NATIONAL REFERENCE STATIONS

BIOGEOCHEMICAL OPERATIONS MANUAL

CSIRO Ocean and Atmosphere Report for IMOS

Version 3.3.1

July 2020

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Document Version Control

Version	Date	Change Description	Author
3.3.1	June 2020	Larval fish procedure updates Nutrient analysis clarification of use of unfiltered samples Links to TSS and flow cytometry reports added	C. Davies
3.3	Aug 2019	Larval fish procedure included Hydrochemistry section updated Microbial section updated	C. Davies
3.2.1	Jan 2019	DOI added to report, required citation added	C. Davies
3.2	Dec 2017	NEW TSS procedure NEW CTD pre checks Appendices moved to separate documentation to allow for easy updates	C. Davies
3.1.2	May 2017	Sampling procedures and post sampling processing for the pooled sample, pigment samples and flow cytometry changed and updated Update of contact details for salinity and nutrients from Val Latham to Stephen Tibben	V. Latham
3.1.1	Mar 2017	Appendix 10 - Setting up the drop net has been added	V. Latham
3.1.1	Mar 2017	Since automation of all BGC data uploading, the section has been updated	C. Davies
3.1	Feb 2017	Added updated comments from Michele Scuza, Ryan Crossing, Marty Hidas Removed the genetics zooplankton sampling and TSS sampling now only from surface sample	V. Latham
3.0	Mar 2016	**NOTE: Draft for Comment** The entire document was reviewed and the following amendments made: • A table of contents added • Sampling procedures for ichthyoplankton introduced to the manual • Existing sampling and post sampling protocol rewritten, with some photos added to the text and links to new videos of sampling and filtering procedures • Improved instructions for labelling samples • Integrated water column name change to pooled depth sample • Most methods rewritten and/or updated to reflect current practices • Contact details for samplers and analysts updated • More information on handling the CTD including two documents in the Appendix on preparing the CTD for the field and processing the data • New procedures for uploading data to AODN (formerly eMII) • More information about data quality including Table of flagging codes	V. Latham
2.2	July 2012		G.W. Critchley

2.0	Sep 2009		G.W. Critchley

Citation

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Contents

1 Introduction

The NRS program started in 2009 with 9 stations, 2 of which were discontinued in 2013.

The active stations are:

- MAI Maria Island, TAS
- KAI Kangaroo Island, SA
- ROT Rottnest Island, WA
- DAR Darwin, NT
- YON Yongala, QLD
- NSI North Stradbroke Island, QLD
- PHB Port Hacking, NSW

The discontinued stations are:

- ESP Esperance, WA (May 2009-July 2013)
- NIN Ningaloo, WA (November 2010-August 2013)

Three of the active stations, MAI, ROT and PHB, build on oceanographic time series data collection sites that go as far back as the early 1940's. IMOS NRS Biogeochemical sampling aims to enhance and expand on the national coverage of time series data.

This manual outlines best-practice techniques in biogeochemical and blue-water oceanography for ensuring the output of reliable, quality data to the end-user community. The aim is for sampling, analytical, and reporting standards to be at least equivalent to: the WOCE (World Ocean Circulation Experiment) and JGOFS (Joint Global Ocean Flux Study) studies.

Procedures for sampling, analyses and data handling are outlined here for the Australian IMOS National Reference Station (NRS) project. Use of these procedures will ensure consistency in sampling and analysis, leading to high quality data gathering.

Monthly collection of biogeochemical data from most NRS sites gathers information on seasonal, annual and long term variability in Australian marine ecosystems.

Moorings are deployed at the NRSs, with instrument arrays at 2 depths, some with a third surface meteorological surface buoy. These record a suite of time series data.

The moored instruments are Sea-Bird™ sensor packs modified by WetLabs™, and as a package they are marketed as Water Quality Meters (WQM's). These packages measure conductivity, temperature, depth, time (UTC & Local), dissolved oxygen, fluorescence and turbidity at two set depths. **NOTE**: The moored sensors will continue to be referred to as WQMs throughout this Version of the manual, but from July 2017 they are being phased out as they reach their end-of-life and replaced with Sea-Bird SBE37s. The SBE37s measure conductivity, temperature and pressure.

Biogeochemical results are used to monitor and assess the performance of the moored WQM's as well as creating an independent suite of data obtained from a number of depths that cannot be sensor determined.

IMOS is a national collaborative research infrastructure, supported by Australian Government. It is operated by a consortium of institutions as an unincorporated joint venture, with the University of Tasmania as Lead Agent. www.imos.org.au.

More information on NRS can be found at
http://imos.org.au/facilities/nationalmooringnetwork/nrs/

2 NRS Locations

The locations of the NRS are shown in Table 1 and Figure 1.

Table 1 – NRS codes, depths and locations

Site	Operator	Station code	State	Start-up Date	Nominal Sonic Depth	Nominal Longitude	Nominal Latitude
Maria Island	CSIRO	MAI	TAS	Oct 1944	90m	148.233333	-42.596667
Kangaroo Island	SARDI	KAI	SA	2008	110m	136.448	-35.836
Rottnest Island	CSIRO	ROT	WA	Apr 1951	50m	115.416667	-32
Darwin	AIMS	DAR	NT	2009	20m	130.7827	-12.417467
Yongala	AIMS	YON	QLD	2007	29m	147.26	-19.306
North Stradbroke Island	CSIRO	NSI	QLD	2008	60m	153.580217	-27.388917
Port Hacking 100	SIMS	PHB	NSW	May 1953	100m	151.25	-34.083333
No longer sampled							
Esperance	CSIRO	ESP	WA	2008	50m	121.85	-33.933333
Ningaloo	AIMS	NIN	WA	2010	50m	113.94665	-21.871733

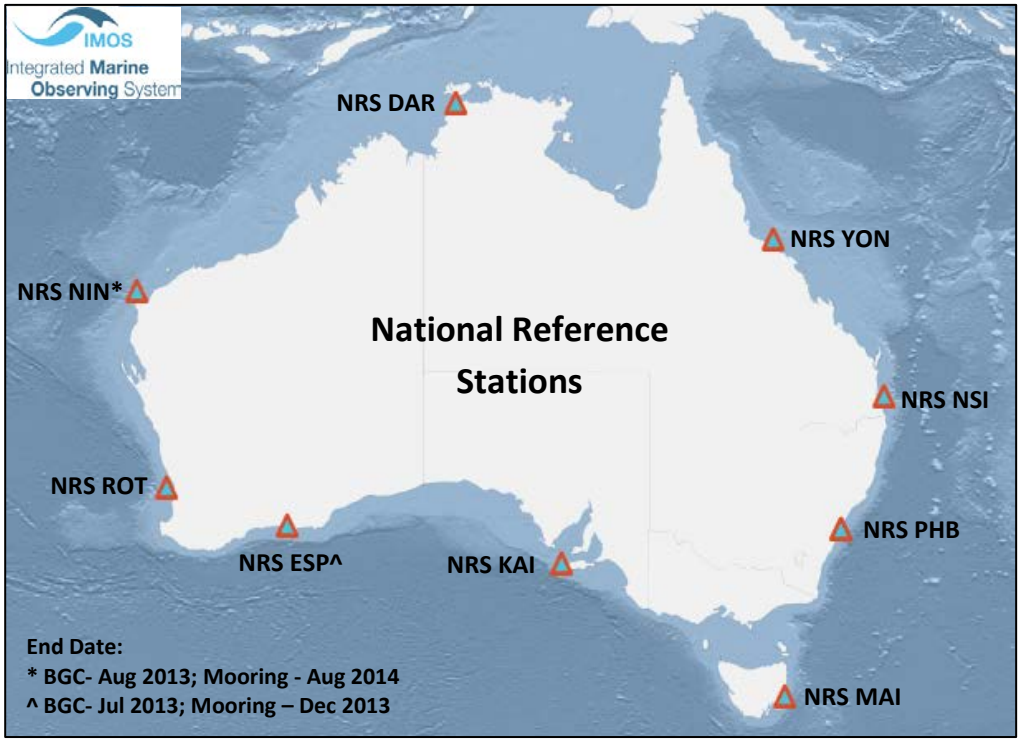


Figure 1 – IMOS NRS Locations

Table 2 – WQM sensor calibration samples

Site	Station code	Sonic Depth	Officer Responsible	Casts for WQM pigment comparisons
				Sampling depths per site (metres)
With WQMs				
Maria Island*	MAI	90m	Dave Hughes	5L at 20m WQM Calibration, 15 litres surface for remote sensing
Kangaroo Island	KAI	110m	Charles James	5L at 20m WQM Calibration, 15 litres surface for remote sensing
Esperance	ESP	50m	Tim Lynch	20 (WQM Calibration only), bottom + 2.5 (WQM Calibration only)
Rottnest Island*	ROT	50m	Dave Hughes	5L at 20m WQM Calibration, 15 litres surface for remote sensing
Ningaloo	NIN	50m	Tim Lynch	20 (WQM Calibration only), bottom + 2.5 (WQM Calibration only)
Darwin	DAR	25m	Craig Steinberg	5L at 20m WQM Calibration, 15 litres surface for remote sensing
Yongala	YON	28.9	Craig Steinberg	5L at 20m WQM Calibration, 15 litres surface for remote sensing
North Stradbroke Island	NSI	60m	Anthony Richardson	5L at 20m WQM Calibration, 15 litres surface for remote sensing
Port Hacking 100*	PHB	100m	Tim Pritchard	5L at 20m WQM Calibration, 15 litres surface for remote sensing

3 Safety Considerations

In order to avoid any potential injury to personnel during the field sampling, and the onshore post–sampling treatment and preservation, ensure that the following points are adhered to:

3.1 Boat operations

When conducting a sampling excursion we have determined that in order to carry out the work safely and efficiently it is essential that there be a minimum vessel crew of three (3):

- A certified coxswain (minimum requirement) or skipper
- 2 persons wholly dedicated to undertaking the sampling requirements
- One of the three staff needs to be licensed to drive a heavy vehicle, if the vessel requires trailering to and from a NRS departure and arrival point.

If, when conducting the sampling there is enough vessel drift which leads to a large wire angle and reaching for the wire is uncomfortable or dangerous, it is recommended that an open hook attached to a short length of rope fastened securely to the rails be used to hold the wire/cable at a comfortable reach and as close to vertical as possible. When slipping the hook around the cable during a Niskin cast, do so below the bottle in order to avoid accidently knocking open an end cap of the bottle, causing sample contamination or loss.

3.2 Personal kit

Each member of the sampling team should wear:

- Steel capped boots or shoes,
- a self-inflating safety vest, and
- gloves.

3.3 Weather considerations

If the coxswain or skipper decides that conditions are not safe to conduct the work, they can, without blame, cancel sampling at any stage of the excursion.

4 Sampling Parameters

Samples are collected to measure the following parameters:

Carbon

- Total Dissolved Inorganic Carbon (TCO2)
- Total Alkalinity (TALK)

Hydrochemistry

- dissolved oxygen– only collected at MAI and ROT
- salinity
- nutrients (Nitrate/nitrite, silicate, phosphate, ammonia, nitrite)
- total, organic and inorganic suspended matter (TSS)

Biological

- Phytoplankton
    - pigment composition / HPLC
    - phytoplankton microscopy (species composition)
    - picoplankton / flow cytometry
- Zooplankton
    - dry weights
    - community composition
    - average size of the zooplankton community

Physical/profiling instrument measurements

- Secchi disk
- CTD with sensors for
    - temperature
    - pressure
    - conductivity
    - fluorescence
    - turbidity
    - dissolved oxygen

Genomic analysis

- Microbial (including phytoplankton)
- Frozen zooplankton

Ichthyoplankton

- net tows at MAI, PHB, NSI, ROT and KAI

5 Gear check list

General

- Field log sheets, clipboard, pencils, permanent markers
- MSDS for formalin and mercuric chloride

Seabird CTD

- Make sure battery has enough charge

Zooplankton drop net

- 12mm silver rope for drop net, length appropriate for station depth
- 2 x white plastic 500mL, 1 x large black jars and labels
- Stopwatch
- Formalin, 100mL

Niskin gear

- 5L Niskin bottles and bronze messengers
- Weight for line

Dissolved oxygen

- DO bottles
- DO reagents in dispensers

Carbon sampling

- Blue box with glass bottles,
- mercuric chloride and dispensing kit

Salinity

- Bottles, inserts, sampling tube in road case

Nutrients

- Esky and icepack, labelled tubes in rack

Integrated sample

- 3L jug,
- 5L carboy or 1L lugol's container,
- Large plastic funnels
- 5mL lugol solution
- Shadecloth

WQM samples

- 1 x 5L carboy
- 1 x 20L carboy
- Eskies and ice

Ichthyoplankton

- Net
- Flowmeter
- 3 x 1.2L containers
- 50 ml Formalin and 100 ml ethanol
- Temp HOBO
- Wash bottle
- Weight

6 Sampling Protocol

The sampling site should be located as close to the mooring as possible. If the vessel drifts then reposition as appropriate.

The sampling order will depend on the site. There are videos available for carbon, salinity and nutrient sampling and for the bottle leak test procedure at https://www.youtube.com/channel/UCXp5vxj2TaCatkura7vMYOA
(NOTE: some procedures have changed in 2017 and the videos have not been updated to reflect those)

6.1 The field sheet

The field sheet should be filled in with arrival time and latitude/longitude co-ordinates from the GPS. As sampling progresses further information such as bottle numbers and sampling times needs to be added to the sheet.

Each NRS site has a unique field sheet which details depths for each type of sample and the order of sampling. A sample copy of the Field Data Sheet is available here. The Field log sheet is to be completed and emailed to MariaNRSBGC@csiro.au and uploaded to the AODN.

6.2 Secchi Disk Observations

- Make the Secchi disk measurement on the lee side of the vessel in order to minimize wind driven surface ripples and, if possible, in the sun.
- Lower the disk until it is just no longer visible, then raise the disk until it becomes visible again.
- The average of these two depths is considered to be the limit of visibility.
- Record the data for Secchi disk depth in metres on the field log sheet.

Do not wear sunglasses – particularly polarised lens type - as this will introduce error in the readings.

6.3 CTD Profiles

A Seabird 19plus V2 Seacat Profiler equipped with dissolved oxygen sensor in addition to the standard sensors of temperature, pressure, fluorescence and conductivity is used to measure a water column profile from surface to 2.5 metres from the bottom.

Before departing, for the field work most importantly check the battery has enough power. The voltage should be above 11.2V. Instructions for checking the battery and other essential pre-run checks can be found in the Pre-run check and field sampling CTD Procedural Guide. Further recommended pre-run checks and maintenance procedures are also contained in this Guide.

Preparation Stage

CTD Pre-Run Check (cheat-sheet)

1. Prepare CTD

- Use ~100mL **1%** Triton-X (T-X) solution to clean the CTD's C/T cell.
- Rinse the cell with ~1L of deionised (DI) water until no T-X bubbles remain.
- Fill the cell with DI water and leave to soak for one hour.

2. Check CTD

- Check physical condition: Cables undamaged, battery cap tightened, bolts/sensor mounts secure.
- Check status: Connect to CTD via SeaTermV2 (Baud = 9600, Parity = None, Bits = 1, Flow Control = None) and input "DS" (Display Status).
- Ensure adequate battery ("vbatt" ≥ 12.0 V) and memory (wipe memory with input "IL").
- Set date and time to UTC ("General" > "Set Date and Time" > input "mmddyyyyhhmmss").

3. Test Zero Conductivity

- After one hour's soak, fill 3 x 600mL PET bottles with DI water.
- Ensure comms with CTD remain active in SeaTerm ("DS") and then up-end one of the 3 bottles into the connected syringe.
- Once DI water begins flowing from the pump's exhaust input "TC" (Test Conductivity) into SeaTerm to initiate conductivity readings in the terminal window. Reading lasts 60s and will require all 3 PET bottles to be used.
- Copy conductivity values into the Pre-Run spreadsheet. Their mean value should be ≤0.001 mS/cm – if not, rinse the cell and repeat the process.

4. Test FLNTU Dark Voltage + DO in Air

- Drain DI water from C/T cell and rest CTD on its side, allowing access to the magnetic switch.
- Cover the FLNTU's **detecting** window with black **electrical** tape (detecting window emits no light when mag. switch "ON").
- With all interior lights off and out of sunlight, turn the mag. switch "ON" and collect ~3 minutes of data.
- Upload cast and extract FLNTU Chl-a voltage, NTU voltage, and DO saturation (%) data into Pre-Check spreadsheet.

5. Fill Fieldsheet Pre-Check Table

- Calculate mean values ($\bar{x}$) and coefficients of variance (CV) for each test and copy these into the NRS sampling fieldsheet.
- Compare these values to those obtained previously, checking for significant deviations.

Deployment

- CTD cast is via a winch line and not a rosette.
- Remove the fluorometer cap and switch the instrument on before deployment.
- Allow one minute surface soak to ensure the pump is working. Tick the check box on the sheet.
- If possible, download the CTD cast to check if it was successful.
- After use, thoroughly rinse the CTD frame and sensors with fresh water.

6.4 Zooplankton sampling method

Equipment:

- drop net with steel collar – refer to Set up of Zooplankton Drop net
- cod end
- 2 white plastic and 1 large black containers
- formalin (100 mL)
- ice and esky

Three samples are collected with the zooplankton drop net:

- two samples are preserved in formalin in the white containers for composition and biomass analysis and one is frozen for molecular work

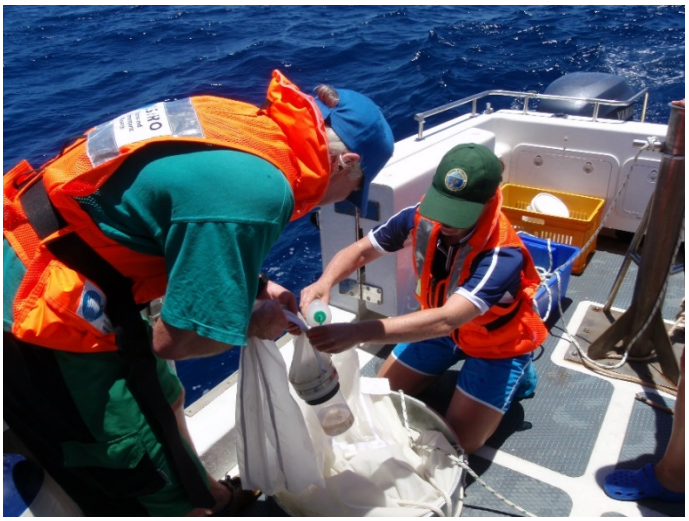
Before the first drop, make sure that the net is wet (spray it down with the deck hose), in order that the net is allowed to fall efficiently through the water column and the cod end is attached.

IMOS NRS ZOOPLANKTON –Preserved in Formalin			
Date	Time	Site Code	Replicate number
Comments			

The net is dropped over the side and must be allowed to free fall at ~1.0 m.s-1. Time the required depth with a stopwatch starting as soon as the collar hits the water, 50m depth = 50 seconds. When the required time is reached haul on the rope to close the net. Record the time, drop time and site ID on the waterproof labels for each sample and on the field sheet.

Haul the net on deck using a winch or pot hauler, the net is no longer sampling so speed is not important. Wash the contents of the net into the cod end using seawater from the deck hose. Reduce the volume in the cod-end as much as possible.

For the first 2 zooplankton samples, empty contents into the white jars, add 50 mL of formalin and the waterproof label. For the 3rd drop empty the sample into the large black jar and leave on ice.



Clearly label both jars by writing site and date on the outside and putting a waterproof label in each one also with date, time of drop and site information.

6.5 Collecting water column samples

These samples are collected using Niskin bottles. The niskins are generally lowered on a weighted line to the correct depths. A series of messengers is attached, the initial messenger at the surface is dropped and triggers the other messengers to close the bottles at the prescribed depth. The CTD and the niskin casts are done separately as none of the boats has a rosette set up. The order of casts may vary depending on station and logistical set up for each boat. The timings of each sample are taken and recorded alongside the data.

Instructions for the use and maintenance is contained in Niskin bottles Information and Maintenance document.

Before taking any samples a leak test should be performed on each bottle.

6.5.1 Leak test

- With the top valve remaining closed, open the Niskin spigot by lining up the metal pin and hole, then push inward

- Visually check for liquid coming out of the spigot, liquid flow indicates a Niskin bottle leak. If the Niskin has leaked, this must be recorded on the field log sheet.

6.5.2 Dissolved Oxygen

General information

Dissolved oxygen samples must be collected first. Pickling reagents should also be located nearby, ready to use.

Sample Collection

- Attach the sampling hose to the Niskin spigot, this is easiest if the pin and hole are not lined up. Open the valve on top of the Niskin bottle

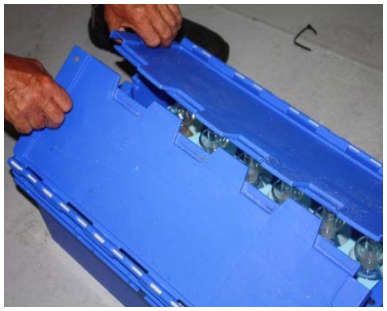
- Open the spigot by lining up pin and pushing inward.
- Repeatedly pinch the tubing to remove the bubbles. Rinse the sample bottle 3 times, pinching the tube to stop flow between rinses to conserve water.

- Insert the tube until it touches the bottom of the flask held at a 45° angle. Slowly allow water to flow into the flask. Do not entrap air in the flask, redo if bubbles form.
- When full, hold the flask upright allowing the water to overflow for roughly 15 seconds (about 2x the volume of the flask). Slowly reduce the flow while retracting the tube from the flask. Flow should be stopped as the tube reaches the surface. Pull the spigot out to stop water flow

- Prime the reagent dispensers by pumping several times into waste containers. Make sure no bubbles are left in the dispensing tip
- Pull up on the dispenser to fill/prime. Insert tip below water level and dispense the reagent 1 followed by reagent 2. Again making sure no bubbles are dispensed.

- After both reagents have been added carefully insert the correct flask lid. The insertion should be done so that no air becomes trapped in the flask
- Tightly hold the lid and invert the flask 20 times, this is to ensure complete capture of the oxygen and the best result possible.

- Gently return the flask to the blue box and fill the conical neck with MilliQ water.
- Close the box lid, as the samples can degrade in the light. Give the bottles another shake approximately 30 minutes after sampling. Recap the reagent dispensers after use.



6.5.3 Carbon

Two types of sample are required for carbon dioxide measurements:

- TCO_2 (total dissolved inorganic carbon) and
- TALK (total alkalinity)

TCO_2 bottles are square with black lids and TALK bottles are round with blue lids. They are supplied in strong boxes for safe transport. Bottles are supplied pre-labelled.

6.5.3.1 Sampling Total Dissolved Inorganic Carbon

As it is a dissolved gas, TCO_2 is sampled as soon as possible after opening the Niskin bottle, usually immediately after dissolved oxygen (DO) sampling.

1. Fit the flexible end of the sampling tube provided, over the Niskin spigot.

2. Start flow through the tube by pushing in the outflow valve. If there is any water flowing from the spigot, note it on the field sheet. Open the upper air bleed and water should flow freely from the spigot. If there are air bubbles in the sampling

tube pinch and release a few times to remove them. Pinch the flexible part of the tube to stop the water flow. Insert the tube to the bottom of the sample bottle and slowly release pressure on the tube to allow water to flow.

3. Fill the bottle to 1/3 full, swirl and invert the bottle to pour the contents out over the sampling tube to rinse both bottle and tube. Do this 3 times. Pinch the tube again then release carefully, trying to avoid creating turbulence in the bottle so as to minimise gas exchange with the sample.

4. Fill the bottle (with the tube still at the bottom) and allow it to overflow with about half the volume of the bottle. Pinch the tube to stop flow and withdraw it carefully from the bottle. The level of sample in the bottle should be as shown in this picture. If it is too full, pour out a little water.

5. Using the pipette provided add 100µL of saturated mercuric chloride solution to the bottle. Keep the pipette tip just above the sample surface. CAUTION: mercuric chloride is toxic. Wash with copious amounts of water if it touches your skin. Please refer to Appendix 1 - Safe work instructions for Handling Mercuric chloride. Screw the cap on tightly and shake the bottle 4 times to distribute the mercuric chloride.

6. Store samples at room temperature in the box provided, and retighten the lids after an hour or so.

6.5.3.2 Sampling Total Alkanity

Alkalinity is sampled immediately after TCO_2 samples are collected.

7. Use the same technique as for TCO_2 sampling. Tip out some water after withdrawing the sampling tube so that the level is as shown here.

8. Using the pipette provided add 100µL of saturated mercuric chloride solution to the bottle. Keep the pipette tip just above the sample surface. CAUTION: mercuric chloride is toxic. Wash with copious amounts of water if it touches your skin. Please refer to Appendix 1 - Safe work instructions for Handling Mercuric chloride. Screw the cap on tightly and shake the bottle 4 times to distribute the mercuric chloride.

9. Store samples at room temperature in the box provided, and retighten the lids after an hour or so.

All TCO_2 and TALK samples must be returned to Hobart in the boxes provided.

Contact information for any queries regarding Carbon sampling

Kate Berry
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart Tas 7001
Phone: (03) 6232 5270 (W)
Mobile: 0408 035 079
Email: kate.berry@csiro.au

6.5.4 Salinity

The next parameter to be taken from the Niskin bottle is salinity. Refer to the field sheet to ensure that the correct salinity bottle number has been entered.

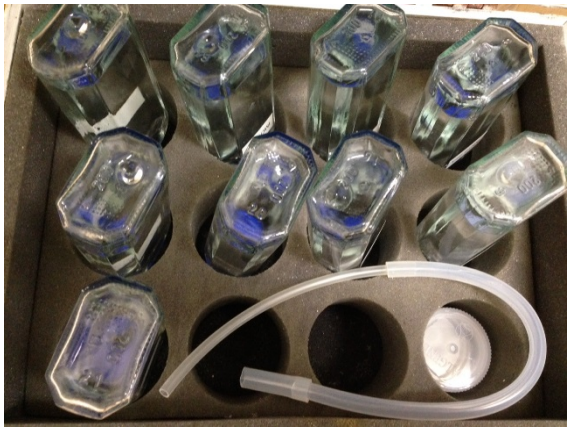
Empty the old sample from the bottle. Attach the sampling tube to the spigot and rinse out the old sample by filling about 1/3 and emptying 3 times.
Make sure any salt crystals in the blue cap or thread are removed
Fill the bottle until it is overflowing, using the tube. Check there are no bubbles

Pour out enough sample to fill the bottle to the level shown. DO NOT OVERFILL

Take a clean dry plastic insert from the container supplied in the carry case

Push the insert into the bottle. If the inserts are wet, rinse with the sample before using

Replace the blue cap and screw tightly to hold the insert in place



Put the bottle back in the carry case upside down

For more information contact:

Stephen Tibben
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart Tas 7001
Phone: (03) 6232 5343 (W)
Email: Stephen.tibben@csiro.au

6.5.5 Nutrients

- Nutrients can be sampled after the gases (oxygen and carbon). Nutrient samples at NRS sites are not filtered during collection as filtering my introduce contamination and particle loads are generally low in oceanic waters. For consistency across the NRS program, nutrient samples at coastal stations are also unfiltered, though particle loads may be significant at times in coastal waters.

- Duplicate samples are collected in single use 30 mL polypropylene tubes with screw caps.

- Tubes should be labelled with a depth or number (starting at 1 for shallowest) as well as the site code (e.g. MAI) and a date. It is protocol to label nutrients on the field sheet with ascending numbers (1, 2, 3 … from shallow to deep).

- Nutrient contamination occurs readily from sample contact with skin, sunscreen, sweat, etc. Do not touch the tubes to the sample spigot and ensure your fingers do not contact the spigot outlet or the inside of the tubes or the caps. Wear latex or vinyl gloves if possible

- Half fill the tubes with sample water, loosely cap the tubes and shake in order to rinse the inside of the tubes and caps. Repeat this a for a total of three rinses before the final fill.

- It is VERY IMPORTANT to leave an air gap of about 2 cm from the top of the tubes for sample expansion upon freezing. The water line should be roughly level with the 30 mL marking.

- Freeze the samples in an upright position as soon as possible and store frozen until analysis. The samples can be kept cool in an esky with ice or freezer packs for a few hours before freezing.

Ammonia contamination

Try not to store samples with biological material as it can contaminate the nutrients with ammonia, especially fish.
Smoke from cigarettes can also cause ammonia contamination.

6.5.6 TSS and Pigments

Collect enough surface (15-20 L) water for 3 times TSS samples and one pigment analysis. Transfer to 20 L carboy.
Collect 5 L of water at 20m and transfer to 5 L carboy for pigment analysis.

6.5.7 Pooled depth sample

Collect the pooled sample in the 5L carboy or alternatively collect sample directly into the 1L lugol's container and preserve on board. Measure equal amounts from all Niskin bottles

sampled at depths less than or equal to 50 metres after sampling for carbon, nutrients and salinity.

Equipment needed: 3L jug, large funnel, 5L carboy or 1L lugol container which should be clean and dry, shade cloth

- Rinse all items with surface water before sampling to remove any dust that may have settled during transportation.
- For sites with 6 depths, measure 500ml of water from each Niskin bottle into the 3L plastic jug and pour into the 5L carboy using the funnel. For YON and DAR measure 1-1.5 L from of each depth or fill the 1L container with the mixed water.
- Preserve on site, add 5mL (measured on shore into a small screw top plastic container) lugols to sample and mix.
- Store the sample in a shaded location (under wetted shade cloth is an option), until sub sampled on shore.

6.5.8 Phytoplankton calibration samples

Phytoplankton (pigments) samples are collected to check the WQM sensor performance. The water collected at the surface will also be used for the TSS samples

Equipment needed: 1 x 5L and 1 x 20L clean carboys, funnel, eskies

- 15L of water is collected at the surface, more than one Niskin bottle of water will be needed.
- 5L is collected at 20m.
- Use a funnel to transfer a small amount of water into the carboy and rinse. Fill the carboy with the remaining water.
- Store the carboys in the eskies with ice or freezer packs.
- Note on the field sheet the time the samples were collected.

6.6 IMOS Marine Microbiome Initiative/AMMBI Microbial and picoplankton sampling

AMMBI = Australian Marine Microbial Biodiversity Initiative, the precursor to the IMOS Marine Microbiome Initiative.

Equipment: 6 x seawater containers (plastic; dark or covered ~4 L capacity, 1 x Funnel (plastic; compatible with seawater containers), Large eski to carry 4 L plastic containers (unless processing directly after sampling)

On station:

1. Conduct Niskin bottle cast(s) to pre-determined depths (x6). Make cast as close to last activity on station as possible.

2. For each depth, rinse funnel and container (labelled with relevant depth) with retrieved seawater (~0.5 L). Discard.

3. Fill container with a minimum of 3 L seawater and store in Eski. Repeat for all depths.

4. Surround containers with ice and if possible keep eski/containers out of direct sunshine to minimise sample temperature shifts prior to sample processing.

6.7 Ichthyoplankton

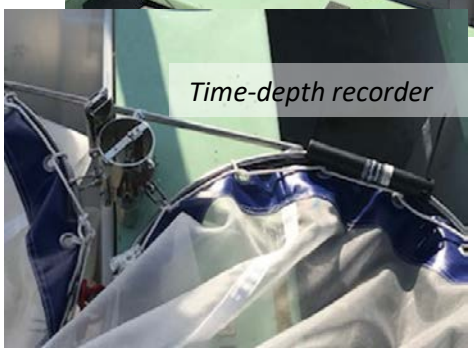
Summary of gear deployment:

- Deployments are meant to be simple, and uncomplicated for a variety of vessels (i.e. from 25 m Ngerin to a 7 m shark cat)
- Tow ~1.5 m/s (~3 knots through the water), sampling obliquely from ~25 m depth to 5 m depth.
- Total deployment time is approximately 12 minutes to sample at least 170 m^3 for each net = 340 m^3 per tow.
- Two tows per trip:
    - 1st tow at the NRS location (e.g. PH100);
    - 2nd tow at spatially distant station within your logistical constraints (e.g. PH50 m)

Don't forget the cod-jars

9 kg lead weight behind bottom bar

TSK attached with shackles and string



Time-depth recorder

Procedure (note: you may need to adjust depending on the vessel, the skipper and swell):

1) Launch Hobo temperature-depth logger prior to departure
2) Fill in field log-sheet and labels – access here.
3) Screw on both 1.2 L white sampling jars to net cod ends and attach the 9 kg weight
4) Check that the TSK flow meter is set to zero
5) Vessel in gear and appropriate course for swell, wind (~1 knots)
6) Deploy over side of boat at 1 knot *through the water (not over ground)* and start stopwatch. Beware that the TSK flow meter may turn rapidly in the wind – a small amount (<40 revs) is not of concern; or you can insert a small string preventer into the meter which is pulled out as you lower.
7) Fully deploy all 35 m rope to start at depth while slow, and bring speed up to 2-3 knots, the goal is a relatively even oblique from 25 to 5 m, ~twice.
8) Adjust this in future tows as necessary, depending on the depth profile from logger; the goal is a relatively even oblique from 25 to 5 m, ~twice. If you have a winch, all the better.
9) To let the net sink, some boats drop into neutral to slow the boat for 30 seconds
10) At ~12 minutes reduce speed, or even into neutral to bring briskly on board
11) With the net frame and depressor on board, then 'tea-bag' the cod ends; inspect the mesh that it is clean. With the full cod jar still attached to the net, pour most of the content back out the mesh just above the clamp

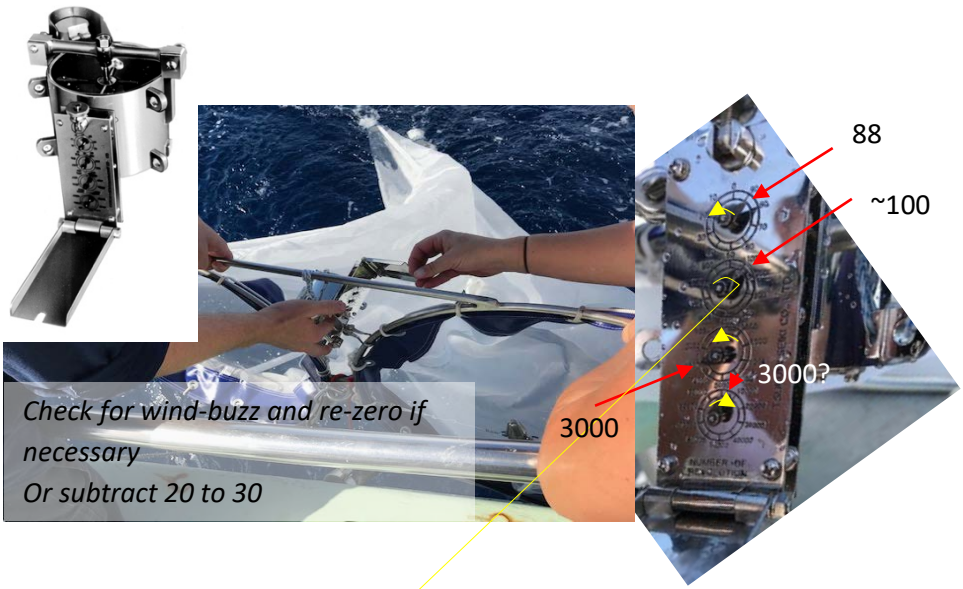
12) Jellyfish or high salp procedure: use a sieve to remove jellyfish or salps, otherwise save up to 4 jars or retain half to a quarter
13) Subsampling: If the sample is very large and there is a need to subsample note down the original total volume and the volume retained on the log sheet
14) For each tow the sample from one cod end will be preserved in ethanol (sample A) and the sample from the other cod end will be preserved in formalin (sample B)
15) Sample A (ethanol, is done first) – shake the zooplankton off the mesh and back into the jar, so that it has minimal water, unscrew the cod-end and fill the jar with 500 mL 95% ethanol (or so that there is *at least four times* the estimated volume of zooplankton)
    a. **Within 24-48 hours**, screw on lid with 300 micron mesh and pour off all ethanol; tap zooplankton back into jar and fill with fresh 500 mL 95% ethanol. Then allow samples to fix for at least two weeks in 95% ethanol.
16) Sample B (formalin) – rinse the zooplankton back into the jar so that it is half full with seawater, and allocate concentrated formaldehyde solution (37%) to make 2% solution (i.e. add 25 mL formaldehyde and top up with water to a total of 500 mL or for a larger plankton sample add 50mL formaldehyde and top up with water to a total of 1 L). As formalin is colourless, you may wish to add a trace of red dye Bengal red for a pinkish solution to remind everyone.
17) Safety: When handling chemicals follow the relevant Material Safety Data Sheet (see http://www.msds.com.au/), your institutions risk assessment and safe work procedures accordingly.
18) If necessary, rinse the open cod end to remove any residual slime and screw on fresh jars, and prepare for next tow.
19) Fill in log sheet remembering flow meter details.

Post sampling:

- Rinse out TSK flow meter with fresh water and allow to dry
- Hobo temperature-depth logger; download logger, prepare depth profile and attach to field sheet to email Paloma: paloma.matis@sims.org.au
- Blast net with the hose to prevent it getting fouled. If necessary some enzymatic detergent for 2 hours will remove dried slime.
- Type up field log sheets and email to Paloma

Description of gear:

TSK flow meter:



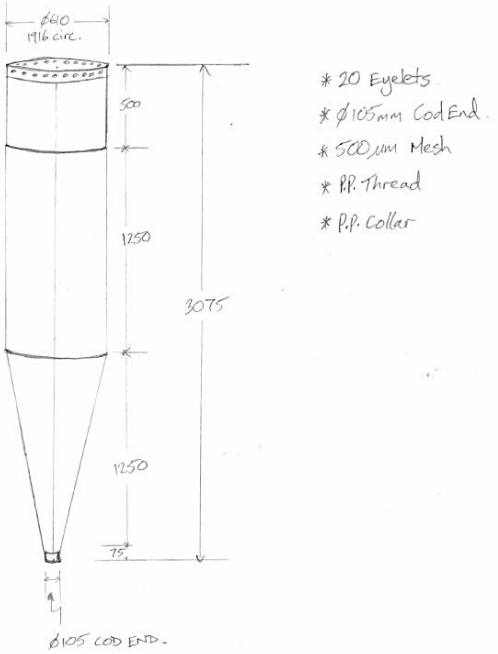
i.e. 3,088 revs, for 12-14 min at 2-3 knots

(Because 88 is ~100 and nowhere near 200 for 3188)

4th dial (10,000s) hardly turns, is not used

Hobo temperature-depth logger:

- To initiate the depth logger you must connect the depth logger to the USB base station and install then run the software (Set units to SI).
- You will then have to set the Logging Interval: to 1 second prior to deployment to get a good measure of what is going on during the tows.
- When downloading the data you will have to go to: Device>Read out>Plot Setup>
- Select series to Plot, then select 'None' on the same page 'Select Internal Logger events to plot' and also select 'None'
- Then below go to Data Assistants>Barometric Compensation Assistant>Process



- Set this to Fluid density: Saltwater and tick the Use a Reference Water Level box and set this to: 0.000 Meters
- On the bottom of this window then select 'Create New Series'. This will then return you to the previous window and you can the press 'Plot' here.

Bongo net: 61 cm diameter bongo frame net made of 12 mm stainless steel rod; 500 micron mesh net

6.8 Preservation and storage of samples

Care should be taken for the following procedures:

- Preserving zooplankton and ichthyoplankton samples with formalin
- Preserving ichthyoplankton samples with ethanol
- Adding mercuric chloride to carbon samples
- handling of Lugols solution during the sampling treatment and preservation
- handling of Glutaraldehyde during the post – sampling treatment and preservation
- handling of Liquid Nitrogen and Dry Ice during the post – sampling treatment and preservation

Carry laminated mini-MSDS sheets for all potentially hazardous materials, on all sampling excursions.

7 Post-sampling activities

There are videos of all these procedures available at:
https://www.youtube.com/channel/UCXp5vxj2TaCatkura7vMYOA
(**NOTE:** not all these videos are relevant as of July 2017 due to new sampling requirements)

Safety Warning for cryovials used to store samples in Dewars containing liquid nitrogen.
They must have an external screw thread with silicon seal because the "pop-on" style lids can become dangerous projectiles when they are removed from the liquid nitrogen.
Each site is provided with the recommended type of vials – 2mL volume tubes for pigment/HPLC samplings and flow cytometry.

Filtering hints:

- Sites will require a 240V heavy duty variable rate vacuum pump with gauges, a catcher vessel (10L bottle or flask or similar) between the pump and the filtration apparatus, and a filtering kit with at least 2 filter holders, of preferably 47 mm diameter. The filtration units supplied hold 4 filtration units of 47 mm diameter, allowing for multiple filtrations to be carried out simultaneously thus minimising processing time during this phase.
- When using the vacuum pump, the pressure should not exceed 5 inch Hg or approx. 100 mm Hg.
- Keep a close watch on the level in the catcher vessel - it may need to be emptied before filtering is completed
- Filter all samples under subdued lighting where possible.

7.1 Summary of samples to be processed in lab

Parameter	Discrete depths	surface	20m WQM	Deep WQM (bottom-2m)	Pooled sample
TSS		yes (3) + blank			
Pigment		yes	yes		
Flow cytometry	yes (3 per AMMBI depth)				
Phytoplankton counts					yes
AMMBI	yes				

7.2 Pooled Depth Sample

If the lugol sample has not been preserved on the boat follow this procedure. Use the 5L carboy of mixed water depths for the phytoplankton counts sample (lugol's).

Preparation of Lugol's solution

The Lugol's solution should be prepared at the laboratory servicing each NRS to avoid shipment of chemicals.

- The solution requires - 100 g potassium iodide, 50 g iodine, 1L distilled water and 100 mL glacial acetic acid
- Dissolve potassium iodide in distilled water, add iodine into the KI solution and dissolve. Slowly add the acid to the solution.
- Store the made up Lugol's solution in a glass container.
- NOTE: Users should use Lugol's solution with concentrations as specified in the recipe above for acidified Lugol's.

Acidified Lugol's is available through Rowe Scientific: Product CL1252.
Commercially available microscope grade Lugol's may also be used (e.g. Sigma 62650) as long as it conforms to the proportions in the recipe; it may need to be acidified before use, by adding glacial acetic acid to 10% of volume.

Preserving the sample

- Rinse the plastic sample bottle, preferably a "PET" Kartell square, wide-mouthed 1000 mL with about 50mL of sample then fill with 1L of sample.
- add 5 mL of Lugol's iodine solution, dispensed via a catalyst dispenser with a cap.
- Replace the plastic insert and the lid and gently mix.
- Seal around the cap and neck with duct tape and clearly label with, site, date and PHYNUM

  Label example **MAI160215 PHYNUMWC**

- The sealed sample bottle is then stored in a black storage bin or similar in a cool environment for shipment to Hobart.

7.3 Total Suspended Matter (TSS)

The surface water is filtered through glass fibre filters to collect three TSS and one blank sample. Use the pre-prepared filters in the Millipore Petri-slides that have been through the procedure in section 10.7.

Perform the following procedure in triplicate.

- Shake the carboy and rinse the 2L measuring cylinder with about 50mL of sample.
- Pour 2L of sample into the measuring cylinder.
- Using clean stainless steel forceps place one of the numbered TSS 47 mm GF/F filter papers on the filter unit and screw on the funnel.
- Record the time and filter number on the log sheet.
- Pour some of the sample onto the filter and start the pump. The volume filtered (1 – 4 L) will depend on location - tropical vs. temperate. Swirl the cylinder to make sure no sediment is left on the bottom of the cylinder.
- Once the sample has finished filtering but before the filter paper is dry, rinse the filter with about 50 mL of MQ to remove residual salt from the filter paper.

- Remove the filter from the filter unit, with vacuum still applied, using clean stainless steel forceps and return it to the numbered petri-slide and **label with the site name using a texta not sticky labels** for example: **MAI 2063**
- As these filters are pre-weighed and pre-treated it is very important that the entire filter is returned. If the edge starts to separate from the rest of the filter, just make sure all pieces of the filter end up in the correctly numbered petri-slide. If this is not possible make a note on the filtering log sheet.
- Store the filters in a fridge and return to Hobart for analysis as soon as possible as the filters can deteriorate.

For the blank perform the following:

- Prepare 50 mL of fresh, filtered seawater using the 0.2 micron syringe and filter provided. The water filtered from the above samples can be used. You may use the syringe repeatedly but only use the filter for one sampling.
- Use a clean filter rig.
- Filter, label and store as per instructions above using this freshly prepared filtered seawater as the sample.

7.4 Pigments for WQM sensor comparisons and remote sensing calibration

The samples collected in the 5L carboy at 20m and the 15L carboy at the surface are filtered for these samples.

- Shake the carboy to mix the sample, rinse 2 x 2L measuring cylinders with about 50mL sample and measure 4L of water from the carboy into measuring cylinders, or less if the water is particularly turbid.
- Using stainless steel forceps place a 47 mm GF/F filter in the filter unit and screw on the top. Pour some water into the filtering unit and start the pump.
- Record the volume filtered, the time and date on the provided log sheet.
- Using clean flat blade forceps, fold the filters into halves/quarters and fold or roll to fit into a 2mL cryovial.
- Label each cryovial, using the special cryopen, with the site, date, PHYPIG
  Label examples:

  MAI160215 PHYPIG surf
  MAI160215 PHYPIG WQM

- If cryovials are unavailable, the folded filter can be wrapped in aluminium foil.
- The samples are stored in a storage Dewar or dry-shipper and should be sent to Hobart for analysis within 2 months of collection

For more detail on shipping contact: Lesley.Clementson@csiro.au

7.5 Preparation of the sample for flow cytometry of picoplankton

These samples are taken from each AMMBI depth and container.

- Using a 50 mL plastic beaker, collect some water from the carboy, rinsing the beaker and mixing the carboy first.
- Use a 1000µL pipette to add 1mL of sample to 3 labelled 2 mL cryovials.
- Add 10 µL of glutaraldehyde (25%) to each cryovial. Wear protective gloves and eyewear when handling gluteraldehyde. Store the glutaraldehyde in the fridge.
- Using the cryopen, label each vial with the site, date, depth and PHYCYT

Label example: **MAI100215 10m PHYCYT 1/3**

- Place these cryovials in a polycarbonate specimen jar to avoid potential contamination of other samples in the liquid nitrogen Dewar during storage.
- Place the polycarbonate jar into the Dewar or dry shipper for transport back to Hobart for analysis.

7.6 Preparation of zooplankton sample for genomics

- Once back on land, pour the "cool blackened jar" sample through the fine mesh screen and concentrate sample to one side using a squirt bottle of water.
- Next rinse lightly with distilled water, and scrape sample out of the mesh container using a metal spatula to avoid contact with any organics. The process of light rinsing to collect the sample against the side may have to be repeated in order to obtain the entire sample. Pressing a paper towel under the mesh may assist in pulling water through gelatinous samples. Eliminate as much water as possible. Don't fill above the white line on tube.
- Place as much of the collected zooplankton sample into one clearly labelled 5 mL cryovial(s), use a cryopen to label according to the coding described in the sample coding section, above.
- Store the vials in at -80 °C prior to transport to Hobart.

7.7 Protocol for IMOS Marine Microbiome Initiative/AMMBI microbial sample processing

The following protocol was provided by Dion Frampton in May 2015 and updated by Jodie van de Kamp in August 2019. Remember to collect water for the picoplankton samples from this water.

Equipment:

- Peristaltic pump, Pump head & tubing with Sterivex luer attachment
- Millipore Sterivex GP 0.22 µm filters (Cat. # SVGPL10RC) – one per sample
- ≥3 x 2 L plastic volumetric cylinders (ideally 1 per depth to be filtered)
- Small snap-lock bags; label with date/station/depth/volume filtered
- Large snap-lock bags big enough to hold 6 x small snap-lock bags with filters

- Small eski containing ice
- Laboratory single-use gloves (e.g. latex)
- 5-10% bleach
- 70% EtOH

Sample processing:

1. Wearing gloves, fill 2 L volumetric cylinders with seawater from each of the depths and label the cylinders accordingly. Place one end of tubing in the volumetric cylinders and pass tubing through pump head ensuring barb/luer lock fitting is attached to the end of the tubing but not with filter attached). Run ~200 mL seawater through tubing (with flow adjusted to approx. 200 mL/min).
2. Refill volumetric cylinders to exactly 2 L of seawater for each depth (making sure that the correct depth seawater is being used).
3. Attach Sterivex filter (labelled: station e.g. MAI; depth e.g. 10 m; date; volume – if different than 2 L) to fitting (luer lock), being careful not to touch lock end, and pass 2 L seawater through filter (as measured by cylinder which the filter should be emptying into). If filter blocks before 2 L has passed through, stop pump and record new volume.
4. After 2 L has passed through filter, let the pump continue for ~1 min to assist in drying filter.
5. Disconnect each filter from tubing, giving each filter several "flicks" to remove as much residual water from inside the filter housing as possible.
6. Cap both ends of filter with the relevant caps (1 x inlet; 1 x outlet) or in an individual snap-lock bag if no caps are available.
7. Put capped filters into one larger snap-lock bag (labelled "AMMBI samples"; MAI; Date) and place on ice in the dark. Store all samples at -80 °C as soon as possible after processing.
8. To clean the pump tubing after use, run 200 mL 5-10% bleach solution through tubing, followed by 200 mL milliQ or distilled water, and lastly 200 mL 70% EtOH (to dry the tubing and prevent biofilm growth between uses).

7.8 Protocol for ichtyoplankton samples

1. If sorting samples preserved in ethanol, begin at step 3. If sorting samples preserved in formalin, filter larvae from sample jar using the 420 um gauge sieve and funnel, draining formalin into waste cube. Fill jar with water and swirl to make sure you have all the plankton loose in the sample and drain into sieve
2. Rinse sample in the sieve to remove chemical residue. Scrape sample back into jar and half fill with tap water
3. Remove label from the sample jar, copy all information (including whether originally fixed in ethanol or formalin) onto a water proof label using a pencil and place into small glass sample vial. Write label information onto the lid of the glass vial
4. Fill in the details of the sample in the sorting log (include whether in formalin or ethanol)
5. Ladle sample into sorting tray

6. Using the microscope and lights, search through the sample in the tray and remove all larval fish and cephalopods. Place removed larvae into the small glass vial with 95% ethanol. Looking through the sample until you are SURE there are no larvae left in the tray (for at least 2 mins)
7. Make notes of the condition of the sample in the sorting log (Is it full of algae? Are the fish in good condition?)
8. Once all the larval fish and cephalopods are in the small glass vial, fill the vial to the top with 95% ethanol and double check it is labelled:
    - Samples originally preserved in formalin: store sample in the cabinet for collection.
    - Samples originally preserved in ethanol: store sample into -20°C freezer (to preserve DNA)
9. Strain off the left-over zooplankton into the 420um sieve, and then place into a 200 mL jar. Make sure the original label goes into this jar and label the lid clearly with the same information using a marker, include "sorted". Record the zooplankton settlement volume (mL) in the sample notes of sorting log
10. Then add preservative to zooplankton – it is important that the samples which were originally stored on formalin go back on formalin and the ethanol samples remain in ethanol

Larval fish identification:

- Sorted larval fish samples will be identified by Tony Miskiewicz who is a taxonomic expert in larval fish identification.
- Sample archive of sorted larval fish (in formalin and 95% ethanol) will then be deposited at the Australian Museum.

7.9 Summary of storage requirements for all samples from a sampling trip

Zooplankton:

- Two 500 mL jars of formalin preserved sample
- 1 (5 mL) cryovial sample for Zooplankton genomics (Zoogen) analysis

Phytoplankton, picoplankton and microbial studies:

- 1 litre of Lugol's preserved sample stored upright, dark and sealed
- 2 (2mL) cryovials of HPLC (pigment) samples- surface and 20m in Dewar
- Up to 18 (2mL) cryovials for flow cytometry (in Dewar)
- Up to 6 capped sterivex filtersin snap-lock bag for microbial genomics analysis

Carbon samples:

- 1 square bottles per specified depth for dissolved inorganic carbon
- 1 round bottle per for Alkalinities

Hydrochemistry:

- 1 salinity bottle per depth stored upside down in road case

- Duplicate 30 mL nutrient tubes stored upright until frozen in -18 °C freezer
- 3 TSS sample and 1 blank filter papers in plastic covers stored in fridge until transportation
- DO samples at selected sites (1 sample per depth)

CTD data:

- Downloaded .hex file of the profile cast if possible

Raw data Log sheets:

- Field log sheet
- Filter sheet
- Ichthyoplankton log sheet

7.10 Handling liquid nitrogen to store samples prior to periodic shipments

- If a storage Dewar is available samples can be stored until it is possible to ship them in the "dry shipper" to Hobart for analysis.
- Storage Dewars need a nearby bulk source of liquid nitrogen for regularly topping up the liquid nitrogen as it evaporates.
- The Dewar must be stored in a well ventilated location to avoid potentially life-threatening build-up of levels of nitrogen gas.
- When transferring the cryovials or containers to or from liquid nitrogen, the wearing of protective gloves and safety glasses or face shield is essential.

7.10.1 Preparing the "dry shipper" Dewar for transporting samples to Hobart

- Liquid nitrogen preserved samples must be transported in a "dry shipper" using priority overnight freight as the freezing capacity of the dry shipper is limited

- The dry shipping Dewar will need to be prepared about 3 days before it is needed.

- Fill the Dewar with liquid nitrogen (the first time it will take quite a lot of liquid nitrogen because the Dewar is hot compared to the liquid nitrogen) then wait for 1 – 2 hours. After this time there should be no loose liquid nitrogen in the Dewar as it will have all been absorbed.

- Re-fill the Dewar and wait 2-4 hours. Check the Dewar, probably all the liquid nitrogen will have been absorbed.

- Fill the Dewar again and leave for 12 – 24 hours. After this time there will probably be some loose liquid nitrogen in the Dewar; this will indicate that the Dewar's absorbent material is fully saturated. If the Dewar is dry, repeat step 3. Return any loose liquid nitrogen to the storage Dewar that is kept in the laboratory.

- The dry shipping liquid nitrogen Dewar will have a working time of about 10 days from when it is saturated with liquid nitrogen. For this reason it is possible to use them not only for shipping samples back to Hobart, but to also use them for freezing samples, in the interim, at remote sampling sites such as Esperance.

- However if something goes wrong or you are delayed or you are over 10 days since saturating the Dewar get it filled with liquid nitrogen and then wait for at least an hour (longer is better).

- If there is still loose liquid nitrogen in the Dewar it will have to be tipped out before the Dewar is taken to the airport. If the liquid nitrogen has been fully absorbed then fill the Dewar with liquid nitrogen again and wait for another 1-2 hours.

- The Dewar must not travel with loose liquid nitrogen in it.

- You will need to make sure the consignment note has "**NOT RESTRICTED as per IATA SPECIAL PROVISION A152**" written on it, otherwise the dry shipping Dewar is considered dangerous goods and the cost of transportation is 3-4 times more or it may be refused carriage.

7.10.2 Shipping samples preserved in liquid nitrogen

If samples are in a storage Dewar move them to the dry shipper. You should have:

- cryovials of pigment (HPLC, phytoplankton) samples
- cryovials for flow cytometry

It's very helpful if you pack each type of sample separately, held on cryo-canes or cryo-sleeves (plastic tubes) or tied in batches inside "knee-hi" stockings.

Package and consignment note must carry the wording:
"**NOT RESTRICTED as per IATA SPECIAL PROVISION A152**"

Please dispatch the dry shipper, preferably by TOLL PRIORITY OVERNIGHT, or TNT Overnight or Australian Air Express (please do not use TOLL-IPEC Priority) to:

Attention: Rasanthi Gunasekera
CSIRO Marine & Atmospheric Research
Castray Esplanade
Hobart TAS 7000
Ph: (03) 62325365 or (03) 62325347
M: 0418316116
Email: rasanthi.gunasekera@csiro.au

Attention: Lesley Clementson
CSIRO Marine & Atmospheric Research
Castray Esplanade
Hobart TAS 7000
Ph: (03) 62325337 or (03) 62325347
M: 0409140230
Email: Lesley.Clementson@csiro.au

Remember, these liquid nitrogen stored samples should always be shipped in dry shipper Dewars – not on dry ice – as they will otherwise rapidly degrade.

7.11 Transport of NRS samples for analysis

Samples should be transported as soon as possible to allow the analysts as much time as possible to complete analysis within the six month reporting deadline specified by IMOS.

Samples not preserved in liquid nitrogen

7.11.1 Zooplankton

The two white 500 mL jars of formalin preserved samples should have the lids taped with electrical tape and collected in the black transport drum provided. Ensure that the transport container is full (use empty jars, vermiculite, newspaper, etc as packing) and store upright.

Except for MAI samples should be sent to:

Attention: Frank Coman
CSIRO Oceans & Atmosphere
Qld Biosciences Precinct
Building 80
Services Road
St Lucia Qld 4067
Ph. 07 3833 5917
Email: frank.coman@csiro.au

7.11.2 Carbon samples

The glass bottles are shipped in the supplied blue boxes every 2 months to:

Attention: Kate Berry
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart TAS 7001
Phone: (03) 6232 5270 (W); (03) 6227 9589 (H)
Email: kate.berry@csiro.au

7.11.3 Phytoplankton samples

The 1 litre bottles of Lugol's preserved sample should be shipped upright in a sealed and dark leak proof container to:

For all sites:
Attention: Claire Davies / Ruth Eriksen
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart Tas 7001

ph: 03 6232 5373
Email: claire.davies@csiro.au

7.11.4 Suspended matter

Filters should be stored in a cool dark place, but not frozen, and shipped in the dry shipper travel case or salinity box to:

Attention: Rasanthi Gunasekera
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart TAS 7000
Ph: (03) 6232 5365 or 6232 5347
M: 0418316116
Email: rasanthi.gunasekera@csiro.au

7.11.5 Microbial genomics samples and frozen zooplankton samples

Filters and cryovials must be stored at -80ºC until ready to send, and sent either monthly or quarterly, packed in dry ice and labelled as per protocol.

Attention: Jodie Van de Kamp
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart TAS 7000
Phone: (03) 6232 5331 (W) or 0409214374
Email: jodie.vandekamp@csiro.au

7.11.6 Salinity

The inverted glass salinity bottles should be sent as soon as possible by overnight freight.

Attention: Stephen Tibben
CSIRO Oceans and Atmosphere
Castray Esplanade
Hobart TAS 7000
Phone: (03) 62325343 (W) or 0447 467 305
Email: Stephen.tibben@csiro.au

7.11.7 Nutrients

The frozen tubes should be shipped every 3 months in an esky containing dry ice.
Most sites use a registered dangerous goods carrier such as World Couriers as dry ice is a dangerous good. Ship to:

Attention: Stephen Tibben
CSIRO Oceans and Atmosphere
Castray Esplanade

Hobart TAS 7000
Phone: (03) 62325343(W)
Mob 0447 467 305
Email: Stephen.tibben@csiro.au

7.11.8 Ichthyoplankton samples

This procedure was compiled by Paloma Matis and Iain Suthers with guidance from Amanda Scholes, the DG trained representative at SIMS.

Ethanol samples: allow samples to fix for at least two weeks in 95% ethanol. Immediately prior to sending pour off ethanol, discard and replace with fresh 20% ethanol. Ethanol at a concentration < 24% is not considered to be dangerous goods under the ADG code. Note: samples will be ok in the reduced concentration for a few days, so only replace with 20% ethanol when you are ready to send them. Samples will be replaced back into 95% ethanol by Paloma upon arrival.

Formalin samples: Formalin at a concentration < 25% is not considered to be a dangerous good under the ADG code. Therefore, samples containing 2% formalin are not dangerous goods. Follow safety procedures according to the SDS for 5-10% formalin.

Formalin and ethanol samples must travel separately using the triple packaging method:

- *Inner packaging*; leak-proof rigid sealed plastic containers (i.e. specimen jar)
- *Secondary packaging*; leak-proof bags able to contain the volume of inner packaging should there be a spill (i.e. suitable sized zip-lock bag)
- *Outer packaging*; a rigid outer container of adequate strength for its capacity, weight and intended use. i.e. lidded drums
- NOTE: A suitable spill absorbent (Vermiculite) should be added to the secondary packaging to contain a spill during transport.
- NOTE: Ensure size of container is suitable to reduce movement of items inside or provide suitable cushioning inside container.
- Samples should be sent road express (samples from Hobart should be sent by air)

Include “Fragile” and “Upright” stickers on outer packaging.

Send samples to:
Professor Iain Suthers/ Dr Paloma Matis,
Upper Campus Store, E26 Bioscience South
LG018 Loading Dock, Via Gate 11 Botany Street,
University of New South Wales, 2052

8 Sampling equipment maintenance

8.1 Zooplankton & ichtyoplankton gear

After each sampling trip, please rinse the entire net and netting with freshwater, dry in the shade and store out of the sun. If necessary, some enzymatic detergent for 2 hours will remove dried slime.

Rinse out the TSK flow meter with fresh water and allow to dry

8.2 Niskin sample bottles and messengers

At the conclusion of a sampling trip, rinse the Niskin bottles with fresh water, inside and out, whilst in the cocked/open position. Leave to dry in a clean environment for approximately 3 days before closing the bottles until their next use.

Wash the Niskin bottle messengers with fresh water and store them in an “open and airy” position or container in order for them to thoroughly dry. If this is not carried out, the messengers will become coated with verdigris and become very stiff and awkward to use.

8.3 Secchi disk

Wash down the secchi disk and rope with freshwater to maintain it in good condition. Again, allow the fish box container open for a while to allow the contents dry out

8.4 Seabird CTD

Ensure that the Seabird CTD’s and protective steel frames are washed down with fresh water, and the detector units are rinsed with reagent grade water. The DO sensor should be stored wetted as recommended by the manufacturer.

9 Data handling, Archival and Retrieval

9.1 CTD data

Raw CTD profile data (files with .hex extension) should be processed with Seabird software according to the ANMN Standardised Profiling CTD Data Processing Procedures to produce text-format (.cnv) files. These are further processed and converted to IMOS-compliant netCDF (.nc) files with the IMOS Matlab Toolbox. Instructions for installing, configuring and running the Toolbox can be found in the Toolbox Wiki at https://github.com/aodn/imos-toolbox/wiki.

Both processed versions (.cnv and .nc) of each profile should be uploaded to **incoming.aodn.org.au** using a File Transfer Protocol (FTP) client. Files should be named according to IMOS conventions, as per the File naming convention for log sheets and raw CTD profiles document, and uploaded to the **ANMN/NRS/directory** (please do not create any sub-directories). Further information on uploading files via FTP can be found in the FTP registration and data upload document.

Please notify AODN (previously eMII) (Marty.Hidas@utas.edu.au , or info@aodn.org.au) of any new uploads, *especially if any previously published file needs to be replaced by an updated version.*

9.2 Field log sheets

The field log sheets used in the field to record the trip are scanned. The information is typed into the standard IMOS log sheet which includes the post sampling information (see Field Sampling Logsheet) by the team responsible for the sampling.

Scanned and typed PDF versions (not MS Word."doc" files) of all logsheets should be emailed to MariaNRSBGC@csiro.au and uploaded to **ANMN/NRS/directory** (please do not create any sub-directories) using a File Transfer Protocol (FTP) software. Instructions for obtaining FTP access are included in the FTP registration and data upload document.

Ichthyoplankton logsheet to be emailed to Paloma Matis paloma.matis@sims.org.au.

Please notify AODN (Marty.Hidas@utas.edu.au, or info@aodn.org.au of any new uploads, *especially if any previously published file needs to be replaced by an updated version*

When changes are made to the field log sheets, new templates are emailed to sites as a word document.

File Naming protocol

Scanned field sheet: IMOS_ANMN-NRS_YYMMDD_NRSSSS_FV00_LOGSHT.pdf

IMOS log sheet: IMOS_ANMN-NRS_YYMMDD_NRSSSS_FV01_LOGSHT.pdf
Where SSS is the site code eg. NRSMAI
The file naming protocol is detailed in the File naming convention for log sheets and raw CTD profiles document. Original hard copies of the field sheets can be kept by the samplers.

9.3 Analytical results (including QA/QC)

The data is entered directly into an Oracle database via a web based data entry interface. The AODN harvests the data from the database automatically and makes the data available through the IMOS data portal. This process applies to zoo- and phytoplankton, flow cytometry, pigments, carbon, TSS, dissolved oxygen, salinity and nutrients.

The analytical log sheets, calculations and results should be retained by the analyst.

The final data is flagged using the IMOS flagging system. Carbon data is assessed using the WOCE flags and these are converted to IMOS flags for the final database.

Analysts are responsible for assessing the quality of the data according the QA/QC procedures used by the lab.

Method detection limits are included with each method in the Analytical methods in section 10.

Table 3 – IMOS flagging system

flag_value	flag_meaning	flag_description
0	No QC performed	The level at which all data enter the working archive. They have not yet been quality controlled
1	Good data	Top quality data in which no malfunctions have been identified and all real features have been verified during the quality control process
2	Probably good data	Good data in which some features (probably real) are present but these are unconfirmed. Code 2 data are also data in which minor malfunctions may be present but these errors are small and/or can be successfully corrected without seriously affecting the overall quality of the data.
3	Bad data that are potentially correctable	Suspect data in which unusual and probably erroneous features are observed
4	Bad data	Obviously erroneous values are observed

flag_value	flag_meaning	flag_description
5	Value changed	Altered by a QC Centre with original values (before the change) preserved in the history record of the profile. AODN discourage the use of this flag. Where data values must be changed (e.g. smoothing of data sets) we strongly prefer that the original data be retained and an additional variable be added to accommodate the interpolated/corrected data values.
6	Below detection limit	used for picoplankton indicating “below detection limit” (previously not used)
7	Not used	Flag 7 is reserved for future use
8	Interpolated value	Indicates that data values are interpolated
9	Missing value	Indicates that the element is missing

9.4 Accessing BGC data from the AODN portal

The web address is: https://portal.aodn.org.au

IMOS data is collected by facilities that are distributed around the country. The marine data collections are wide ranging, and all data collections are available in full to the public. Data covers a wide range of parameters in different ocean environments collected from ocean-going ships, robots, moorings and other platforms.

The portal is an evolving tool and there is online help documentation (http://help.aodn.org.au) complete and up to date for users.

If you are still having trouble accessing the data, please contact info@aodn.org.au

10 Analytical Methods

10.1 Zooplankton

Laboratory processing at CMAR Dutton Park

Dry Weight Analysis:

- The sample is drained of liquid by pouring it through a plastic plate with holes attached to an aspirator. A fine mesh (smaller than 100 µm) is placed over it to retain the sample. The sample is rinsed with distilled water
- The sample is then scraped off the mesh (e.g. plastic knife) and placed on pre-weighed (to at least 3 decimal places) numbered pieces of aluminium foil. Numbering can be accomplished using the indentation left by a ball point pen without ink
- The sample is dried (40-70ºC) over night (or for 24 hours) in an oven until dry
- The aluminium dish and sample is then re-weighed and recorded

Community Composition:

- Analysis of the composition of the zooplankton community will be performed on the second formalin preserved sample. It is done using a dissecting microscope for easy to identify larger species and a compound microscope for identifying smaller difficult to identify species based on their appendages
- Identification will be guided by the library of taxonomic keys we have assembled. Unknown specimens will be digitally photographed and sent for confirmation to expert collaborators
- Copepods will be identified to species-level where possible
- Other zooplankton groups will be identified to the highest taxonomic level possible
- Quality control of the zooplankton identification will be maintained by annual taxonomic training with our national and international network of collaborators

Zooplankton size spectra:

- This will be performed on the same sample as for the zooplankton community composition and subsequent to the microscopic analysis
- We will separate the sample into 2 size classes before scanning (<1 mm and >1 mm)
- We will scan the sample with our existing EPSON high performance scanner
- We will analyse the scanned image using ZooImage software (customised in CSIRO by Nick Mortimer)

Archiving

The formalin-preserved samples for zooplankton community analysis will be archived in proponal phenoxitol at Dutton Park before microscopic analysis. Proponal phenoxitol is safer for using in the laboratory but formalin remains the initial preservative of choice for fixing zooplankton samples

Zooplankton Data Reporting/units:

- Dry Weights (expressed as mg per m3)
- Community composition (expressed as species per m3)
- Average size (in µm) of the zooplankton community

10.2 Phytoplankton

Population

Phytoplankton identification/cell counts

- The samples will be transferred to 1 L measuring cylinders (volume recorded) and allowed to settle for at least 24 hours.

- After this time approximately 900 mL will be siphoned off and the remaining sample will be transferred to a 100 mL measuring cylinder and again allowed to settle for at least 24 hours.

- After this time approximately 90 mL will be siphoned off, the final volume recorded and thoroughly mixed before a 1 mL aliquot will be taken

- The aliquot will be placed in a Sedgwick Rafter counting chamber and examined under an Olympus IX71 inverted microscope with phase contrast facility, DP70 camera and AnalySIS imaging software.

- The counting method is based on Hötzel, G and Croome, R. (1998.).

Precision of phytoplankton data (QA)
Staff from the same laboratory should conduct a 'blind' recount of the same sample once per sample trip. Recounts should yield precisions (density of top 10 species) that average ± 10% of initial count. Failure to meet this target indicates a need to review procedures.

Accuracy of phytoplankton data (QC)
A minimum of 5% of samples should be split and analysed independently by a separate laboratory (and analyst). If accuracy is less than ± 20% for the estimated means of any of the top 10 taxa then methods and training should be reviewed.

PLEASE NOTE Recipe for Lugol's solution: (100 g potassium iodide, 50 g iodine, 1L distilled water, 100 mL glacial acetic acid.) Store Lugol's solution in a dark ventilated container.

10.3 Pigments

HPLC pigments from the "water column" and for WQM Calibration.

Phytoplankton pigments - Samples will be analysed by HPLC at CMAR with the established analytical procedure for pigment analysis using HPLC as follows:

- All extraction procedures should be done under subdued lighting conditions.
- Cut frozen filters into 3 or 4 pieces and place in a clean 10 mL centrifuge tube (wipe blades of scissors clean with a tissue between samples).
- Add 3 mL of 100% acetone, cover tube with parafilm and vortex for ≈ 30 seconds.
- Tubes are then placed in an ice-water/ultrasonic bath and the filter and acetone are sonicated for 15 minutes.
- Store the tubes at 4°C for ≈ 18 hours or overnight.
- Add 0.2 mL MilliQ water to each tube and sonicate in an ice-water bath for another 15 minutes.
- Transfer filter and solvent quantitatively to a "Biorad" column containing a small GF/F filter acting as a plug.
- The centrifuge tubes are rinsed with 2 x 0.5 mL 90:10 acetone:MilliQ water, which is quantitatively transferred to the respective "Biorad" columns. Each "Biorad" column is fitted into a clean 10 mL centrifuge tube and centrifuged for 5 minutes at 2500 rpm.
- Record volume of extract in each centrifuge tube.
- Wash "Anatop" filter with 1 mL of 100% acetone three times and dry filter by passing air from the syringe through filter (remove filter from syringe before drawing up air).
- Take up about 0.5 mL extract from centrifuge tube in syringe, place filter on syringe and push 0.5 mL sample through filter to waste. Take up about 1.0 mL extract from centrifuge tube in a syringe.
- Place filter on syringe and push 1.0 mL sample through filter into amber sample vial.
- Only fill vial to 3/4 full. (Wipe tip of syringe on filter between extract and acetone wash).
- Repeat syringe and filter wash step in between samples.
- Note: "Anatop" filters can be used for ≈ 25 samples. They should be washed as described above between samples and washed 3 times with acetone between batches.

- Sample vials are then placed in the auto sampler holders for the HPLC analysis to take place.

10.4 Flow cytometry

Flow cytometry analyses:

From July 2009 until June 2014 the flow cytometry samples were analysed exclusively by Dr. Paul Thomson – initially using a FACSCalibur (BD Biosciences, USA) instrument located at the Australian Antarctic Division (AAD) followed by a FacsCanto II (BD Biosciences, USA) instrument located at the University of Western Australia (UWA) Centre for Microscopy and Critical Analysis (CMCA). During late 2016, CSIRO Oceans & Atmosphere acquired a CytoSub (CytoBuoy, Netherlands) flow cytometer which has been used to analyse the IMOS flow cytometry samples from mid-2016 onward. Unlike the BD instruments which are ideally laboratory-bound due to their sensitivity to external motion, the CytoSub is designed to be interchangeable between laboratory, ship-board and submersible sample analysis modes. These modes potentially give users the ability to make *in situ* or "on location" analyses and compare these with analysis of fixed and stored samples – the standard method for IMOS samples. The CytoSub is primarily located in the BC2/PC2 Algal Laboratory at CSIRO's Battery Point, Tasmania site – the same laboratory that processes all Maria Island NRS samples and also receives IMOS samples from all other NRSs for pigment, microscopy, microbial and cell analysis.

With this change of instruments, it is important to calibrate between instruments to ensure consistency of data. To this end, selected IMOS samples collected between 2013 and 2016 were analysed in parallel using both the FacsCanto II and the CytoSub instruments and the data compared. This comparison explains the observed differences between instruments and to ascertain if, and to what extent, conversion factors could be applied when using the IMOS NRS flow cytometry data set across the temporal range where results from both instruments have been reported – here.

Prior to July 2017, the IMOS NRS flow cytometry samples were taken from one pooled sample (in triplicate) per NRS per sampling effort. The pooled sample has been referred to as an integrated depth sample, although this term is incorrect as the sample consists of a sub-sample from several discreet depth samples being mixed and then an aliquot being taken from this mixed sample, i.e. not truly integrating the whole of the water column over the sampled depth range. As each NRS has a different depth profile (Table 1), this sample consisted of both different depths and a different overall number of depths depending on the NRS. Since July 2017 the flow cytometry samples have consisted of sub-samples (in triplicate) being taken directly from discreet depth water samples (Table 1). Depending on the NRS, this means between three and six discreet depth samples are measured.
In parallel with the discreet depth sampling protocol, the collection of pooled samples continued between August 2017 and December 2018 for the Maria Island (MAI) NRS only. The comparison provided in this report serves to use these MAI samples to determine the degree of similarity between the pooled and discreet depth approaches and gives some

context as to the range of measurements covered over the comparison study time period. – here.

Picoplankton analysis by flow cytometry

Photosynthetic picoplankton (Prochlorococcus, Synechococcus and picoeukaryotes) were enumerated by flow cytometry. Composite seawater samples from surface waters to a maximum depth of 50 m were collected from IMOS National Reference Stations every 1 to 3 months, depending on location. Subsamples of 1 ml were fixed in EM grade glutaraldehyde (0.25 % final concentration) for 15 min and quick frozen in liquid nitrogen until analysis (Marie et al. 1999). Samples were thawed at 37oC and 1 µm fluorescent beads (Molecular Probes) were added as an internal standard. Samples were analysed using a FACSCANTO II (Becton Dickinson) flow cytometer fitted with a 488 nm laser on high throughput mode at a flow rate of 60 µl min-1 for 2 min (Patten et al. 2011). Prochlorococcus, Synechococcus and picoeukaryotes were discriminated in scatter plots of red and orange autofluorescence of chlorophyll and the accessory pigment phycoerythrin (Marie et al. 1999).

References:

Marie D, Partensky F, Vaulot D, Brussard C (1999). Enumeration of phytoplankton, bacteria, and viruses in marine samples. In: Robinson JPEA (ed) Current protocols in cytometry, suppl 10. John Wiley & Sons, Inc, New York, pp 11.11.11–11.11.15

Patten, N.L., Wyatt, A.S.J., Lowe, R.J., Waite, A.M (2011). Uptake of picophytoplankton, bacterioplankton and virioplankton by a fringing coral reef community (Ningaloo Reef, Australia). Coral Reefs, 30:555–567

Units for Phytoplankton – population, pigments, flow cytometry

For optical phytoplankton study

- Phytoplankton – counts will be expressed in cells per litre
- Estimated phytoplankton biomass in mL L-1.

For HPLC pigments.

- in µg.L-1 (or mg m-3)

For Flow Cytometry

- to be notified

Are there alternate SI units to report these parameters in? NO

10.5 Total CO2 and Alkalinity

Samples are returned to CSIRO Hobart for analyses using techniques developed for measurements in ocean waters on CO2/CLIVAR sections. The accuracy of the methods is checked against certified reference material from the Scripps Institution of Oceanography for each series of about twenty sample analysed. Detailed analytical procedures are provided in Dickson et al (2007).

Carbon Parameters

Total dissolved inorganic carbon (TCO2), also known as DIC or CT
Precision and accuracy estimate: ±1 µmol kg-1

Total (titration) alkalinity (TALK)
Precision and accuracy estimate: ±2 µmol kg-1

Total dissolved inorganic carbon:

Total dissolved carbon dioxide in seawater is:

$TCO2 = [CO2]+[HCO3^-]+[CO3^=]$

Carbon dioxide dissolved in seawater is analysed by acidifying the seawater to convert bicarbonate and carbonate to CO2, extracting the CO2 from the solution by bubbling with high purity nitrogen (>99.995%), and trapping and quantifying the amount of CO2 using a UIC model 5011 coulometer.

A SOMMA system is used to extract the CO2 and follows the procedure described in detail by Johnson et al (1993) and Dickson et al (2007). The SOMMA loads seawater from a sample bottle into a calibrated pipette thermostated at a constant temperature of 20°C. The sample in the pipette is then dispensed into a stripping chamber to which 1 mL of a 10% (v/v) solution of phosphoric acid has been added. The stripping chamber has a glass frit at the base and this is used to bubble nitrogen carrier gas through the sample and strip the CO2 from the sample. The CO2 in the carrier gas stream flows into the cathode compartment of a coulometer cell where it is quantitatively trapped in an ethanolamine solution. The absorbed CO2 reacts to form hydroxyethylcarbamic acid, causing a change in the colour of the cell solution due to the presence of a thymolphthalein pH indicator in the solution. Base is generated at the cell cathode, until the solution colour returns to its starting point. The efficiency of the coulometric method is determined by injecting known amounts of pure CO2 (>99.99%). Accuracy is checked by analyzing certified reference seawater from the Scripps Institution of Oceanography.

For each series of sample analyses, the general procedure is:

- The coulometer cell is setup by adding UIC Coulometric Inc. solutions to the cathode and anode compartments, with the platinum cathode and silver anode connected to the coulometer. The gas stream from the SOMMA system is connected to the coulometer cell.

- The power to the cathode and anode of the cell is switched on, followed by a series of injections of pure CO2 to condition the cell solution. The pure CO2 is added by switching an inline gas sampling valve with two loops of known volume (1.5 and 2.2.mL at 21.7°C).

- Gas calibrations are next run to determine the efficiency of the cell. Values of between 99.5 ± 0.1% efficiency are considered suitable to begin sample analyses. Checks are also made to ensure there is a consistent blank and no evidence of leaks in the system.

- A test seawater sample is analysed, followed by a certified reference material. If the certified reference material is within 2 µmol kg-1, the analysis of samples proceeds.

- All samples are placed in a water bath (20°C) to ensure a constant temperature. The salinity of the samples is measured by the SOMMA system and used with the temperature of the sample to sample density Concentrations are in units of µmol kg-1

- Samples are analysed in batches of about 20 to 25 before a new cell and solution is required.

- For quality control, two to three reference material analyses are made with each batch of samples.

Total alkalinity method:

An automated open-cell potentiometric titration is used to measure total alkalinity. The titrations are performed using a Metrohm automated burette to deliver acid titrant, and a combination Metrohm reference/glass pH electrode to track the progress of the titration. Sample volumes of 100mL are measured using a Metrohm dosino burette.

The volumes delivered by the burettes are calibrated every six to twelve months by weighing volumes of deionised water dispensed by the burettes at 20° and applying an air buoyancy correction (Dickson et al 2007). The pH electrode responses are checked by comparison with Tris and Aminopyridine buffers in synthetic seawater (Dickson et al 2007). Electrodes with responses within 100 ± 0.3% of the Nernst slope of the electrode are used for titrations. The e.m.f. of the electrodes is recorded to ±0.1mV.

The 0.1N HCl titrant contains 0.6 mol/kg sodium chloride to approximate the ionic strength of seawater. The normality of each batch of titrant is measured by coulometry and is known to better than ±0.03%. The density of the titrant, which is used to calculate the total alkalinity, is measured with an Anton Parr density meters over a range of temperatures near 20°C and is known to better than ±0.01%.

A non-linear fitting routine, written in IDL, is used to calculate TA. The routine is similar to the computation described in Johansson and Wedborg (1982) and Dickson et al. (2007). Comparison of the routine used here with a calculated TA result for data published in Dickson et al (2007) and using a different non-linear fitting procedure agree within ±0.01%.

- Samples stored in sealed glass bottles are placed in a thermostated water bath and brought to a temperature of 20°C prior to analysis.

- A 100mL volume of sample is pipetted into a water jacketed (20°C) glass beaker for analysis and the sample mixed with a stir bar.

- A 0.1N solution of hydrochloric acid (HCl) titrant is added to the sample to adjust the pH of the seawater to about 3.5. The sample is then stirred for 10 minutes to degas CO2.

- The titration proceeds by adding small increments of the hydrochloric acid titrant until the pH reaches about 3.0. The amounts of acid added and the associated change in e.m.f. of a pH electrode used to monitor the progress of the titration are recorded. About 20 data points are collected.

- The total alkalinity is calculated using a non linear least squares technique.

References:

Dickson, A. G., Sabine, C. L. and Christian, J. R. (2007) Guide for best practices in ocean CO2 measurements. PICES Special Publication 3, 191pp.

Johansson, O. and Wedborg, M., (1982) On the evaluation of potentiometric titrations of seawater with hydrochloric acid, Oceanologica Acta 5:209–218

Johnson, K.M., Wills, K.D., Butler, D.B., Johnson, W.K. and Wong, C.S. (1993) Coulometric total carbon dioxide analysis for marine studies: maximizing the performance of an automated continuous gas extraction system and coulometric detector. Marine Chemistry 44: 167–187.

10.6 Genomics (molecular) analyses

Samples for genomics are returned to the Marine Microbiome Initiative Facility at CSIRO, Hobart for DNA extraction and archiving of DNA.

Zooplankton - DNA is extracted from zooplankton biomass in cryovials using the method established by Berry et al. (2019). In brief, zooplankton samples are homogenised and a 20 µl subsample of the resulting slurry is extracted and purified using the DNeasy® Blood and Tissue Kit (QIAGEN) following the manufacturers tissue protocol and a 2 x 100 µl elution in AE buffer. Extracts are archived at -80 °C.

Microbial & phytoplankton – DNA is extracted and purified from sterivex filters using the method described in Brown et al. (2018), using the PowerWater® Sterivex® DNA Isolation Kit (QIAGEN) following a modified version of the manufacturer's instructions and eluted in 80 µl 0.1 x TE. An aliquot of the DNA extracts are sent to the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) for next generation sequencing. Remaining extracts are archived at -80 °C.

Genomics (Molecular)

Genomics analysis, (next generation sequencing and bioinformatic analysis), of microbial DNA from IMOS NRS samples is conducted as part of the Australian Microbiome (AM) project (https://www.australianmicrobiome.com/).

Protocols for genomics analysis can be found here:
https://www.australianmicrobiome.com/protocols/

Genomics data is available from the AM data portal:
https://data.bioplatforms.com/organization/about/australian-microbiome

References:

Berry TE, Saunders BJ, Coghlan ML, Stat M, Jarman S, et al. (2019) Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. PLOS Genetics 15(2): e1007943. https://doi.org/10.1371/journal.pgen.1007943

Brown, Mark; Van De Kamp, Jodie; Ostrowski, Martin; Seymour, Justin; Ingleton, Tim; Messer, Lauren; et al. Systematic, continental scale temporal monitoring of marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative. Nature Scientific Data. 2018; 5(180130):1-10.
https://doi.org/10.1038/sdata.2018.130

10.7 Total Suspended Solids (TSS)

Filter preparation:

- Filters for TSM analysis are prepared in the following manner prior to field sampling.
- Place individual 47 mm GF/F filters on a sheet of aluminium foil and cover with another sheet of foil.
- Place in muffle furnace and set temperature to 450°C.
- Once the furnace has reached 450°C, leave it at this temperature for approximately 1 hour and then turn the furnace off.
- When furnace is cool remove filters.
- Rinse filters in Milli-Q water for 1 hour then remove each filter from the water using forceps and place on a clean numbered glass petri dish which contains 3 small balls of aluminium foil.
- Place petri dishes on a tray (a shallow cake tin is ideal) cover with a sheet of aluminium foil and place in an oven at 75°C for approximately 3 hours.
- Remove from oven and let cool for around 15 minutes.
- Weigh each filter, record weight on sheet and return to the same petri dish.
- Return petri dishes to the oven at 75°C for approximately another 2 hours.
- Remove, cool and weigh again.
- Generally after 2 weighings, the filters should have reached constant weight. If there is more than 0.2 mg difference between the first and second weighing, repeat the drying/weighing process.

- Once the filters have reached constant weight store in the appropriately numbered Millipore Petri-slides until required. On TSM log sheet record the number of the Petri-slide along with the weight of the filter stored in the Petri-slide.
- Always do the initial and final post-sampling weighing of the filters on the same balance.

After the NRS Samplers have collected the Suspended Matter sample in the field:

- Place filters in glass petri dishes, each labelled with the same number as that on the petri slide from which each filter came. Each petri dish will contain 3 small balls of aluminium foil on which the filter will sit
- Place petri dishes on a tray (cake tin), cover with a sheet of aluminium foil and place in an oven at 75^{o}C for approximately 3 hours.
- Remove from oven and let cool (around 15 minutes).
- Weigh each filter, record weight on the TSM log sheet against the same number and return the filter to the same petri dish.
- Return petri dishes to the oven at 75^{o}C for approximately another 1-2 hours.
- Remove, cool and weigh again.
- Generally after 2 weighings, the filters should have reached constant weight. If there is more than 0.2 mg difference between the first and second weighing, repeat the drying/weighing process.
- Determine the TSM weight by subtraction of the pre- filtration weight from the post-filtration weight.
- Take note of the sample volume that was filtered through the filter.
- Calculate the weight per volume (Total).
- Return filters to glass petri dishes and place petri dishes on the floor of a muffle furnace (note the position of each of the numbered dishes as the numbering on the dishes will be removed during the muffling process). Cover the dishes loosely with a sheet of aluminium foil and program the muffle furnace to 450^{o}C. After the furnace has reached this temperature, wait 3 hours before programming the temperature of the furnace to 20^{o}C. When the furnace has reached 20^{o}C, remove the dishes and filters and weigh immediately.
- Determine the weight of the inorganic fraction by subtraction of the pre- filtration weight from the post-filtration muffled weight. Calculate the weight per volume.
- Determine the weight of the organic fraction by subtraction of the inorganic fraction weight from the total TSM weight. Calculate the weight per volume
- This analytical procedure is also followed for the “seawater blank” that was carried out at the time the suspended solid sample for the same station was filtered.
- As mentioned in the sample filtration procedure there is a need to have a “ blank” filter for comparison to the actual sample filters. The procedure for filtering the blanks is described in detail in the sample treatment section. Basically it is just necessary to prepare and send off an extra filter in a petri dish for each station to use as a blank at each sampling.

Since the start of the NRS sites there has been issues with the collection of the TSM blank sample, often resulting in values higher than the corresponding TSM samples. This has in

turn caused issues if the sample TSM value is corrected for the blank, as negative TSM values are then recorded which we know are not correct. From July 2017, we have implemented quite specific instructions and provided filtration equipment for the collection of the blank sample in an effort to make the collection consistent across all sites and potentially reduce the value of the TSM blank to a consistently low value. Further details relating to use of the TSS data can be found here.

10.8 Nutrient analyses

IMOS nutrient samples are measured on a Seal AA3HR segmented flow auto-analyser fitted with 1 cm flow-cells for colorimetric measurement of dissolved organic phosphate, nitrate plus nitrite, reactive silicate and nitrite. A JASCO FP2020 fluorescence detector is used for the measurement of ammonia.

The analyses of phosphate, nitrate plus nitrite, silicate and nitrite by the Hobart hydrochemistry group is based on the following manuscript:

Rees, C., L. Pender, K. Sherrin, C. Schwanger, P. Hughes, S. Tibben, A. Marouchos, and M. Rayner. (2018) "*Methods for reproducible shipboard SFA nutrient measurement using RMNS and automated data processing*." Limnol. Oceanogr: Methods, 17(1): pp. 25-41. doi:10.1002/lom3.10294

The analysis of ammonia is a based on the following method:
Seal AutoAnalyzer Applications method no G-327-05 Rev. 4.

Method detection limits and precision are determined for each run using standards. Accuracy is determined using KANSO reference material of nutrients in seawater (RMNS).

Detection limits are:

- Silicate: 0.2 uM
- Nitrate + Nitrite(NOx): 0.1 uM
- Phosphate: 0.05 uM
- Ammonia: 0.05 uM

All analyses are conducted on unfiltered samples that have been stored frozen until analysis. Our accuracy and precision is limited by the need for transportation and freezing of the samples. Our instrumentation can measure more accurately than these detection limits with freshly collected samples.

10.9 Salinity

IMOS samples are analysed using the Guildline Autosal 8400B salinometer. The instrument measures a conductivity ratio which is converted to practical salinity units using the practical salinity scale formula. IAPSO international seawater standards made by Ocean Scientific International Laboratories (OSIL) are used to calibrate the salinometer each time it is used.

Salinity accuracy and precision is ± 0.002 PSU

Operating procedure

- Salinity measurements are made in a constant temperature room capable of maintaining temperature ideally within +/- 1°C. Samples are allowed 24 hours to equilibrate to lab temperature.

- The cell is flushed with an old standard until stable and then a new standard is opened and measured until 2 stable readings (agreeing within ±0.00002 displayed conductivity ratio digits, i.e ±0.0004 PSU) are achieved. The Rs vernier scale is adjusted if necessary. It should not move by more than 10 from the last calibration or analysis should not proceed. The conductivity ratio reading should be exactly twice the K15 value on the seawater standard label.

- The analysis is recorded and calculated using OSIL acquisition software.

- Shake sample gently to avoid making bubbles. Fill and flush the cell two or three times and take readings when two consecutive measurements agree to +/- 0.00004 conductivity units.

- Continue reading samples unless the original standby value has drifted more than +/-1 unit. If this occurs run an IAPSO standard is used to quantitate the drift.

Salinity Units

Salinity used to be expressed as parts per thousand, however since its derivation from a conductivity ratio has become an equivalent unit-less number, it is sometimes referred to in practical salinity units (PSU) e.g. 34.432 PSU

10.10 Dissolved oxygen

IMOS samples are measured using the current Scripps Institute of Oceanography (SIO) method based on a whole-bottle modified-Winkler titration of Carpenter (1965) with modifications by Culberson *et al* (1991). Manganese chloride followed by the alkaline iodide, is added to the sample, and the precipitated manganous hydroxide is distributed evenly throughout the bottle by shaking. At this stage, the dissolved oxygen oxidizes an equivalent amount of Mn(II) to Mn(IV). The sample is then acidified, converting the Mn back to the divalent state and liberating two moles of Iodine per mole of the original dissolved oxygen (O_2) content of the water. The Iodine is then titrated with standardized thiosulphate solution. The thiosulphate concentration is determined against a precisely-known oxidizing agent, KIO_3. The tri-iodide ion strongly absorbs at a wavelength of λ = 365 nm, and thus changes in the UV absorption are measured until a turning point and plateau indicate that the endpoint has been reached.

Detection limit

The accuracy of this method is better than ±0.5 $\mu molL^{-1}$.

10.11 Ichthyoplankton

To be updated when method is available

11 References

World Ocean Circulation Experiment – Operations Manual, Volume3; WHP Office Report WHPO 91-1 – WOCE Report No. 68/91, Revision1.
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Heron AC (1982) A vertical free fall plankton net with no mouth obstructions. *Limnology & Oceanography*: 380-383
Hotzel, G and Croome, R. (1998.) A Phytoplankton Methods Manual for Australian Rivers. Occasional Paper 18/98, Land and Water Resources Research and Development Corporation, Canberra. 52pp

Appendix 1 - Safe work instructions for Handling Mercuric chloride

Oceans and Atmosphere

Safe Work Instruction (SWI)

Title	**Use and Management of Mercuric Chloride ($HgCl_2$) Saturated Solution for Preserving Water Samples**
Issued by	*Kate Berry, Biogeochemist, Oceans and Atmosphere, Hobart*
Application	Preservation of water samples for inhibition of biological activity for storage of samples before chemical analysis. This activity may take place on site or on small boats or ships This SWI should be followed by all staff undertaking this procedure.
Authorisation	*Only staff with training in science (diploma minimum) or fieldwork experience may use this substance.*
Hazards	*S7 POISON and TOXIC 6.1 DG CLASSIFICATION* Hazard Phrases: **H300** Fatal if swallowed **H310** Fatal in contact with skin **H330** Fatal if inhaled **H315** Causes skin irritation **H319** Causes serious eye irritation **H341** Suspected of causing genetic defects **H361** Suspected of damaging fertility or the unborn child **H373** May cause damage to organs through prolonged or repeated exposure **H401** Toxic to aquatic life **H411** Toxic to aquatic life with long lasting effects
Personal Protective Equipment (PPE)	*Lab coat, safety glasses or goggles, enclosed footwear, long sleeved shirt and long trousers, and gloves. Gloves can be nitrile or butyl – see Glove Selection Chart.* Be aware that contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants.
Emergency Information	**Response** **P301+P310** IF SWALLOWED: Immediately call 000, a POISON CENTER or doctor/physician. **P304+P340** IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. **P308+P313** IF exposed or concerned: Get medical advice/attention. **P330** Rinse mouth. *NOTE: Spill kit available in laboratory 2GD.38 at CSIRO in Hobart. For spills on small boats or ships, mop up as much as possible with paper towels and triple bag waste for chemical waste disposal. Rinse deck with copious amounts of water.*

DEFINITIONS		
CAS No	**%[weight]**	**Name**
7487-94-7	7	mercuric chloride $HgCl_2$
7732-18-5	93	water H_2O

Further Information

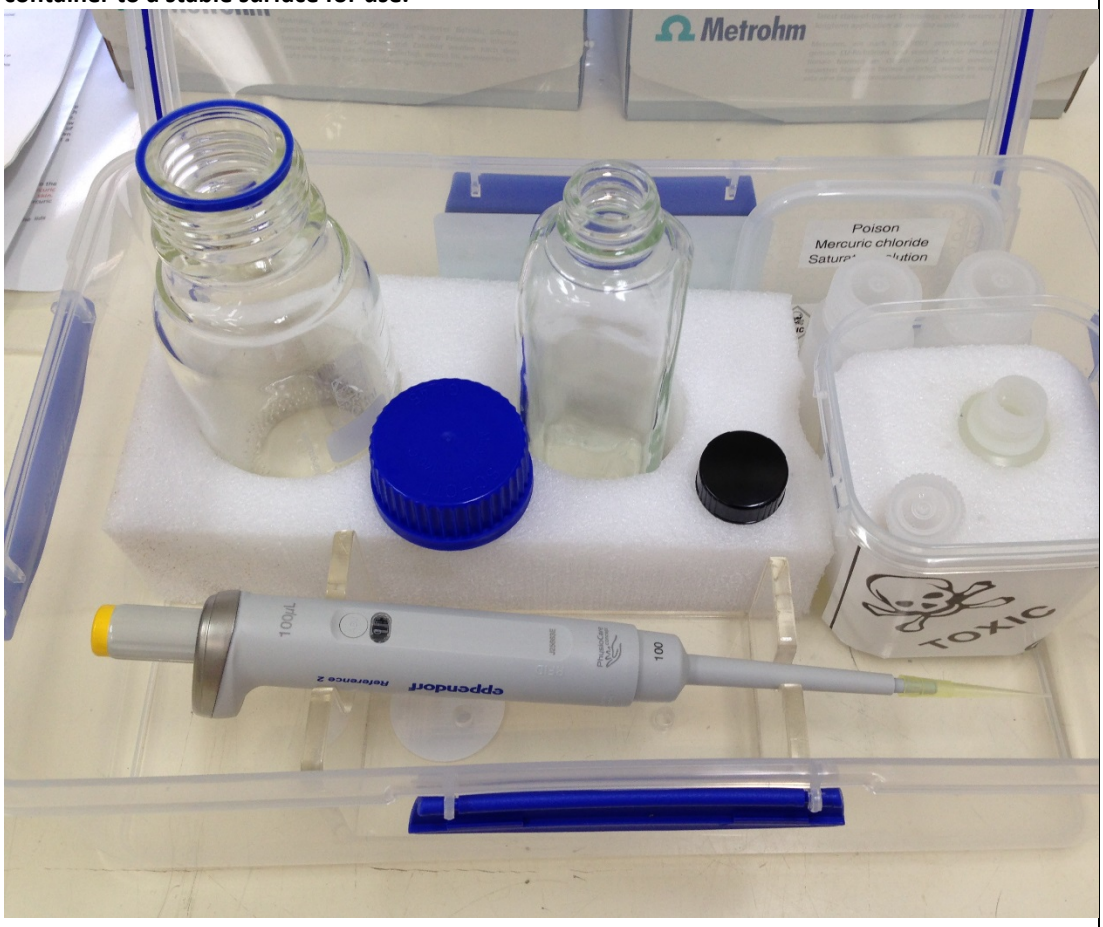
Precautionary statement(s) Prevention
P201 Obtain special instructions before use.
P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P262 Do not get in eyes, on skin, or on clothing.
P270 Do not eat, drink or smoke when using this product.
Precautionary statement(s) Response
P301+P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P308+P313 IF exposed or concerned: Get medical advice/attention.
P330 Rinse mouth.
Precautionary statement(s) Storage
P403+P233 Store in a well-ventilated place. Keep container tightly closed.
P405 Store locked up.
Precautionary statement(s) Disposal
P501 Dispose of contents/container in accordance with local regulations.

Version 1	Title	Name	Date
Consulted with *(group/team/users)*	Research Project Officer HSE Advisor HSE Advisor	**Heidi Franklin** **Jill Cooper** **Barb Vaschina**	15/12/2015 11/01/2016 28/01/2016
Cleared by	HSE Advisor *		
Approved by	Manager/Supervisor	**Craig Neill**	
Next Review			

INSTRUCTIONS

1. **Mercuric chloride saturated solution must be stored in a locked drawer or cabinet as it is an S7 poison.**

2. **Use a sturdy container to securely hold the mercuric chloride solution (enclosed in its own box), two sample bottles and a 100µL pipette (see photo). This setup provides secondary containment of the mercuric chloride solution in case of spillage. Secure all components to the bottom of the container with Velcro or double sided tape to ensure their stability in rough seas. Secure the container to a stable surface for use.**



3. **Before taking water samples, put on PPE including safety glasses, appropriate clothing, gloves and enclosed shoes as outlined above. Water sampling is generally easier and safer with two people taking part, one taking the samples and one adding the mercuric chloride solution.**

4. **Securely position each full sample bottle within the sampling container and unscrew the lid.**

5. **Using the pipette provided add 100µl of saturated mercuric chloride solution to the sample bottle. Keep the pipette tip just above the water surface to prevent cross contamination of samples. Do not allow the pipette to leave the containment of the plastic container. Never wave it around.**

6. **Screw the sample bottle lid on tightly and invert the bottle 4 times to distribute the mercuric chloride.**

7. **After adding mercuric chloride to all samples, discard the pipette tip into the vial provided. Put lids on the mercuric chloride bottle and box, and on the outer container. The mercuric chloride is now well secured for transport.**

8. **If mercuric chloride is spilled, or it is suspected that droplets of solution are in the container, rinse everything thoroughly with copious amounts of water.**

9. **Return the mercuric chloride solution bottle within its small box to the locked drawer or cabinet.**

10. **Sample bottles do not require extra labelling for mercuric chloride; under current GHS legislation preserved samples are not deemed to be hazardous substances.**

	Use and Management of Mercuric Chloride ($HgCl_2$) for Preserving Samples
Title	**Use and Management of Mercuric Chloride ($HgCl_2$) Saturated Solution for Preserving Water Samples**
Issued by	*Oceans and Atmosphere Hobart*
Application	*Who – members of ocean carbon team, samplers in Hobart IMOS team, other people taking seawater samples for analysis of carbon parameters* *What – addition of mercuric chloride solution to water samples* *Where – laboratory and field use for poisoning*

Persons signing this form acknowledge that they understand the instructions listed therein and will comply with them.

NAME	**SIGNATURE**	**DATE**