Census of Antarctic Marine Life (CAML)
DRAFT Uniform Sampling Protocols – Pelagic Realm

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Aim
The uniform sampling protocols are being prepared by the CAML Scientific Steering Committee to provide a baseline of minimum acceptable standards in projects coordinated by CAML. These uniform protocols are provided as a guideline for the various researchers, vessels and locations included in the CAML project. They are not intended to be entirely prescriptive, but parts of the sampling and processing protocols should be followed strictly to allow quantitative comparison between regions, and with past and future surveys. CAML is as much a quantitative as well as a qualitative census. At the same time, new sampling systems are encouraged that will collected specimens that have been missed previously or poorly sampled, e.g. gelatinous zooplankton and cephalopods. At the very least, the protocols provide the baseline of a minimum standard for the publication of metadata on the SCAR-MarBIN data portal to OBIS. Where the protocols cannot be met, for example where a non-standard net is fitted following damage to standard gear, this should be noted as an exception to the uniform protocols.

For some realms, there will be overlap in the sampling protocols. For example, demersal fish are included in the pelagic realm for convenience, however many may occupy the benthic habitat. Microbes may be pelagic; however they are cross-referenced in a separate set of protocols.

Introduction
The pelagic realm is always moving, forming part of a dynamic oceanographic system. The system is subject to climatic and ocean variability. This drives the variability in sea ice cover, which in turn determines the structure of the pelagic ecosystem in the Antarctic. The geostrophic currents, and the frontal systems separating them into different water masses, are important aspects of the environment of pelagic organisms.

The epipelagic and mesopelagic communities support the top predators - whales, seals, fish and seabirds - that have been the subject of huge exploitation in the past and are still affected by commercial fisheries and their bycatch. Together with the "top down" impact of whaling and fisheries, the "bottom up" impact of global climate change is shaping the pelagic ecosystems that we see today in Antarctica. These affect the extent and timing of the annual sea ice cycle, the ecology of the pelagic ecosystem and the ecosystem services from Antarctica to the global ocean system.

To monitor the ongoing and future status of the Antarctic pelagic realm, it is not only critical that we continue to collect new data, but that we clearly document how it was collected, and ideally that we use similar equipment and methodology. Collection data should include the date, time (GMT) and georeference of the sample, together with relevant information on the depth and duration of a tow, type of sampling gear including dimensions (e.g. mouth area and mesh size) and volume filtered. Georeferencing of the sample pinpoints the location of the sample, however additional information in relation to fronts, upwellings and other oceanographic phenomena may be highly relevant. Where possible, oceanographic sampling or profiling, e.g. CTD, should be conducted concurrently. In the unique situation of tissue samples removed from collections, the tissue should be accompanied with full description of its origin, including the species, author, date of the description and location of voucher/type specimen (This information is often indicated by a species code, with a relational link to another database). If the organism is undescribed, reference to a voucher specimen is imperative.

1. Main groups of organisms in the Antarctic pelagic realm

One of the challenges of working in the pelagic realm is that it encompasses organisms spanning orders or magnitude in size, from the microscopic plankton to nektonic mammal tens of meters long. This document is intended to focus on the metazoan zooplankton, fish and cephalopods and ignores the heterotrophic protozoans that also important grazers on primary production. Separate protocols have been developed for protists. Similarly, separate protocols have been developed for the continuous plankton record which uses separate sampling and processing methods to conventional zooplankton sampling. Operationally, we are considering the groups that can generally be sampled by plankton nets. These are often separated into size groups that reflect to some extent their trophic position.

Microzooplankton
Passively moved by a current.
Size 20–200 μm

Mesozooplankton
Passively moved by a current, little mobility against currents, but capable of large vertical migrations on a diel cycle.
Size 0.2–20.0 mm

Macrozooplankton
Passively moved by a current, some limited mobility against currents, can be diel migrators.
Size 20–200+ mm

Micronekton
Capable of swimming against a current.
Size 10–100 mm

Nekton
Capable of swimming against a current, and capable of extended geographic migrations.
Size > 100 mm

Demersal (as sampled in daytime)

On or near the bottom; may migrate vertically into the water column at night and may include merozooplankton. Included in the pelagic realm for convenience, although some organisms may be oriented more to the seafloor than the water column. Larger forms are capable of swimming against a current.

2. General methods of collection

For the purposes of CAML sampling, collection from the pelagic realm has been grouped according to the sampler employed. Below, we list the conventional samplers such as nets with specified mesh size and flow meters, yielding a *quantitative sample*. These samples are comparable between different areas, times and vessels of deployment. In Appendix I, we list additional methods of gathering information, yielding useful but not necessarily quantitative data. The various methods may provide a window on more than one group of animals. There is overlap in the organisms sampled by the various gears. For example, bongo nets may capture micro-nektonic fish as a bycatch of the target zooplankton, especially when deployed at night.

2.1 Key to abbreviations

LHPR	Longhurst Hardy Plankton Recorder (This is a cod end that can be attached to RMT, IKMT or semi rigid nets – not commonly used), **variable mesh size 333-505 µm or 1.55mm**
RMT	Rectangular Midwater Trawl **RMT1 – 300µm with nominal 1 m^2 mouth, RMT8 – 4.5mm with nominal 8 m^2 mouth,** often equipped with multiple nets, electronic sensors and both sizes run in tandem on a single deployment. The RMT1+8 is a the principal sampling system in CCAMLR protocols, and has been used extensively in Antarctic waters since the BIOMASS survey.
IKMT	Isaacs-Kidd Midwater Trawl (previously a common alternative to the RMT for krill and macro zooplankton) mesh size **4.5mm**
CPR	Continuous Plankton Recorder, mesh size **270µm** see separate description and protocols
WP2	Probably the most common conical net. Mesh size **200µm** and **0.57 m** diameter (0.25 m^2) mouth normally
Bongo	A pair of conical nets pulled from a central bridle, variable mesh, typically 64 µm for 20 cm, and 333 or 505 µm for 50, 60 or 70 cm mouth diameter.
Norpac	North Pacific Net mesh size **110 and 330µm, mouth diameter 45cm,**often as a bongo net as used in the Japanese Antarctic Research Expeditions JARE
Reeve	Reeve net are conical nets with 30-110 litre cod ends for capturing live and delicate zooplankton **variable mesh 50-500µm, mouth variable 0.25-1m^2**
Multinet	Multiple system available in 3 sizes, 0.125 & 0.25 m^2 mouths with 5 nets or 0.5 m^2 with 9 nets, **variable mesh typically 100-500µm,** electronic sensors and pre-programmed autonomous opening/closing possible
NIPR-Net	National Institute of Polar Research net/pump for under ice sampling, a cylinder of 24 cm x 57.5 cm fitted with a motorised propeller that draws water into a small net fitted on the end, **variable mesh usually 100 µm**
MOCNESS	Multiple Opening/Closing Net and Environmental Sampling System, available in 0.25 and 1 m^2 with 10 nets or 10 m^2 with 5 nets, **variable mesh typically 64-500µm, except for MOC-10 where up to 5 mm mesh employed**
IYGPT	International Young Gadoid Pelagic Trawl **reducing mesh, 100mm at front, then 80, 40, 20 and 10mm in cod end**

AGASSIZ or Beam Trawl	These are normally used for catching fish near the sea floor **mesh size is variable, e.g. 10 -50 mm**

For additional details on specific gear, see Wiebe & Benfield, 2003

2.2. Conventional quantitative samplers

Basic common systems are highlighted in **bold** text

Group	Gear Type	Comments
Microzooplankton	**Water bottles** – Niskin Water pumps **Ice cores** **Umbrella nets**	Suitable for fragile organisms Overlap with microbe protocols Sea ice micro zooplankton
Mesozooplankton	Multinet MOCNESS Bongo nets – as **WP2** or Norpac **Plankton nets – WP2**, Norpac, **RMT 1** LHPR **CPR** Light traps, umbrella nets, NIPR-net	Depth-stratified samples from vertical or oblique tows. Depth-stratified samples from oblique tows Oblique or vertical tows Mainly vertical tows As part of RMT1+8 Horizontal tows See separate protocols Under ice sampling
Macrozooplankton	**RMT 1+8** IKMT Multinet or MOCNESS LHPR Light traps, umbrella nets	Stratified oblique* and horizontal tows – overlap with CCAMLR-IPY Krill survey protocols Oblique and horizontal tows Oblique and horizontal tows Horizontal tows Under ice sampling
Micronekton (including fish larvae)	LHPR **RMT 1+8** IKMT	Overlap with CCAMLR-IPY krill survey protocols

	Multinet or MOCNESS **Bongo nets**	Depth-stratified sampling* Oblique, vertical or depth-stratified sampling*
Nekton	**RMT 1+8** RMT 25 **IYGPT** IKMT AGASSIZ **Commercial trawls** Squid Jigs or similar	Overlap with CCAMLR-IPY krill survey protocols (See also Benthic Protocols)
Demersal	Epibenthic sleds Commercial bottom trawls Longlines	Cameras may be fitted Special dispensation required (See also Benthic Protocols)

* Stratified depths are likely to be 0–200 m, 200–500 m, 500–1000 m and 1000–2000 m (or deeper, depending on the winch capability). The upper 200 m is sometimes even more finely divided depending on project goals (e.g. 0-20m, 20-50 m, 50-100 m, 100-200 m). These recommended strata are all based on discussions amongst Antarctic zooplankton and krill biologists.

3. Specific Sampling and Processing Protocols – This section describes detailed handling and processing of samples onboard ship. Groups covered in this section include, microzooplankton, meso- and macrozooplankton, Antarctic krill (*Euphausia superba*) gelatinous zooplankton and how to collect live zooplankton. See separate protocols for fish and cephalopods.

3.1 Protists/microzooplankton - possibly as defined by microbe group (see also the microbial/prostistan protocols.

Protists: With the exception of radiolarians and foraminiferans, many of which can be effectively sampled as integrated or depth stratified collections by fine-meshed plankton nets, all other protozoans are best collected at specific depths by Niskin bottles. Pumps tend to damage the naked protozoans and should not be used for them. Rapid preservation of samples is critical due to the tight coupling between predators, and for some naked groups it is critical that bottles be drained without introduction of air or excess disturbance. Depending on the target group and type of processing planned, preservatives vary between gluteraldehyde, formalin, Bouin's solution, Acid Lugol's solution, and Utermol's solution. Some analyses required concentration onto filters and subsequent storage at -80°C or in liquid nitrogen. Additional details on protist sampling and preservation are under development at ICOMM (International Census of Marine Microbes - http://icomm.mbl.edu/)

Metazoans: Few metazoans (or their eggs) occur smaller than 40 μm and can therefore be collected adequately with fine mesh nets of 45 or 50 μm mesh. Because microzooplankton is generally very abundant, mouth sizes for such nets are small, typically of mouth area less than 0.25 m2 and as small as 0.01 m2. They can be deployed vertically as well as obliquely on mini-Bongo nets. Fine mesh nets clog easily and it is critical that such nets have a larger aspect ratio than typical for conical nets, that flow meters are employed to assess clogging, and that they be hauled/towed slower and over shorter distances than coarser meshed-nets (to

accounted for their limited filtration efficiency and offset their tendency to clog). Metazoan microzooplankton is dominated primarily by copepod nauplii and early copepodite developmental stages of smaller-bodied copepod species. A notable exception occurs for poecilostomid copepods such as *Oncaea*, for which even the adults pass through a 200 µm mesh. In general, the crustacean microzooplankton are amenable to sampling with pumps, but this size class can include significant numbers of larvaceans (=appendicularians) that can be severely damaged by pumps. Technically, Niskin bottles samples can be employed to assess metazoan zooplankton, but unless sample volumes are large, counts are low and lack the statistical confidence of nets or pump samples.

3.2 Meso-, macrozooplankton (non-gelatinous) and krill.

Protocols established by the international Biological Investigation of Marine Antarctic Systems and Stocks (BIOMASS) programme in the 1980s and by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) have dominated the sampling in the Southern Ocean and Antarctic waters in recent decades. Other zooplankton sampling and processing have also been established and used in other regions, e.g. the UNESCO "Zooplankton sampling" manual (UNESCO 1968), Omori and Ikeda's (1984) "Methods in Marine Zooplankton Ecology", the "ICES Zooplankton Methodology Manual" (Harris et al. 2000) and the protocols established by the Census of Marine Zooplankton (CMarZ). These various protocols were consulted, along with the recent deliberations of the SCOR Working Group 115 "Standards for the Survey and Analysis of Plankton" in developing the CAML protocols and recommending the following sampling systems, mesh sizes and processing methods.

3.2.1 Recommended sampling gear and mesh sizes

The more important issue for zooplankton sampling is not so much the choice of net but the choice of mesh. As long as we know the net dimensions (mouth area and length) and the net is fitted with a **functional calibrated flowmeter**, then comparisons can be made between nets and data sets. A number of different types of plankton nets have used around Antarctica, such as bongo nets, WP2, Norpac or other conical net, as well as midwater trawls and multiple-net systems.

During the international BIOMASS programme, the principal sampling system was the Rectangular Midwater Trawl combination net or RMT1+8 (Baker et al. 1973). The RMT8 has a nominal mouth area of $8m^2$ mouth area and 4.5 mm (4,500µm) mesh, which was used primarily for catching Antarctic krill, *Euphausia superba*. The last 1.8 m of the net usually has a mesh of 1.5 mm and the cod end mesh is 0.85 mm. The RMT1 has a $1m^2$ nominal mouth area and 300µm mesh and was ideal for collecting krill larvae. The RMT1+8 also proved useful for collecting meso- and macrozooplankton and studying zooplankton ecology. The RMT1+8 has remained the first choice in most surveys since BIOMASS. It was used during the 2000 CCAMLR Krill Synoptic Survey (Watkins et al. 2004). The RMT1+8 system is one of the recommended net systems for CAML to allow comparison with past and likely future surveys, and is the preferred net for use during the CCAMLR-IPY survey in 2007/08.

Meso-zooplankton

Low tech, single strata: WP2 nets either singly or as a bongo net with a preferable mouth area of $0.25m^2$ each equipped with 200, 300 (333?) or 500 µm mesh are recommended. A twin or bongo arrangements allows either replicate samples or samples with different mesh (often one

333 and one 500 µm). There should be no bridles in front of the mouth, but calibrated flowmeters should be fitted in the mouth of each net. The Japanese JARE expeditions have used a twin Norpac net system fitted with 110 and 330µm mesh annually since 1972. Nets can be towed obliquely or vertically and at the recommended towing speed of 2 knots (1 m s^{-1}). Oblique tows have the advantage of integrating larger volumes per collection, but may not fish all depth layers evenly. Vertical nets typically integrate the water column more evenly, but fish smaller volumes of water, and are therefore more susceptible to patchiness in the environment. When nets are deployed for vertical operation, it is critical that they are configured such that the flowmeter does not spin while the net is lowered in "non-collecting" mode. This is generally accomplished by using semi-rigidly mounted meters that can only spin/record in one direction (i.e. when moving forward), or rigging the net so that it envelops/entraps the flowmeter during descent, thereby preventing its recording.
High-tech, stratified sampling: The Multinet, the 2 smaller MOCNESS and the RMT1 are all excellent tools for sampling mesozooplankton when equipped with 200 to 500 µm mesh, and have the advantage of capturing multiple strata in a single deployment. All provide real-time feedback on their depth, speed, volume being filtered per strata, and optionally the physical environment (T, S, fluorescence, transmittance). All can be fished in oblique mode (typically at 2 knots) to provide large volumes filtered per strata, but only the multinet can be used for vertical collections, and this can be a serious consideration when working within ice-covered or deeper waters. Vertical hauls are commonly made a slower speeds of ~0.5 m s^{-1}.

Macro-zooplankton and Antarctic krill
The Rectangular Midwater Trawl RMT 1+8 system can be towed obliquely or horizontally. This system has an advantage of not having bridles in the front of the mouths, plus the RMT 8 provides a substatiantially large mouth area than conventional plankton nets and is usually supplied with black mesh to reduce its visibility and reduce net avoidance. The CCAMLR protocol recommends double-oblique hauls from the surface to 200m and back to the surface using an RMT 1+8 or RMT 8. However, CCAMLR will also accept krill data collected by other nets during IPY as long as the data can be properly quantified. Horizontal tows with an opening-closing RMT system are useful for identifying patches or layers detected by hydroacoustics. Flowmeters must be employed and the recommended towing speed is 2 knots (1 m s^{-1}). Note: the actual mouth areas of the RMT1+8 are dependent on towing speed and trajectory. When calculating the volume filtered, the equations of Roe et al. (1980) and Pommeranz et al. (1982) should be consulted for horizontal and oblique tows, respectively.

Ocean basin-scale, rapid survey,
Continuous Plankton Recorder (CPR) will be used to sample long transects within survey areas or on transects to and from survey areas. The CPR uses 270µm silk mesh (see CPR protocols) Phyto- and zooplankton analysis will be conducted as well as phytoplankton colour indexing (PCI) of the mesh. See separate sampling and processing protocols http://www.caml.aq/pelagic/documents/Pelagic-Plankton-Sampling.pdf

Live and delicate zooplankton
In principal, any of the nets described above can be used to collect live and/or delicate zooplankton, but success is generally higher when one uses finer meshed nets (e.g. 50-150 µm and hauls the nets slower (i.e. 0.1-0.5 m s-1) than typically used for routine sampling. Both these factors reduce the damage to animals when they are pushed against the net's mesh, and tumble down it toward the cod-end. For crustaceans this reduces the damage to the fragile setae on appendages, while for gelatinous zooplankton is reduces the filtration pressure which can extrude portion of the animals through the mesh. Larger volume cod-end, and cod-ends

without filtering windows also improve the quality of the collections by reducing the turbulence and crowding in the cod-end (and hence mechanical damage). Large mouth plankton nets with large non-filtering cod ends, such as the Reeve net (Reeve 1981), pulled very slowly (0.1-0.2 m s^{-1}) have proven the optimal net-based design for collecting fragile zooplankton and live plankton for photography and observational studies. Once on board it is critical that animals be diluted and stored in seawater of similar temperature and salinity as that of their collection depth. Nonetheless, there are limits to net-based designs and for several gelatinous groups it is virtually impossible to prevent some damage during collection, and this renders subsequent measurements and observations suspect. For the most fragile zooplankton, especially ctenophores, the ideal collection and observational tools are scuba divers in shallow waters. ROVs or submersibles become the tools of choice in deeper waters, and when equipped with high definitions camera and appropriate collection tools they can capture even the most delicate zooplankers. Although generally semi-quantitative (e.g. Raskoff, 2001; Raskoff et al., 2005), these technologies can be made fully quantitative by determining the volume of the field of view for a given size of organism (i.e. smaller animals can only be identified close to the camera, while larger animals are noticed farther away), then determining the volume of water observed by knowing the velocity of the water passing through the field of view (e.g. Robison et al., 1998; Silguero and Robison, 2000). Fore more on ROV collecting tools see http://www.oceanexplorer.noaa.gov/technology/tools/suction/s_d_sampler.html.

Under ice sampling

Sampling below sea-ice, either pack ice or fast ice, is particularly difficult and we know relatively little about the zooplankton that lives immediately under and below the ice. Trawling in pack ice is difficult and usually restricted to leads and polynyas (open areas of water in the ice) and the zooplankton collected in leads can be different from that collected or observed under the ice. A number of umbrella nets have been developed that can be deployed and retrieved in a closed state through holes in the ice and open when below the ice (e.g. Kirkwood and Burton, 1987; Macaulay and Daly, 1987). An alternative method is the NIPR-net (Fukuchi et al, 1979) which draws water though a cylinder 24cm in diameter in a small net fixed on the end. An electric powered propeller is fitted inside the cylinder. Push nets have also been used by divers under the ice - see review by Wiebe and Benfield (2003). Provided open space can be maintained around a vessel in the ice, vertical nets and the multi-net are the most appropriate sampling tools.

Trawl Logbook

Complete details of each trawl, net haul or other sample collection should be recorded in a logbook, hard copy or electronically. Minimum details to record include:

- Ship name, cruise name or number
- Station and trawl numbers, especially if more than one haul per station.
- Date of sampling in Universal Time Constant (UTC), also known as Greenwich Mean Time (GMT)
- Time of sampling in UTC-GMT including time net in water, time out of water and opening and closing times for start and end of sampling for nets with opening closing mechanisms.
- Latitude and longitude at start and end of sampling.
- Depth of sampling
- Type of net and mesh size
- Type of haul: e.g. oblique, vertical, horizontal

- Wire out and angle is useful
- Ship's speed, both speed through the water and speed over ground is useful
- Flowmeter at start and end of sampling, flowmeter ID or calibrations (e.g. counts or turns per metre)
- Sea and weather conditions
- Anything else that may be useful for or have affected sampling, e.g. gear failure, notes on other sampling or data recorded.

3.2.2 Handling and processing of samples on ship

See Appendix II for a list of laboratory and chemical supplies which are recommended for processing and preserving the samples. These have been modified from the CMarZ protocols.

Basic procedures

- If the catch is large, regardless of species, either weigh the cod end or measure the total drained sample volume. RMT8 nets can produce large catches. Record total wet weight in the trawl log book. This is useful information if sub-sampling is required.
- Antarctic krill *Euphausia supera* and salps, mainly *Salpa thompsoni,* collected in the RMT1+8 nets that can be used for the purpose of the CCAMLR-IPY Krill Survey should be removed entirely or a suitable sized sub-sample taken and preserved in formaldehyde or Steedman's according the CCAMLR-IPY sampling protocols. The recommended sampling and processing protocols are available at the CCAMLR website (www.ccamlr.org) and also on the CAML website http://www.caml.aq/pelagic/index.html. If a sub-sample is taken of krill or salps, then determine the weight or volume of all krill/salps. Take care to wash or remove other zooplankton as best possible and return to the main catch. Although the preferred sampling net for CCAMLR-IPY Survey is the RMT8, any data on krill and salps collected by nets other than the RMT1+8 would be welcomed by CCAMLR. **Note: CCAMLR cannot accept krill samples for analysis as it does not have the appropriate facilities. However, CCAMLR will accept any data on distribution and abundance of krill and salps, as well as length measurement and classification of maturity stage of individual krill. Please refer to the CCAMLR-2000 Survey protocols and CCAMLR's *Scientific Observers Manual*, which describes the krill sex and maturation stages and how to measure krill, available on the CCAMLR and CAML websites.**
- Specimens taken for DNA analysis and barcoding should be processed according the "Sampling protocols for CAML barcoding studies" located on the CAML website at http://www.caml.aq/barcoding/index.html.
- Soft bodied plankton, e.g. ctenophores may need to be identified as far as possible (species, genera or family), counted and weighed prior to preservation as these animals often fall apart when preserved, making later identification difficult or impossible. Large jellyfish, i.e. too large to properly preserve (these are often members of the Coronatae) should be identified as far as possible, counted, weighed. Tissue for DNA and high quality photographs will also aid identification. See detailed protocols below for sampling and processing gelatinous zooplankton.
- Adult or juvenile fish and squid are often collected in plankton nets and should be processed according to the fish and cephalopod protocols.
- The catch should be carefully washed into the sample jar using filtered seawater. The sample can also be concentrated in a sieve, with mesh the same or finer than the plankton net, before washing into the sample jar. The sieve for RMT8 samples should

match or be less than the cod mesh, usually 0.85mm. Preserve the samples with 5% buffered formalin, i.e. add 5 ml of concentrate formalin (buffered with borax) for each 100 ml of seawater or volume of the jar and top up with seawater if required. Add a water proof label to the jar with catch details written in pencil. Also record details on the outside of the jar using sticky labels or waterproof permanent marker, and also recorded in a log book. 5% formalin is equivalent to 2% formaldehyde.

- With the advent of high-resolution and inexpensive digital camera's, it can prove useful to photograph each sample in a shallow tray with the sample label and a scale bar visible to provide a qualitative record of the collection's composition.

Preservation

Please see Appendix III about preservative and general preservation procedures.
The amount of zooplankton in a jar should not exceed one third of the volume of the jar, otherwise they may not preserve properly. As noted above, concentrate formalin is 37-40% formaldehyde solution. It is acidic and needs to be buffered with borax or the samples will be destroyed. Buffered formalin should be prepared in advance. Large volumes of 20 litres or more can take 1-3 months to buffer. Do not attempt to buffer at the time of preserving the zooplankton as the correct pH may not be achieved. Check the pH regularly afterwards to ensure that it does not drop below pH 7. Attempt to adjust the pH with small quantities of borax back to pH 8.
If possible a replicate sample or quanitative subsamples should periodically be preserved in 95-100% ethanol for molecular sequencing. This procedure requires that samples are drained of all salt water, rinsed with ethanol to remove remaining salt, and then stored in 95-100% ethanol. The ethanol should be completely replaced with fresh ethanol 24-72 hours after initial preservation. Ethanol samples have a "shelf life" for molecular work, and the quality of the DNA degrades over time. Efforts should be made to have any sequencing completed within 1 year of preservation.

Labelling

Waterproof paper or card should be used inside the all jars. 100% cotton rag (laundry paper) can be used but we recommend checking the integrity of the label in water before use. Details should be recorded in pencil and not ink. Minimum details to record are: ship name, cruise name or number, date and time in UTC (GMT), station and tow number, latitude and longitude, net type and mesh, jar number if more than one jar used per sample, and the collectors name. Also record the preservative used if more than one type has been used on the voyage. *Local or ship time is not recommended, and can be confusing as Antarctic vessels often vary their ship time as they cross longitudes and can also operate one or two hours ahead of true longitude time.* Day and night periods can be calculated from UTC time and longitude. Details should also be recorded externally on the jar.

Sample splitting

Ideally, it would be useful to preserve catches in their entirety. Some nets such as the RMT8 can produce large samples. Samples can be sub-sampled by taking a known weight or volume or by splitting the catch using a plankton splitter. Motoda or Folsom splitters are recommended (Omori and Ikeda, 1984). These split samples in half and further splits can also be made, if the catch is still large. Note: both splitters are rarely made accurately to produce two equal halves. The percentage volumes of the two portions should therefore be measured so that precise aliquots can be recorded. CCAMLR protocols recommend the Folsom splitter.

Laboratory logbook

Details of all procedures, such as shipboard identifications, sample or sub-sample weights/volumes, removal of specimens for other uses (and where they go), photographs taken, catch description and size/number of sample jars used, should be recorded in detail in a logbook. This can be in addition to, or part of, the trawl logbook and can be hard copy or recorded on computer*. It is important to record where specimens go, and to whom, if removed from the catch so that details of species identification, numbers and weight can be confirmed and added to the census database. Remember, CAML is about gathering both quantitative and qualitative data, as well as finding new species.

3.3 Gelatinous zooplankton

As indicated above under collection, handling and preservation protocols, the gelatinous zooplankton present a multitude of problems not encountered when working with crustacean zooplankton. Some groups such as the ctenophores are difficult to collect intact even when abundant in the water column, and virtually impossible to preserve due to their watery composition. Colonial siphonophores are often fragmented during collection, and generally completely dissociate upon preservation. Larvaceans/appendicularians contain extremely thin tissue layers and degrade rapidly upon collection with standard nets, so should be preserved immediately upon collection. All can be severely damaged during sample handling post-preservation. Through ideal, the financial and/or logistical complications of using divers, ROVs or submersibles prevents their widespread use. These complications impede our ability to adequately characterize these zooplankters, despite there potential importance in ecosystem function.

For smaller forms, the best advice is to employ finer meshed mesozooplankton nets, with larger cod-ends, and that ideally some sorting/identification of samples occurs pre-preservation. This must be traded off against potential degradation of the samples during pre-processing, a concern that can be reduced my maintaining the samples as cold as practical. Quality of the preserved material is critical to the subsequent ability to identify it.
For larger, more robust scyphomedusae, the MOCNESS and RMT nets may be the only way to sample enough water to accurately determine the density of these relatively rare species. Regrettably, there is no simple and single solution to working with all gelatinous groups.

3.4 Zooplankton Photography

Photography of zooplankton is best done on living material that reflect the natural colour and transparency of the organisms, and these are best collected as noted previously. Larger organism can be maintained and imaged in phototanks or plankton kreisels (Raskoff et al. 2003) using standard (35 mm) camera technology. Although digital photography using "street" cameras mounted to microscopes is relatively affordable, quality photographs require the highest quality optical and camera system. Except for very large specimens, clear pictures are generally only possible while the ship is on station, with engine and ship vibration/movement at a minimum. Good photographs require substantial effort, and duties of those responsible for photographs should be allocated accordingly.

3.5 Fish sampling

See separate fish sampling protocols. These apply to fish collected by both pelagic and demersal sampling methods on the CAML website. [Weblink]

3.6 Cephalopod Sampling

See separate cephalopod sampling protocols. These apply to fish collected by both pelagic and demersal sampling methods on the CAML website. [Weblink]

3.7 DNA Barcoding protocols

Separate DNA barcoding protocols have been developed. DNA Barcoding and other molecular techniques will be useful for establish the identity of species, especially gelatinous zooplankton. The protocols can be found at the following websites. [Weblink]

4. References – *work in progress*

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Appendix I.

Additional Methods of Gathering Information

CTD-Bottle rosettes
Provides basic oceanographic profiles of salinity, temperature, as well as oxygen, light profiles and fluorometry to help explain horizontal and vertical distribution patterns. The CTD also provides a platform for water bottles for sampling protist and nutrients and also serve as a platform for camera/video systems.

Acoustics
Passive acoustics monitor the activities of whales, using hydrophones, moorings and acoustic curtains. Active acoustics assess the stocks of pelagic species eg. CCAMLR surveys of krill using split-beam echosounders with 38, 120 and 200 kHz and EK500 (can be quantified in a relative framework).

Moorings and drifters
Passive acquisition of data eg. AUDOUS. Images may be collected this way.

Images and video identification systems
Important for gelatinous zooplankton that cannot be properly captured or preserved by conventional techniques. Provides information on function and movement.

Remotely Operated Vehicles
Vehicles gather data and images, may have pumps and arms to grab specimens. *ROV Isis* deployed to provide information on vertical migration of scattering layer with diel cycle.

Higher Predators
Body parts of prey in the stomachs of their predators indicate trophic pathways and energy flow in the ecosystem. For example, squid beaks from whale, seal and seabird stomachs; fish otoliths seal and seabird stomachs. Barcoding of tissue may be used to identify stomach contents, by reference to a library of known organisms.

Detection of aggregations
Organisms in the pelagic zone may aggregate with oceanographic phenomena. Aggregation patterns may be detected by satellite information and location of fishing vessels. Nutrient data indicates highly productive areas, sometimes shown by sea surface colour and temperature. Aggregations of seabirds indicate feeding areas. The distribution of sea ice is visible on NOAA synthetic aperture radar.

Biologgers
Biologger packs and cameras on seals and whales provide data eg. CTD thermal structure of the water column. Areas inaccessible by ships can be sampled, eg. under permanent pack ice.

Commercial fishing vessels
Commercial gear may sample species that are not caught by other methods. Intensive and repetitive sampling in a location may yield rare species. Catch per unit effort data assists in stock assessment. Fisheries and CCAMLR surveys eg. ground fish surveys at South Georgia, Kerguelen, and Heard Island provide abundance and distribution data, possibly stock

assessment. Historical records of the exploitation of whales, seals, seabirds and krill indicate the level of human disturbance of the pelagic ecosystem.

Tourist vessels
Observations may assist on some projects eg. seabird distribution, whale movements.

Parasites
The identity and distribution of parasites on their pelagic hosts provide population-level information and elucidate trophic pathways. See separate protocols promised by Eric Hochberg.

Tissue samples
Samples of tissue from pelagic organisms provide information on gene sequence (barcoding using Coenzyme 1), stable isotopes, heavy metals, lipids, fatty acids, calcification (erosion due to ocean acidification), UV effects.

Swath mappers and bathymetric data
Bathymetrical data show dropoffs and canyons that may be important for the distribution of pelagic organisms. For example, eggs and larvae may be located in relation to canyons.

Appendix II.

The following laboratory and chemical supplies are recommended for processing and preserving the samples and have been modified from the CMarZ protocols.

Ethanol – 95-100% non-denatured grade see the CAML Barcoding protocols http://www.caml.aq/barcoding/index.html.
Formalin* – buffered with sodium tetraborate (borax) at 2.5g per 100 ml (Appendix II). Note: 100% formalin is normally 37-40% w/v formaldehyde solution.
Steedman's Solution* – special buffered formaldehyde solution required for CCAMLR (Appendices II and III)
Gloves and Googles* – for handling chemicals
Material Safety Data Sheets* – for ethanol (UN 1170) and formaldehyde solution (UN 2209)
Plankton splitter – Motoda Box Splitter or Folsom Splitter (CCAMLR recommended) (see Omori and Ikeda, 1984). Note: calibrate or determine the aliquot volumes of the splitters as they are not always equal.
Sample jars and tubes – various sizes
Funnels
Sieves – fitted with mesh the same or smaller than the plankton net used for collection.
Cap Sieve
Buckets
Forceps – general forceps, fine watchmaker forceps (e.g. INOX No.4 or 5), fine-tip feather light stork-bill forceps (these are soft and will not crush the specimens)
Sorting dishes – small to large. Photographic developing trays or coloured cat litter trays are useful.
Spoons – various sizes for gelatinous and delicate zooplankton
High resolution camera – useful for photographic zooplankton, especially gelatinous or other zooplankton that do not preserve well
Balance or scales – for weighing catches and to aid in subsampling. Some ships can use electronic balances if conditions are smooth. Old-fashioned kitchen scales or spring (fishing) scales are also useful.
Internal labels – 100% cotton rag or waterproof paper/card.
External labels (sticky)
Electrical tape – sealing lid on jars tubes
Pencil – for logbooks, and internal labels
Fine tip permanent marker – external labelling
Log books – sampling/trawl log book and laboratory log book (see below)
Sampling protocols

***** Formaldehyde (or formalin) is a carcinogen and appropriate protective clothing, lab coat or coveralls, gloves and goggles should be worn. Preserve the samples and handle the chemicals in a well ventilated area or fume cabinet.

Appendix III.

General notes on preservatives

Buffered formalin.
Formalin concentrate is usually purchased as 37 to 40% w/v or v/v formaldehyde solution. It is acidic and will eventually destroy samples and dissolve the shells of calcareous organisms such as pteropods. Formalin and needs to be buffered with sodium tetraborate (borax) at the rate of 2.5g for 100ml of formalin, or 500g in a 20 litre drum. Buffered formalin should be prepared in advance. Large volumes of 20 litres or more can take 1-3 months to buffer or cure and regular shaking of the drum is recommended to ensure curing is complete. There is often an excess of borax settling out. The pH should be about 8, close to the pH of seawater and formalin which has bee buffered properly will have a more stable pH in the preserved samples. Do not attempt to buffer at the time of preserving the zooplankton as the correct pH may not be achieved and may vary overtime. Buffered formalin may denature and solidify as paraformaldehyde if kept very cold, e.g. on deck or in an external cabinet in polar conditions. The preservative is rendered useless if this occurs as it needs to be reheated and acidified to reform the formaldehyde and this is a dangerous practice. A 5% formalin solution is equivalent to 2% formaldehyde.

Steedman's Solution
Steedman's Solution (Steeman 1976) is a formalin with propylene glycol (1,2-propanediol) and propylene phenoxytol (1-phenoxy-2-proponal, phenoxypropanol, propylene glycol phenyl ether CAS 770-35-4). The propylene glycol keeps crustacean shells soft making it easier to straighten them for length measurements – very useful for taking body length measurements of krill. Propylene phenoxytol acts as a narcotizing agent or relaxant (Steedman 1976). Phenoxytol (2-phenoxyethanol, ethylene glycol phenyl ether CAS 122-99-6) can act as a relaxant but is also antimicrobial agent, often used in food and cosmetics, and can be substituted for propylene phenoxytol in the same proportion.

Stock solution (per litre)

Buffered formalin (40% w/v formaldehyde)	500ml
Propylene glycol	450ml
Propylene phenoxytol	50ml

Steedman's solution is usually used as a 10% solution in sweater, equivalent to 5% formalin. Steedman's solution will not solidify in old conditions.

Formaldehyde may be released from preserved specimens and should be avoided. Processing or counting of zooplankton should be done in a fume extraction system or cabinet. Also samples can be placed in a weak solution of Steedman's without formaldehyde, or just in filtered seawater during processing.

Processing solution (per litre)

Propylene glycol	22.5ml
Propylene phenoxytol	2.55ml
Filtered seawater	975ml

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