during hauling. The net mouth angle is remarkably constant during hauling within the speed ranges given above. When the net reaches maximum depth, the winch should be stopped for about 30 seconds to allow the net to stabilize before starting to retrieve the net. If the net is hauled from the stern of the ship, then the propeller of the ship should be stopped when the net reaches a depth of 15 to 20 m; this is to minimize the effects of the propeller action on the net operation and avoid damage to the samples. The total time of the net haul from surface to bottom to surface should be 40 minutes. The use of a real-time TDR is highly recommended to maintain a smooth net trajectory and control the maximum fishing depth. Calibrated flowmeters should be used to give a measure of net speed during the haul as well as the total distance travelled. The flowmeter should be mounted outside the net opening to avoid clogging, which may reduce efficiency. The dependence of mouth angle to the vertical of net speed has been investigated for the RMT system. The formula of Pommeranz et al., (1982) should be used to calculate the filtered water volume for oblique hauls (if horizontal hauls are used, then the formulas of Roe et al., (1980), should be used). Data from these sensors can be logged on to a computer, preferably at the ship's bridge, for later determination of trawl profile and calculation of the water volume sampled. In addition to the flowmeter and TDR, the trawl should be fitted with a CTD to collect information on temperature, conductivity and depth.

# Sampling frequency and protocol

It is recommended that nets for biological data collection are deployed along transects at a subset of stations along a pre-defined 20 nm grid, although recognising the time demands of sorting large catches, it is not practicable to recommend every 20 nm station is sampled in such circumstances. The minimum rate of net sampling should be two stations per 60 nm of transect. Where a haul brings up no Antarctic Krill, ideally the next station on the 20 nm grid should be sampled. Where transects include onshore and offshore regions, at least one haul should be conducted in each region.

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# Target trawls

Directed or targeted net sampling effort is necessary to reduce the uncertainty associated with the delineation of krill in the acoustic data record. Such target net hauls will be carried out both day and night and should, as a general rule, be undertaken when significant changes in the acoustic scattering structures, or marks, are observed that are consistent with the identification of ‘swarms’. After the target net haul the vessel will return directly by the shortest route to the point on the acoustic transect line where the vessel broke off from, and continue the acoustic transect from that point (see Fig. 1).

It is recommended that nets for targeted sampling effort are carried out at a minimum rate of one per day. The contents of target haul catches must be sorted in the same way as for standard hauls. Specifically, this requires the measurement of a random subsample of at least 100 krill for length frequency, sex and maturity stage; and to record bycatch (see the following section for details).

A diagram of a diagram of a transport system

Description automatically generated with medium confidence

Fig. 1: Illustration of potential configuration of a target trawl and return to the transect break-point

# Observer sampling and subsampling

For the purposes of this document, we focus principally on sampling for determination of krill length-frequency and ground-truthing the acoustics. We acknowledge that vessels undertaking acoustic and biological surveys may have additional objectives to consider which may require a more detailed laboratory sampling procedure. A description of laboratory sampling protocols is available on <http://archive.ccamlr.org/pu/E/sc/ipy/RMT8protocol.pdf> or alternative procedures may be followed, noting the minimum requirements set out below for krill length-frequency measurement and bycatch quantification.

# Krill length-frequency measurements, sex and stage determination

The minimum requirement is to measure all krill caught when the catch abundance is fewer than 100 individuals, or to take a subsample of at least 100 krill when the catch is larger than 100 individuals. A subsample must be obtained following the protocol described in WG-KFO-2023, Appendix D, and summarized below:

Take 1 to 3 x 1 Litre samples of krill, according to availability. Place your subsamples into a bucket and mix gently; if required add some seawater to prevent damage to the krill during mixing.

From this bucket, fill one graduated measuring jug to the ~150 ml mark and transfer it to a bucket previously filled with cool surface seawater to prevent degradation of the krill. The 100 ml size is suggested as this should contain approximately 100 krill, however as krill size is variable, this 150 ml subsample could be adjusted appropriately.

Take a second sample with the ladle and fill another graduated measuring jug to the ~50–100 ml mark, and transfer it to a second bucket previously filled with cool surface seawater.

In the laboratory, place the bucket with the ~150 ml krill, when possible, on ice and store he bucket with the ~50–100 ml subsample in a refrigerator. Measure the length, sex, and stage for all the krill from the 150 ml subsample. (If the number of krill is below 100, process all krill from the ~50–100 ml subsample.)

# Bycatch quantification

In order to quantify the bycatch of fish and invertebrates, the observer should collect a maximum 25 kg sample of krill from a point on the vessel where no pre-sorting of the catch has occurred. Sort through this sample, identify all bycatch species and record the number and total weight for each species.

# Data and information to be reported

Record information in accordance with the Acoustic Survey Metadata form (tabs Set and Haul Details, Haul Catch, Krill Biological) and submit the form to the CCAMLR Secretariat.

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