Census of Antarctic Marine Life (CAML)

Uniform Sampling Protocols – Pelagic – Continuous Plankton Recorder (CPR)

Dr Graham Hosie - February 2006

GENERAL SAMPLING PROTOCOL
(Extracted and modified from Hosie et al. (2003) Progress in Oceanography, **58**, 263-283)

The principal survey area is from about 0° to 160°E and south of approximately 48°S to the Antarctic coast representing an area of ~23 × 10^6 km^2. The RSV *Aurora Australis* deploys CPRs on each voyage, either on routine supply routes or on dedicated research cruises between Australia and the Antarctic stations. Sampling usually commences just north of the anticipated SAF (~48–50°S) and finishes at the ice edge or near the continent. CPRs are not used when sea-ice is present. Sampling on return routes normally commences soon after leaving a station or clearing the ice edge. For much of the year, when ice cover is extensive, there are just two tows on each of the southward and homeward legs. Volunteers amongst the expedition members are used on board most voyages and are provided with four prepared cassettes for loading into the CPR body. Units are serviced on return to the Australian Antarctic Division. In high summer, January–February, the ice cover is minimal, allowing a greater spatial coverage for sampling. A dedicated CPR person participates in at least one voyage during that period so that the CPR can be serviced and cassettes reloaded with new plankton mesh allowing up to 10 or more tows to be completed. In January–February 1998, 11 tows were completed between Hobart and Mawson station producing 2365 nautical miles of continuous records on the southward leg and 1367 miles on the return leg. On each Japanese Antarctic Research Expedition (JARE) voyage, tows commence around 45°S on 110°E, close to December 6, and finish at 60°S or the ice edge. Northward tows on 150°E start around 12–14 March at 63°S. Tows on the southward and northward routes are normally 300 nautical miles long to allow for net and CTD sampling at routine annual stations set at intervals of 5° latitude. When possible, additional tows eastward along 63°S between 80 and 150°E from 4–9 March are also conducted. This is an area which has exhibited a strong longitudinal difference in zooplankton composition and other biological features. Sampling on board *Polarstern* is the same as described for *Aurora*

Australis, and the same applies for other research vessels used opportunistically. The *Aurora Australis* has an automatic data-logging system that records various oceanographic, meteorological and navigational data, e.g. temperature, salinity/conductivity via a thermosalinograph (SeaBird SBE21), fluorescence (Turner Design TD 10), high-resolution water temperature, wind speed and direction, solar radiation, photosynthetically active radiation (PAR), UV radiation, and barometric pressure. An Ashtec 3DF GPS provides data on attitude and position of the ship. Table 1 shows environmental data routinely recorded and available for comparison. The seawater intake for the oceanographic measurements is at 7 m depth. This is close to the average towing depth of 8 to 10 m for CPRs deployed from *Aurora Australis* as determined by a miniature time/depth recorder placed inside a unit. On dedicated cruises, hydroacoustic data are also collected, using 12, 38, 120 and 200 kHz frequencies. The last three frequencies are used to survey the distribution and biomass of krill. *Shirase* has a similar underway environmental system that records water temperature, salinity, fluorescence, oxygen, OPC and GPS data etc. Similar systems are used on other vessels.

Table 1
Typical environmental data recorded by ships' automated systems. * indicates data commonly recorded by all survey ships.

Sea-water temperature*
Salinity & conductivity*
Fluorescence*
Light—Photosynthetically Active Radiation (μ-Einstein m_2 s_1)
—Solar Radiation (Watts m_2)
UV (Watts m_2)
UV-B (MED/HR (Minimum erythema dose per hour))
Wind speed & direction
Barometric pressure
Hydroacoustics—12, 38, 120, 200 kHz
Optical Plankton Counter (*Shirase*)

CPR silks are cut into sections representing five nautical miles. A computer program written in Visual Basic is used to determine the length and number of sections to be cut. The programme enables time-stamped position and selected environmental data, recorded at one-minute intervals on the ships' data logging systems, to be assembled into data sections of five nautical miles each. Environmental data are averaged for the same sections and subsequently linked with the zooplankton data through the latitude and longitude assigned to the end of each section. This program is used in both the Australian Antarctic Division and Japanese laboratories. The five nautical mile segments were chosen in order to make comparisons at a higher resolution in the vicinity of frontal zones, but the data can be assembled into still larger bins, e.g. ten nautical miles as used by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS). After the silks have been cut, large specimens easily identified by eye (e.g. euphausiids) are removed and counted and the remaining species identified and counted using a microscope. Zooplankton analyses are conducted at the Australian Antarctic Division and the National Institute of Polar Research, Tokyo, laboratories, and will also be conducted at the Alfred Wegener Institute, Bremerhaven.

Occasionally, the CPR fails, either at the start of a run or part way through a run, the silk can be damaged or poor preservation of the silks has occurred after the tow. Partial runs are still usable. Severely damage silks are discarded and poorly preserved silks are also not processed because of likely loss of specimens. An examination of the silks by microscopy usually shows poor preservation of the plankton. However, damaged silks and poor preservations is rare and overall we generally have about a 90-95% success rate.

CPR SAMPLING DURING CAML

CPRs to be deployed during CAML will be the AAD designed Type II Mark V units and the older Type II Mark II or III designs. Differences are mainly in construction of material and ease of use, but either wise perform exactly the same. Units will be supplied by Australia, Japan (AAD design), or possibly SAHFOS (older design). Silk will be supplied from the same locations. Sampling will not vary from the current protocols of the SO-CPR Survey as described above, although runs can be shortened or interrupted to accommodate other sampling. However, short runs of less than 120 nautical miles are not ideal as it makes processing of silks difficult.

Post-cruise processing is still to be confirmed, but likely to be processed in the three main laboratories at AAD, NIPR, and AWI, and by a select group of analysts. The CPR is not ideal for sampling gelatinous zooplankton and some species or developmental stages are difficult to identify after damage or distortion through trapping on the silk or preservation. New genetic techniques have been developed for the identification of plankton from formalin-fixed CPR samples, using the principles of the antigen retrieval technique (hydrolysis, alkali conditions and high temperatures) to break methylene bridges and remove methylol groups, followed by application of PCR on mtDNA 16s rRNA gene sequences (Kirby and Reid, 2001; Kirby and Lindley, 2005). This technique and developing new molecular techniques, or other suitable gene sequences, will probably be used to help identify difficult material. Where appropriate, unknown species will also be sent to the appropriate taxonomic specialists.

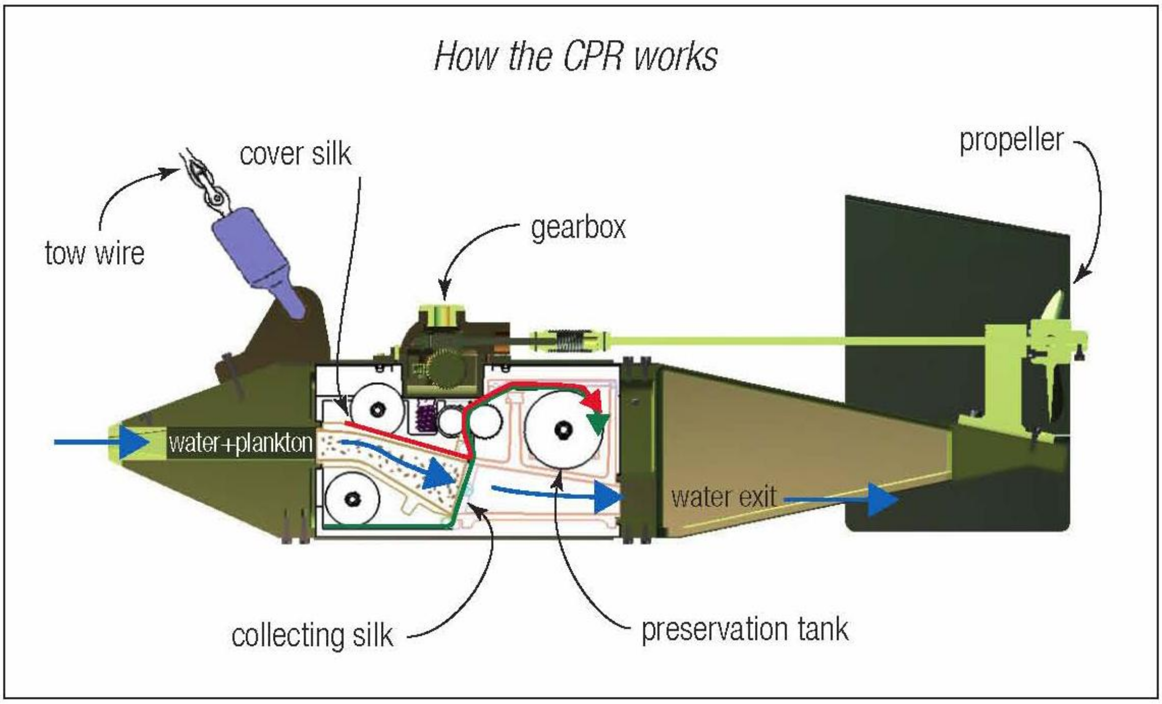
Hosie, G.W., Fukuchi, M. and Kawaguchi, S. (2003) Development of the Southern Ocean Continuous Plankton Recorder Survey. Progress in Oceanography **58** (2-4), 263-283

Kirby, R.R. and Lindley, J.A. (2005) Molecular analysis of Continuous Plankton Recorder samples, an examination of echinoderm larvae in the North Sea. Journal Marine Biological Association of the United Kingdom. **85**, 451–459

Kirby, R.R. and Reid, P.C., (2001) PCR from the CPR offers a historical perspective on marine population ecology. Journal of the Marine Biological Association of the United Kingdom. **81**, 539–540.

HOW THE CPR WORKS

The CPR is a self-contained mechanical automatic sampler towed 100 m behind the ship at about 10 m depth. It is towed at normal ship's speed and can operate in nearly all sea conditions. As the CPR is towed along, water and plankton enter a small 1.25 x 1.25 cm aperture in the nose cone, which then expands into a wider collecting tunnel, slowing down the water flow. Plankton are then trapped between two sheets of 270µm mesh silk, 6.1 m long x 15cm wide, loaded in a removable cassette. The silk is the same type used since Sir Alister Hardy's first CPR deployment. All CPR surveys source their silk, pre-cut and marked, from SAHFOS to ensure standardisation between surveys. The silk and plankton "sandwich" are wound on to a take-up spool inside a formalin preservation chamber, all driven by passing water turning an external propeller. Regardless of the speed of the ship, the sheets of silk are advanced at a fixed rate of 1cm for every nautical mile travelled. Each tow lasts approximately 450 nautical miles. Cassettes can be quickly changed allowing continuous sampling over long transects. Each CPR unit usually has 3 internal cassettes.



INSTRUCTIONS FOR USE OF CONTINUOUS PLANKTON RECORDER ON RSV *AURORA AUSTRALIS*

Loading the inside mechanism:

- Remove the 4 nuts on the left hand side (1) of the external mechanism using the allen keys provided. Pull off the side plate.
- Loosen the nuts on the plate on the top side of the external mechanism until the plate is able to be moved back and forth about a centimetre each way.
- Remove internal mechanism from its small box. With marking pen provided, draw a line across the silk (5) along the edge of the storage tank gasket (the line is shown dotted at A). Mark 'START' on silk.
- Slide mechanism into external casing - but not right home, making sure the locking tabs (2) are clear of the bottom rail.
- Slowly press the internal mechanism home. Slide the top plate forward until you feel the gears have adequately meshed. Remember that the gears do not have to be meshed too tightly. Rotate the propeller shaft a few times, keeping your hand on the coupling (6). You will be able to feel if the gears are meshed correctly. The right hand side plate can be removed to ensure the gears are properly meshed. Tighten the nuts on the top plate of the external mechanism.
- When the internal mechanism is correctly fitted and the gears have meshed, drop the locking tabs (2), making sure these are vertical.
- Replace the side plate. Tighten the 4 nuts. You may need to smear the nuts occasionally with Loctite (provided in kit) to prevent seizing.

Unloading the inside mechanism on completion of a tow:

- Remove the 4 nuts on the left hand side (1) of the external mechanism using the allen keys provided. Pull off the side plate.
- Swing locking tabs (2) on inside mechanism clear of the bottom rail of the casing.
- Slide inside mechanism out of casing.
- Mark silk as above. Label with 'FINISH'. Record in the log the number of the segment of silk at or near the top of the tunnel. (See: **Filling in the logbook**).
- If the machine is to be shot again with the same inside mechanism, do not disturb the wire on the fusee (9) and drum (10). If this wire is displaced, a new internal unit must be loaded. (NB Re-shooting the same CPR is not a usual practise, but if the vessel has to stop unexpectedly, then the CPR unit may have to be brought aboard. In this situation, ensure that the silk is marked 'FINISH', advance the silk about 5-10 cm and remark 'START'.)
- Withdraw front locking rod (12) and open lid of storage tank (7).
- Slacken clips (8) on side of mechanism, and pull the two ends of the mechanism apart to separate the rollers.
- Wind all the silk onto the spool in the storage tank by turning both the fusee (9) and drum (10) anticlockwise. It does not matter if the fusee wire comes adrift at this stage.
- Unscrew the spindle cap (11) and withdraw the storage spool spindle.
- Lift the storage spool out of the tank. Secure the silk from unwinding with two rubber bands, one at each edge. Place each used silk in a 2 litre jar provided, add 100 ml of buffered formaldehyde, and fill with seawater. Include a waterproof

zooplankton label inside the jar and label the top of the jar with the run number CPR 1, CPR 2, etc, the number of the internal mechanism, and the date and position of deployment.
- Close the storage tank lid, reinsert the locking rod and tighten the clips holding the two parts of the mechanism together. If you wish, you may remove the fusee wire and discard it. Immerse the internal mechanism in fresh water for an hour, then remove and allow to air dry. Thoroughly spray with CRC.

Running the CPR:

- The CPR normally runs for 450 nautical miles, or about 37 hours if towed at 12 knots. It can be towed between speeds of 3 and 25 knots. This will leave enough silk unused to make a number of turns around the collecting spindle to protect the samples collected. **Remember that the attached map shows only an approximation of the proposed ship's track, which is likely to be different during the voyage!**
- The port Gilson winch is used for towing the CPR. Approximately 80-100 m is paid out. A mark has been painted on the wire, but is probably long gone. Ask the crew to re-mark the wire when the CPR is first deployed.
- The CPR unit is reasonably robust but not by comparison to several thousand tonnes of icebreaker. To avoid any damage of the CPR against the stern of the ship during deployment or retrieval, ensure that the gantry is all the way aft. This is more of a problem during retrieval as the CPR surges back and forth in the ship's wash. In recent years the ship has been slowed somewhat during retrieval.
  This is not entirely necessary though, and may be up to the Master's discretion. Once sufficient wire has been let out, so that the unit is below the stern (10-15 m), then the gantry can be brought in board and the ship can proceed.

- The internal mechanism has a storage tank filled with a small amount of buffered formaldehyde; ~ 70 ml at 40% w/v concentration prior to use, and much diluted but to an unknown concentration during the tow. Please be careful when loading and unloading the unit to avoid any possible exposure to fumes. This is not a problem out on deck, but it is best to work under the port side fume hood of the wet lab when processing the silk at the end of a tow, i.e. when removing the silk roll for preservation and storage. More importantly, the CPR will be dripping a substantial amount of water as it is brought on board. This water will be contaminated with some portion of formaldehyde. To avoid being splashed/sprayed by this water do not stand under or near the CPR as it is brought in board by the gantry, until it is lowered close the deck, eg waist height.

Filling in the logbook:

- Fill in the log form for each CPR deployment. You can get positions, times etc directly from ship's log located on the bridge. It is important to update the log every few hours, especially noting any changes in the ship's course. **See example given!**

- Make sure that the internal mechanism number (1, 2, 3 or 4 etc), located on the top of the mechanism, is recorded in the log for each deployment - each mechanism has its own peculiarities, they are not all exactly 2" segment = 5 nautical miles.
- Note in the log the number of the segment of silk at or near the top of the tunnel, as well as marking the top of the silk. **IMPORTANT: Remember to mark the silk before and after the tow! However, if the unit has to be brought on board for whatever reason (eg if the ship stops), it is still necessary to mark the silk if the unit is to be redeployed again!**
- If an internal mechanism has failed (jammed, silk piled on, drive wire broken) record all details so the likely cause of the problem can be assessed.
- If the external mechanism becomes damaged in any way then do not continue the program. Minor scrapes and scratches are not important but obviously any damage likely to impair the performance or hydrodynamics of the CPR is obviously serious.

If there are any problems or doubts then call, fax or e-mail either of us.

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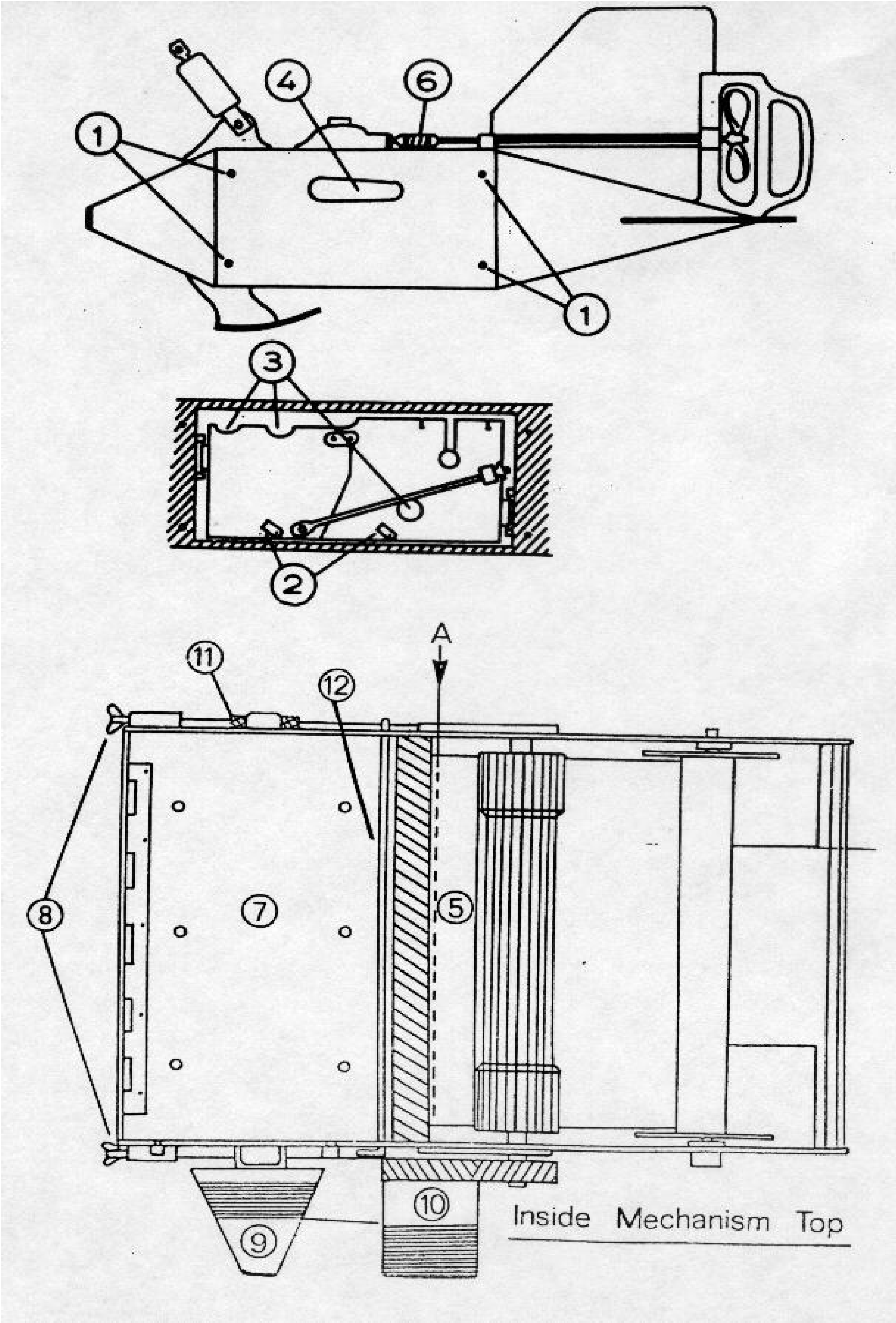
CONTINUOUS PLANKTON RECORDER

Please note: The following diagrams refer to an older model of a CPR and some of the features shown here do not apply to the current model.


1
4
6
1
3
2
A
11
12
8
7
5
9
10
Inside Mechanism Top

SOPEM - LOG FORM FOR CONTINUOUS PLANKTON RECORDER

Please return this log form after completion of tow to:

DR GRAHAM HOSIE
AUSTRALIAN ANTARCTIC DIVISION
CHANNEL HIGHWAY, KINGSTON, TASMANIA
7050

14·31

CPR # 1

Outside Mechanism No. ......-...... Name Of Ship.... Aurora Australis .... Route From.... Hobart ....

Inside Mechanism No. ......2...... Master .... Peter Pearson .... To .... Casey ....

Writer of Log .... Karin & John ....

DATE	TIME (GMT)	DISTANCE	OPERATIONS Shot	OPERATIONS A/C	OPERATIONS Haul	POSITION Latitude & Longitude	COURSE	REMARKS	General Remarks on Weather Conditions During Tow
25/1/00	2155		✓			49° 32.872'S 140° 35.851'E	219°T		Overcast; Calm. 30kt SW
		132.03							
26/1/00	0900	—		✓		51° 18.5'S 138° 31.5'E	220°T		Moderate swell/sea.
		168.35							
27/1/00	0000	—		✓		53° 2[illegible].9'S 135° 2[illegible].4'E	220°T		Moderate swell/rough sea.
27/1/00	0955	113.93			✓	54° 52.572'S 133° 25.181'E	220°T		Moderate/rough sea.
		414.31nm						Segment #91 at top of tunnel.	

EXAMPLE