CCAMLR

Commission for the Conservation of Antarctic Marine Living Resources
Commission pour la conservation de la faune et la flore marines de l'Antarctique
Комиссия по сохранению морских живых ресурсов Антарктики
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Protocols for trawl sampling, recording of biological data, and hydrography for the 2019 International synoptic krill survey in Area 48

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Abstract

Experience gained through participation in international programmes like BIOMASS and the CCAMLR 2000 Survey has demonstrated that standardization of equipment and methods is one of the most crucial steps for any successful work during the field sampling period and later analytical work. The following net sampling and laboratory protocols are based on the protocols developed for the CCAMLR 2000 Survey. The aim is to facilitate a joint understanding of the field and laboratory work in order for participants that carry out the upcoming International Krill Synoptic Survey 2019 to collect comparable and high-quality data. This will hopefully enable the establishment of a uniform and valuable database of comparable quality with data obtained through the CCAMLR 2000 Survey and other more recent surveys that have been conducted within the region of CCAMLR Area 48.

Objectives

There are two primary objectives for the net/trawl 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 length-frequency distribution patterns and maturity stages as well as regional recruitment indices.

These two objectives require two different sampling strategies which will be outlined below

Standard procedure for biological sampling with net/trawl gear

Biological sampling with trawls and nets during the survey will be performed independent of time of day. Trawls/nets deployed for the sampling of macrozooplankton including krill, should as standard be lowered to approximately 200 m depth or to 20 m (or 10 m with short nets like the RMT8) above the bottom on the shelf where bottom depth could be less than 200 m. The above depth range is considered the best compromise between the time available for sampling and the likely vertical depth range of krill during the 24-hour cycle.

It is important to attach a trawl-eye, or other depth-, speed/flow sensors (e.g. CTDs, Time Depth Recorders), to monitor trawl performance, (e.g. Scanmar, Marport or similar manufacturer). Data from these sensors can be logged on a computer, preferably at the ships bridge for later determination of trawl profile and calculation of volume of water sampled.

Standard Gear – the RMT8

The CCAMLR Working Group (WG-EMM-99) recommended the use of a standard type of net to avoid potential variation in catchability and selectivity of nets. Particularly this was an advice for the CCAMLR 2000 survey event. In many ways still, the most appropriate type of net presently available is the RMT8/RMT8+1 (Rectangular Midwater Trawl; Baker et al., 1973). This gear is a combined macro- and mesozooplankton sampling device; a mesozooplankton net mounted on top of the macrozooplankton net. It was agreed at WG-EMM-99 that this net should be used as the standard net for both target and random hauls during the CCAMLR 2000 survey. Alternative gear, such as an equivalent IKMT type of net of 8 to 10 m^2 mouth opening, shall only be used if the RMT8/RMT8+1 net is lost or damaged to such a degree that it cannot be repaired. The net should preferably have a mesh size of 3 to 4 mm.

This page details the protocol for the RMT8 sampling. There are also separate details for processing the zooplankton from the RMT1. The extended use of multiple net versions of the RMT1 and RMT8 systems are not considered here.

Countries that have been monitoring the krill stock in particular survey regions for years (e.g. UK), are encouraged to use their RMT8 and standard sampling strategy also during their International Krill Synoptic Survey 2019 survey contribution. The following sampling strategy will depend on whether a ship has an opening/closing RMT8 or not. The RMT8 and RMT1 (see below) are included for completeness, to allow for the use of nets and associated protocols that were a standard during the CCAMLR 2000 survey for those countries that still rely on their use.

- Ships with an opening and closing RMT8 system will undertake a station net haul at around local midnight, during the day these ships will undertake a targeted net haul if suitable targets are detected.

- Ships without an opening and closing RMT8 system will undertake a station net haul at around local midday and at around local midnight.

Station net tows shall be carried out in the dark period of each day (around local midnight). The timing of the midnight sample is constrained by the period of darkness. A table of sampling times that take account of the changes in length and time of the hours of darkness (due to variation in date and position) will be produced for each ship. A midday station net haul may also be carried out if target fishing has not taken place since daybreak (see Target net haul protocol).

At each station a quantitative standard double oblique tow will be conducted from the surface down to 200 m (or to within 10 m of the bottom at stations shallower than 200 m). Such a 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 avoids damage of 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 essential to maintain a smooth net trajectory and control the maximum fishing depth. Calibrated flowmeters will 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 the 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).

It is realized that few of those that have committed to the survey have access to this type of net.

Standard Gear – the Macroplankton trawl

On Norwegian research vessels 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 macrozooplankton, particularly krill, amphipods, mesopelagic fish and shrimps in the Arctic and Antarctic. This trawl has also been used during the Norwegian South-Orkney surveys conducted with the fishing vessels FV Saga Sea and FV Juvel (cf. Krafft et al., 2018). This trawl will also give improved quantitative estimates of various types of jellyfish, overall the schyphozoan medusae, but potentially also siphonophores and salps. It should also be used when ground-truthing acoustic scattering layers for the type of organisms they contain. Particularly this is the case, when the scattering structures are potentially of zooplankton origin. This standard survey trawl is 42 m long, with a 36 m^2 mouth opening, constructed of 7 mm (stretched) diamond shaped meshes from mouth to rear.

During shooting/deployment it is recommended to decrease ship speed in order to reduce trawl fishing on its way to maximum depth. After reaching maximum depth, the winch should be stopped for about 30 seconds to allow the trawl to stabilize before starting to

haule/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 various national pelagic trawls

Several of the vessels (Chinese, Korean, Ukrainian) participating in the planned survey will be using their own commercial trawls. These vessels are however, encouraged to adopt the sampling procedures as outlined for the scientific vessels that use either an RMT8, a Macroplankton trawl with pelagic trawl doors or a beam rigged Macroplankton trawl.

It is recommended also for these vessels to attach a trawl-eye, depth- and speed sensors to monitor trawl performance, (e.g. Scanmar, Marport or similar manufacturer). Data from these sensors should preferably be logged on a computer at the ships bridge for later determination of trawl profile and calculation of volume of water sampled.

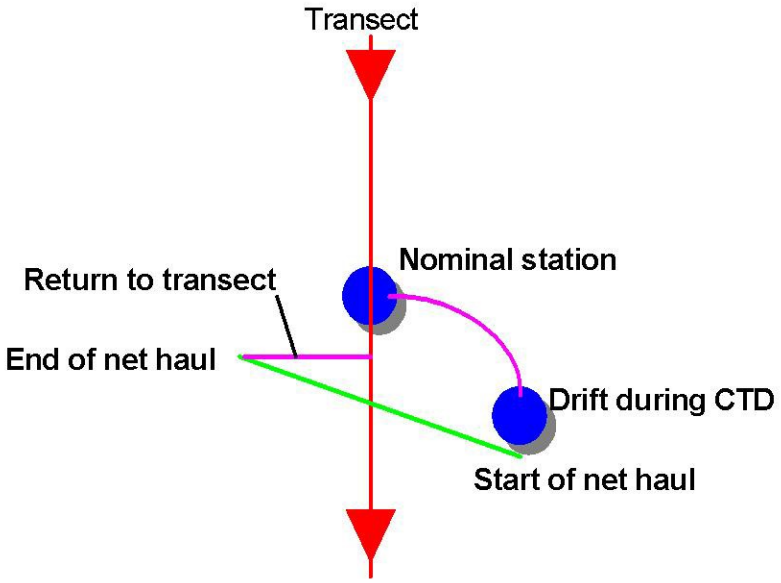
Target net hauls

Directed or targeted net sampling effort will be necessary to reduce the uncertainty associated with the delineation of krill in the acoustic data record. This sampling would be directed at a variety of acoustics registrations or "acoustic morphs", some presumed to be krill and some presumed not to be krill. Such target net hauls will, contrary to the 2000 survey, be carried out both day and night, and should as a general rule be undertaken when significant changes in the acoustic scattering structures are observed.

For ships that plan to use an opening and closing RMT8 net system, the following target fishing strategy can be adopted during the daytime acoustic survey period, but is not obligatory:

1. From the time of local apparent sunrise to local apparent noon conduct a directed tow if an acoustic registration of interest is detected and a reasonable chance of sampling exists.
2. If the directed tow is conducted between local apparent sunrise and three hours before local apparent noon, delay the CTD cast until local apparent noon.
3. If a directed tow is conducted in the three-hour period before local apparent noon, conduct the CTD cast at the same locality.
4. If no suitable acoustic registrations are detected by local apparent noon, conduct a standard oblique tow in conjunction with a CTD cast at the midday station.

After the net haul the vessel will return directly by the shortest route to the acoustic transect line and continue the acoustic transect.



Laboratory sampling and subsampling

Samples from RMT8/Macroplankton trawl can range from a few grams to several kilograms in weight. The total volume of the net catch should be measured (total drained sample volume), but also weighed. For catches with a total volume of less than 1 litre/~1kg the total sample should be sorted. The **minimum requirement** is that all the krill and salp specimens shall be counted and measured, and number of each taxonomic category weighed, immediately after the catch. If possible, the rest of the zooplankton should either be identified to the species level and counted, weighed and/or stored in 4% buffered formalin solution for later analyses.

Samples that are too large to be sorted completely are subsampled volumetrically or by weight immediately after the catch, but first after analyzing and handling the larger components of the catch, e.g. fish, jellyfish or cephalopods if present. Due to differences in catch composition, subsampling must be carried out differently:

- if the sample size is larger than 1 litre and the sample mainly consists of krill: first the total drained sample volume (or weight) must be determined and recorded, afterwards a 1 litre subsample (or a weight based subsample), is taken randomly from the total samples and all krill and salp specimens are counted from this subsample (for measurements see below). For the remaining zooplankton fraction see above.

- if the sample size is larger than 1 litre and the sample mainly consists of salps: first the total drained sample volume (or weight) must be determined and recorded, afterwards all krill have to be sorted from the total sample, counted and measured (for measurements see below). Finally, a 1 litre subsample (or a weight based

subsample), is taken randomly from the total samples and all salp specimens are counted from this subsample. For the remaining zooplankton fraction see above.

Subsampling of catches should be undertaken on samples suspended in sufficient seawater to ensure mixing. Total volume of sample and seawater should be recorded, the sample should then be well mixed and a known volume of sample/seawater mixture withdrawn rapidly. The subsample volume (or weight) should be recorded and the ratio of sample to subsample calculated and recorded. As an alternative approach total wet weight of catch, weight of subsample and weight of sorted specimens within each taxonomic group could be obtained.

Measurements of krill

Numeric

The reductions in sample size must be recorded properly to allow the extrapolation from the subsample to the total sample size for each of the sample components (krill, salps, and other macrozooplankton). These data together with information on fishing depth and filtered water volume will allow the necessary standardization of krill densities and length density data (per m^2 or per 1000 m^3).

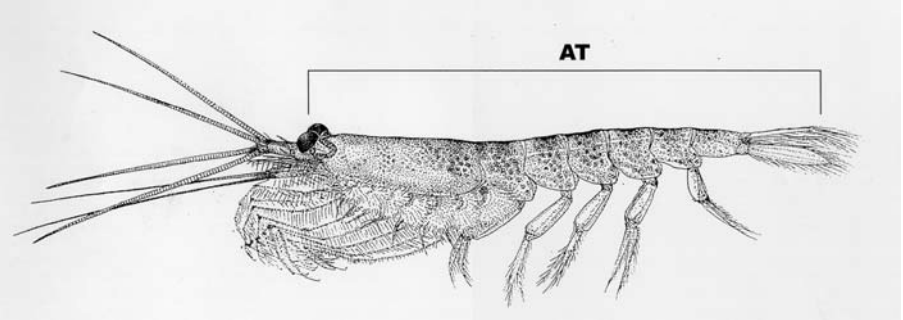
Volume and Weight

Total volume or wet weight of krill in the sample (or subsample) if not both, should be measured. **Participants should use a displacement volume or a wet weight with an accuracy of 0.1 grams or better.** The method should be consistent throughout the cruise.

If time and resources permit data on length-weight relationships of krill should be established for different parts of the survey area (e.g. for each statistical Subarea) and separately for 3 groups of krill (adult males, gravid females and all other krill - see Morris et al., 1988 for further details of this classification). If this is to be conducted on the ship, then about 10 specimens per length class should be pooled and weighted. The station number must be recorded for each of the 10-specimen-measurement.

Length Measurements

The standard length measurement is total length as defined by the Discovery method (AT) from the anterior margin of the eye to the tip of the telson without the terminal spines. The standard unit is given in mm below, with an accuracy of 1 mm size classes. All measurement on each vessel should preferably be done by one person to remove observer variation (see Watkins et al. 1986). Samples which contain less than 150 krill are used in total for length measurements and maturity stage identification. For larger krill catches a minimum of 150 krill must be measured and staged.



Maturity Stages

Krill sex and maturity stages should be identified using the classification of Makarov and Denys (1981, BIOMASS Handbook): Males are separated into three sub adult stages: MIIA1 (petasma vesicles are not divided, but appear as a small “bump” or “bubble” at the root), MIIA2 (petasma has developed the “bubble” to a split with one or two “fingers”), and MIIA3 (petasma root with two short “fingers” and an incipient formation of “wings” on the opposite hold), and two adult stages: MIIIA (petasma fully developed, with swollen “fingers” and with a “wing” overlap, ductus ejaculatori are also visible ventrally, but these are sealed and spermatophores cannot be squeezed out), and MIIIB (petasma as for MIIIA, ductus ejaculatori has spermatophores that can be pressed out, or with the duct passage open where spermatophores are already deposited). Females were separated into one sub adult stage: FIIB (thelycum is small and colorless), and five adult stages: FIIIA (thelycum is fully developed for spawning, red-pigmented and strongly chitinized), FIIIB (thelycum as FIIIA but fertilized with spermatophores), FIIIC (also with spermatophores, mature eggs or large ovaries visible under carapace, but carapace is not swollen), FIIID (with spermatophores, carapace is swollen and this swelling extends into the first abdominal segment), and FIIIE (fully spawned, the ovaries are small and the carapace is hollow). Juveniles, unlike all other stages, have no visible sexual characteristics (no visible petasma or thelycum).

Conduct the compulsory length measurements and weights on freshly caught material. Preferably, staging of krill can be made on fresh material as well, but if not possible, and taxonomic competence is not available to undertake such analyses, samples should be fixated in 4% formalin for onshore taxonomic and demographic examination.

Measurements of salps

If possible all salps should be removed from samples smaller than 1 litre and counted. From larger samples a random subsample of 1 litre should be taken (see above). Please note the different species *Salpa thompsoni* and *Ihlea racovitzai* as well as the different forms (aggregate/solitary). If possible a minimum of 100 specimens per species should be measured. The internal body length (see SL Figure 1 and Foxton 1966), should be measured to the mm below with an accuracy of 1 mm size classes.

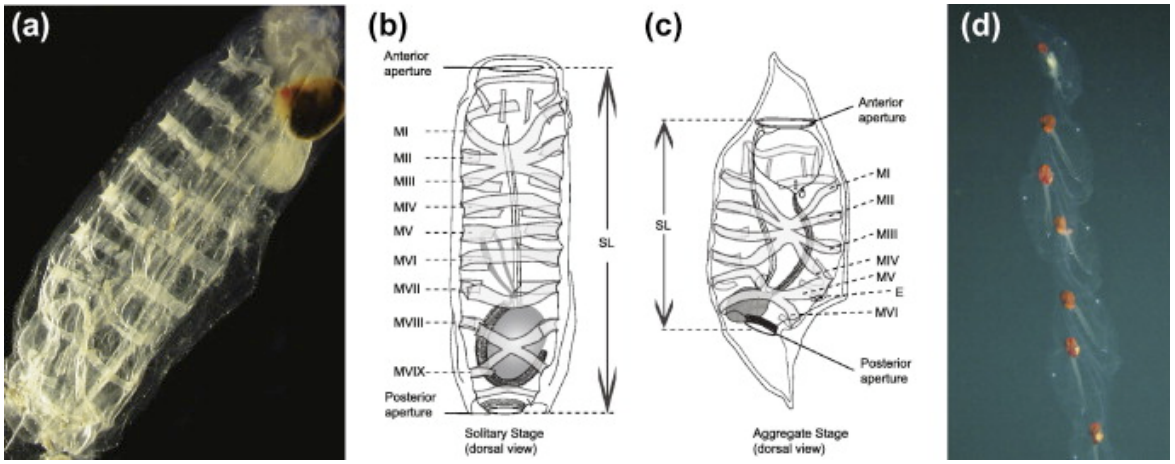


Figure 1. a) a solitary (asexual) salp, b) line drawing of a solitary salp indicating muscle bands and apertures c) line drawing of an aggregate (sexual) salp indicating muscle bands and apertures d) a chain of aggregate salps (Loeb & Santora 2012, SOKI Wiki, 2014c).

Other zooplankton from the RMT8 and the Macrozooplankton trawl samples

If possible, all other macro-zooplankton should be identified to the species level, either from fresh material immediately after the catch or from preserved samples. Special attention should be given to other euphausiid species and early life history stages of fish species. After sorting the larger organisms from the sample/subsample, the smaller constituents (e.g. krill larvae) should be sorted using dissecting microscopes. In case of high krill larvae abundance further splitting into subsamples might be necessary.

If possible a full sample or quantitative subsample of other zooplankton should be preserved.

If possible, length measurements should be carried out for key species like *Thysanoessa macrura* (tip of the rostrum to tip of the telson, mm below, 1 mm size class) and other euphausiid or fish species.

Preservation of krill

It is important that samples of krill are preserved for checking or future studies. It is recommended that if sufficient krill are available then the following strategy should be adopted.

1. a sample of 50 krill should be preserved in ethanol for genetic studies. A minimum of 90% ethanol with a volume 10 times the volume of krill is recommended
2. a quantitative subsample of krill which have not been processed should be preserved in formalin as a back-up data set if possible.
3. the krill that have been measured and staged should be preserved in formalin.

Preservation of other zooplankton

A quantitative subsample of other zooplankton should also be preserved in formalin when possible.

Data Entry

All relevant data should be entered onto computer prior to the termination of the cruise. Example electronic data sheets are provided below.

Net haul data entry table

This table will be available in Excel spreadsheet formats

Cruise :	KRILL AREA 48 Survey 2019
Ship name :	aaaaaaaaaaaa
Net Type (RMT8, IKMT):	xxxx
Mesh Size :	x.x (in mm)
SubArea (48.1, 48.2, 48.3):	
Transect Number :	nn
Station Number :	nnn
Event Number (unique identifier):	nnn
Date (yymmdd):	yymmdd
Start Time, net in water (GMT):	hhmm
End Time, net out of water (GMT) :	hhmm
Haul Duration :	mmm (in minutes)
Latitude :	-ddmmss (no decimal point)
Longitude :	-dddmmss (no decimal point)
Type of haul (random or target identification) :	RA ID
Net Track (oblique, double oblique, horizontal) :	OB DO HO
Flowmeter start :	xxxxxx
Flowmeter end :	xxxxxx
Flowmeter distance (m) :	mmmm

Filtered Water Volume (m^3):	mmmmm (in m3), no decimal point
Fishing Depth min (m):	mmm
Fishing Depth max (m):	mmm
Bottom Depth :	mmmm
Wind Speed (in knots):	kk
Wind direction (in degree):	ddd
Sample size (in ml) :	nnnnnn
Subsample size (in ml) :	nnnn
Comments :	

Net sample data entry form

Event No = nnn	No in total sample	No in subsample	No measured for length	Weight (g wet wt)
Euphausia superba				
Salpa thompsoni				
Ihlea racovitzai				
Thysanoessa macrura				
Thysanoessa vicina				
Euphausia frigida				
Euphausia triacantha				
Euphausia valentini				
Others				
Others				
Others				
Others				
Others				

Krill length frequency data entry form

Cruise:		Ship:		Station:		Event:		No. measured:		No in sample:		
Length	Juvenile	M2A1	M2A2	M2A3	M3A	M3B	F2B	F3A	F3B	F3C	F3D	F3E
17												
18												
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Zooplankton sampling protocols for Synoptic Survey RMT1 Samples

Preservation of samples

- Upon retrieval of the gear ensure that all zooplankton adhering to the net is washed down with seawater into the cod end liner.
- Once cod end liner has been brought into laboratory gently invert the cod- end liner over a tray or bucket and wash out the contents. Concentrate the catch by filtration through a sieve of mesh size smaller than the net mesh (i.e. < 330 µm).
- A measure of the displacement volume for the whole catch is required. This can be achieved by placing the drained catch contents in a known volume of sea- water and noting the increase equivalent to the displacement volume of the catch.
- Place catch or appropriate sub-sample into a jar and preserve with 10% (v:v) formaldehyde solution. Ensure that enough preservative is used to fix the catch. As a guide we have found that a ratio of 3-4:1, preservative:catch, is generally adequate to ensure the good condition of most taxa in the sample, at least in the short-term. Do not use less preservative than this.
- Make out a record of the following station and catch details in pencil on a waterproof paper label:

Date, Start Time, End Time (GMT), Position (Lat Long)
Station, Transect Number (Event Number), Sample Volume Fraction preserved if subsample
Flow reading (Volume swept.)

Place label IN the sample jar and store safely.

Ancillary Information on hauls

We know that the RMT1+8 presents a variable mouth area dependent on speed through the water (Pommeranz et al. 1982). The net must be equipped with a calibrated digital or analog version flowmeter, but a log could alternatively be kept of wire-out against time and also of distance travelled by the ship.

Sorting on board and preserving samples

Notwithstanding decisions as to whether samples are sorted onboard ship or back in the home laboratory we need to ensure that the same degree of taxonomic rigour is applied to the samples by all participating nations. We can generate a sample sorting list of those species that we expect to encounter, but if identification problems are experienced then examples of those representative taxa should be set aside for general examination/opinion of other workers. Even if samples are sorted onboard ship then it is still vitally important that the samples (or representative subsamples) are preserved for later examination.

CTD protocol:

General requirements

All CTD sensors (in particular conductivity) should be calibrated before and after the cruise at the factory. Standard should be annual factory calibration, but preferrably at least once close in time (within 1-2 months) to the survey, especially if no salinity samples are taken during the survey.

Sampling frequency: set by TIME (as fast as possible, e.g. 24Hz for SeaBird Electronics SBE911+ CTD systems, 2 or 4 Hz for SBE Seacats, 1Hz for SAIV AS sensors)

Variables to cover (with preferred units): in situ temperature (ITS-90, deg C), conductivity (S/m), Pressure (dbar).
Additional variables that are desirable: fluorescence, PAR, oxygen concentration (µmol/l, µmol/kg, ml/l, mg/l; provide calibration information!).

Required information

For each cast, information to be noted down:

- date & time (UTC)
- position at start, bottom, and end, if possible throughout the cast (for SBE: append to every scan; lat/lon, degrees & decimal minutes)
- bottom depth (in meter)
- operator
- ship
- sensor type incl. serial number for each component
- software used e.g. for SBE instruments incl version number
- deployment method (winch, trawl)
- meteorological conditions (barometric air pressure at sea level, wind speed & direction (degrees), air temperature (deg C), cloud cover (in 10th)
- sampling log of type of water sample taken and sample number (see below for further details)

Deployment routines

1. Deployment when CTD winch with communications cable and water sampling rosette are available:

- If pumped system with automatic pump: start data acquisition just before the CTD leaves the deck; if not pumped system/pump started manually: start data acquisition when CTD in water.
- Soak for 1 minute at 10 m, bring back up to surface (ie just submerged, rosette/CTD frame not breaking through the sea surface), leave there for 3 minutes.
- Lower CTD to max depth. Rest at max depth for at least 1 minute (preferrably longer).
- Lowering speed: between 0.8 and 1 m/s. If LADCP is mounted, 0.7 m/s. Take care not to hit the bottom with the CTD but stop 5-10 m above ground, depending on conditions (steepness of topography, drift speed of the ship).
- Deployment depth: 1000 m or bottom if shallower. If time does not allow full depth/1000 m casts, limit depth to 750 m.

- Water samples for nutrient samples (nitrite, nitrate, phosphate, silicate), biological sampling (Chl a and phaeophytin) and salinity (calibration of conductivity sensor) at the following depths: (bottom if full water column cast), 1000 m, 800 m, 500 m, 400 m, 300 m – nutrients and salinity; 150 m, 125 m, 100 m, 75 m, 50 m, 30 m, 20 m, 10 m, 5 m – nutrients, biology and salinity.
  Take samples on upcast: stop CTD at sampling depth (do NOT fire ”on the fly”!), wait at least 1 minute, preferrably 2 minutes, then fire Niskin bottle. Flushing time (i.e. wait before closing the bottle) should be longer in calm conditions and for big rosette systems or Niskin bottles with small openings.

2. Deployment when only CTD winch is available (no water samples):

- Soak for 1 minutes at 10 m, bring back up to surface (ie just submerged), leave there for 3 minutes, then lower to max depth. Rest at max depth for at least 1 minute.
- Follow routines above for data acquistion, or as appropriate for systems with internal logging.
- Lowering speed: between 0.8 and 1 m/s. If LADCP is mounted, 0.7 m/s.
- Deployment depth: 1000 m or bottom if shallower. If time does not allow full depth/1000 m casts, limit depth to 750 m.

3. Deployment when mounted on trawl:

Record lowering speed, trawl speed, maximum depth, length of cast. Watch out for maximum depth rating of the mounted CTD sensor.

Water sampling for nutrients, biology and salinity under scenario *2.* & *3.*: Take water samples from 20 m depth.

After the cast:

1. Taking water samples for nutrients and Chl a/Phaeo

- Use lab gloves
- Use appropriate sample bottles
- Note down on the sampling log cast number/station number, Niskin bottle number, sample bottle number.

2. Taking water samples for salinity calibration:

- Use appropriate sample bottle!! With plastic insert.
- Rinse bottle and bottle cap (but not insert!) three times. Dry off bottle neck (in- and outside) and cap with paper before closing the bottle. Pay special attention to the thread on the bottle ad in the cap.
- Note down on the sampling log cast number/station number, Niskin bottle number, sample bottle number.

3. Data recoverty and processing

- for SBE systems with CTD winch and communications cable: run SBE processing routines following IMR procedures, save all files (raw and processed) in appropriate server place with backup

- for SBE systems with internal logging: recover the data from the instrument after each cast, run appropriate SBE processing routines, save all files (raw and processed) in appropriate server place with backup
- for other systems with internal logging (e.g. SAIV AS CTDs): recover the data from the instrument after each cast, export raw data into ASCII format, save all files (raw and exported) in appropriate server place with backup

Data requirements

Submission of data:

- log with all station and deployment information and sampling log
- sensor details with sensor type, serial number, and calibration information
- raw data
- for SBE CTDs: configuration files
- processed data files if available, with information about processing steps; use downcast in case of winch deployments

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