IMOS Integrated Marine Observing System

NATIONAL REFERENCE STATIONS

BIOGEOCHEMICAL OPERATIONS MANUAL

CSIRO Environment Report for IMOS

Version 5

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Document Version Control

Version	Date	Change Description	Author
5	September 2025	Updates to the depths sampled for flow cytometry and microbial samples. Introduction of microbial blanks and bacterial analysis. Standardisation of order of hydrochemistry sampling.	C. Davies
4.0	June 2023	Stopping of larval fish – add text Update citation Update detection limits for pigments Transport details and contacts removed to separate document for easier updating	C. Davies
3.3.1	June 2020	Larval fish procedure updated Nutrient analysis comment added to highlight samples are unfiltered	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	V. Latham

		• 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	
2.2	July 2012		G.W. Critchley
2.0	Sep 2009		G.W. Critchley

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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
- VBM Victorian Bonney Mooring, VIC (started April 2024)

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™ instruments, measuring conductivity, temperature and pressure at two depths. Up until 2017 WetLabs™ Water Quality Meters (WQM's) were used which included sensors measuring dissolved oxygen, fluorescence and turbidity. 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 here.

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	148.23333	-42.596667
Kangaroo Island	SARDI	KAI	SA	2008	110m	136.448	-35.836
Rottnest Island	CSIRO	ROT	WA	Apr 1951	50m	115.41667	-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	153.58022	-27.388917
Port Hacking 100	SIMS	PHB	NSW	May 1953	100m	151.25	-34.083333
Bonney Mooring	Deakin	VBM	VIC	April 2024	100m	141.271	-38.4086
No longer sampled							
Esperance	CSIRO	ESP	WA	2008	50m	121.85	-33.933333
Ningaloo	AIMS	NIN	WA	2010	50m	113.94665	-21.871733

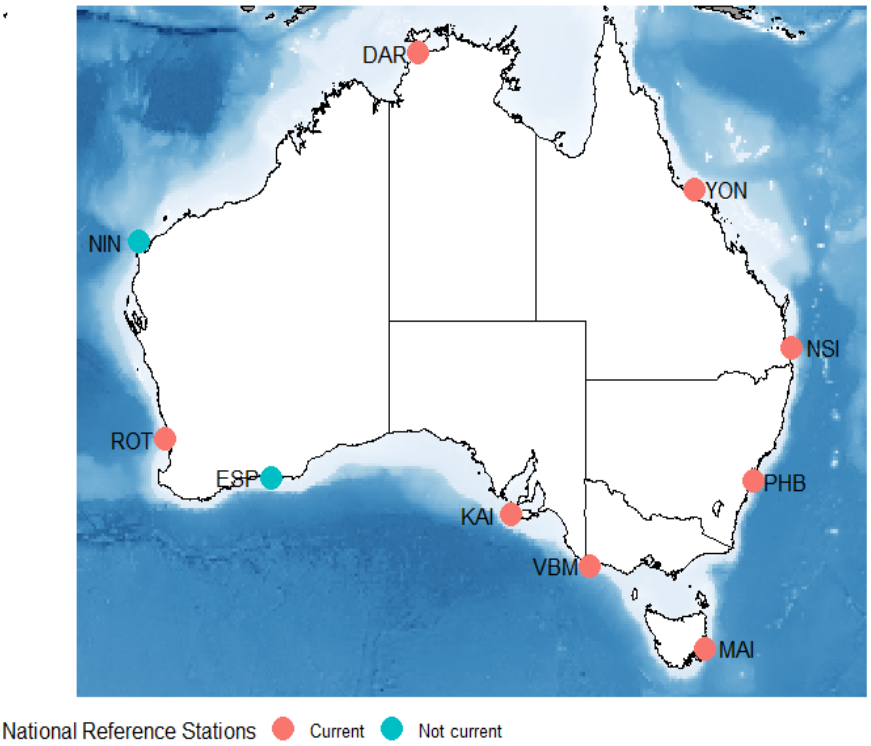


Figure 1 – IMOS NRS Locations

Table 2 – WQM sensor calibration samples

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

3 Safety Considerations

Each station should complete a risk assessment prior to any sampling trip. 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

It is recommended that the minimum number of crew to safely and efficiently manage the sampling trip is three, four would be preferable. These would include a certified coxswain or skipper. One of the persons may need 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,
- sun protection where required,
- 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
- nutrients (Nitrate/nitrite, silicate, phosphate, ammonia, nitrite)
- salinity
- 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)
- Zooplankton bulk biomass

5 Gear check list for boat

General

- Field log sheets, clipboard, pencils, permanent markers, nitrile and/or vinyl gloves
- MSDS for formalin and mercuric chloride

Seabird CTD

- Make sure battery has enough charge and complete pre-trip checks

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
- Formaldehyde 37%, 100mL

Niskin gear

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

Dissolved oxygen (Maria and Rottnest Islands only)

- DO flasks and DO reagent waste bottles
- DO reagents and 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
- 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

5.1 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.

5.2 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.

5.3 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 ap 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 Field sheet 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

- 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.

5.4 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
- formaldehyde 37% (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

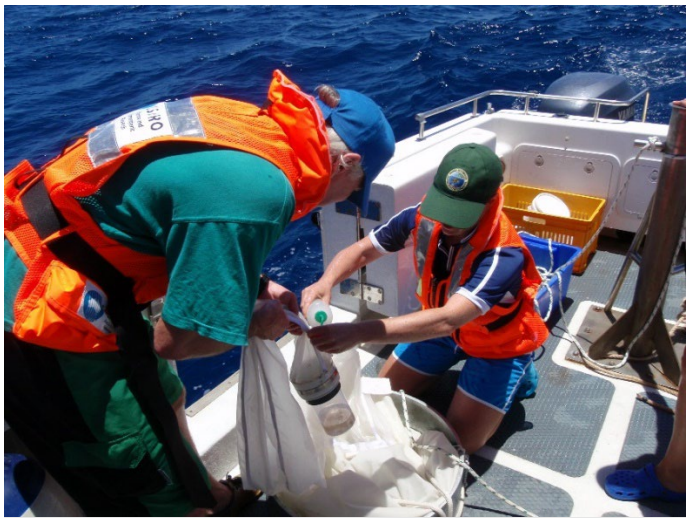
Before the first drop, make sure that the net is wet (spray it down with the deck hose), in order that the net is allowed to fall efficiently through the water column and the cod end is attached.

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.

5.5 Collecting water column samples

These samples are collected using Niskin bottles. 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.

5.5.1 Leak test

- With the top valve remaining closed, open the Niskin spigot by lining up the metal pin and hole, then push inward. Avoid touching spigot tip if possible, wearing vinyl gloves is recommended for this step if this cannot be avoided.

- 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.

5.5.2 Sampling order

The established sampling order for water column samples follows the GO-SHIP Repeat Hydrography Manual, a globally recognized reference for oceanographic best practice (Hood, 2010). The recommended order has been widely adopted and implemented across international research vessels and throughout the oceanographic community and is designed to minimise environmental contamination by prioritising the collection of the most sensitive samples first. Dissolved oxygen (DO) and dissolved inorganic carbon (DIC) are gases; therefore, are always collected first. Nutrients are often collected after gases as they are more susceptible to contamination followed by less sensitive salinity and total alkalinity (TA) (Swift, 2005).

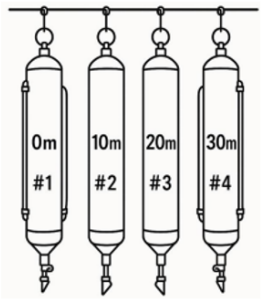
The sampling order outlined in this manual follows GO-SHIP recommendation, with slight modification to accommodate the smaller-scale sampling for NRS sites. TA is brought forward and collected before nutrients to minimise sampler exposure to the toxic reagent mercuric chloride ($HgCl_2$), which is used for DIC and TA, particularly when working on unstable sampling platform such as small boat. The sampling order for NRS sites is

---- *Analytes in this dashed box must be collected together once a Niskin bottle is opened before moving to the next Niskin bottle*
**Please note that only Rottnest Island (ROT) and Maria Island (MAI) are collecting DO samples.*

Based on this sampling order, it is recommended that sampler complete the DO, DIC, and TA together on one Niskin bottle before opening the next Niskin bottle. This approach ensures that the most sensitive samples (DO and DIC) are collected promptly after the bottle is opened. At the same time, this also allows poisoner to handle $HgCl_2$ (for DIC and TA) in a single, continuous period per deployment which minimises overall exposure time and avoids repeated handling.

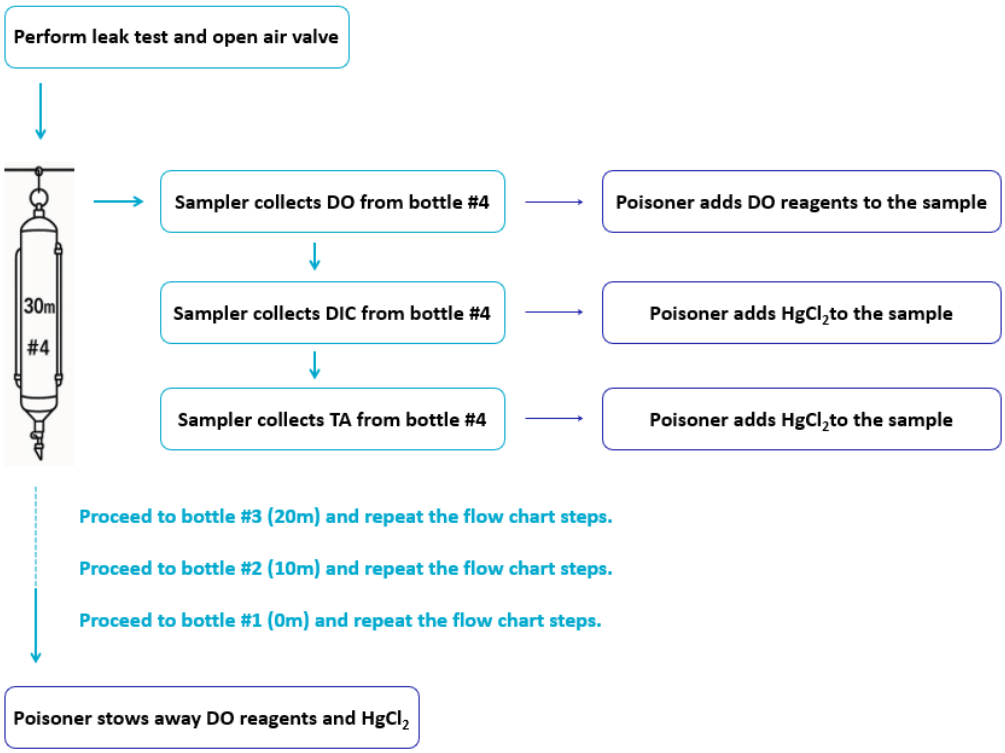
A detailed example of NRS sampling order and steps (including poisoning) are outlined below.

Number of Niskin bottle : 4
Depths for Collection: 0m, 10m, 20m, 30m
Personnel required: one sampler, one poisoner

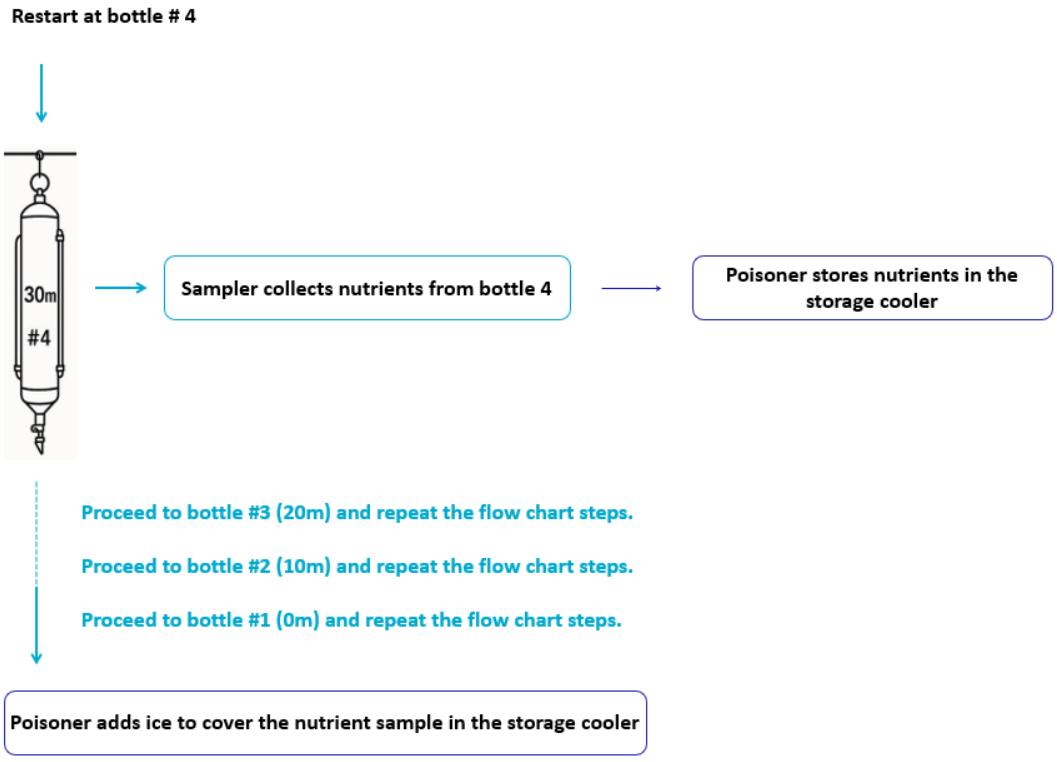


**Please note that it is recommended by GO-SHIP to collect the deepest depth sample first to ensure sample integrity and prevent contamination*

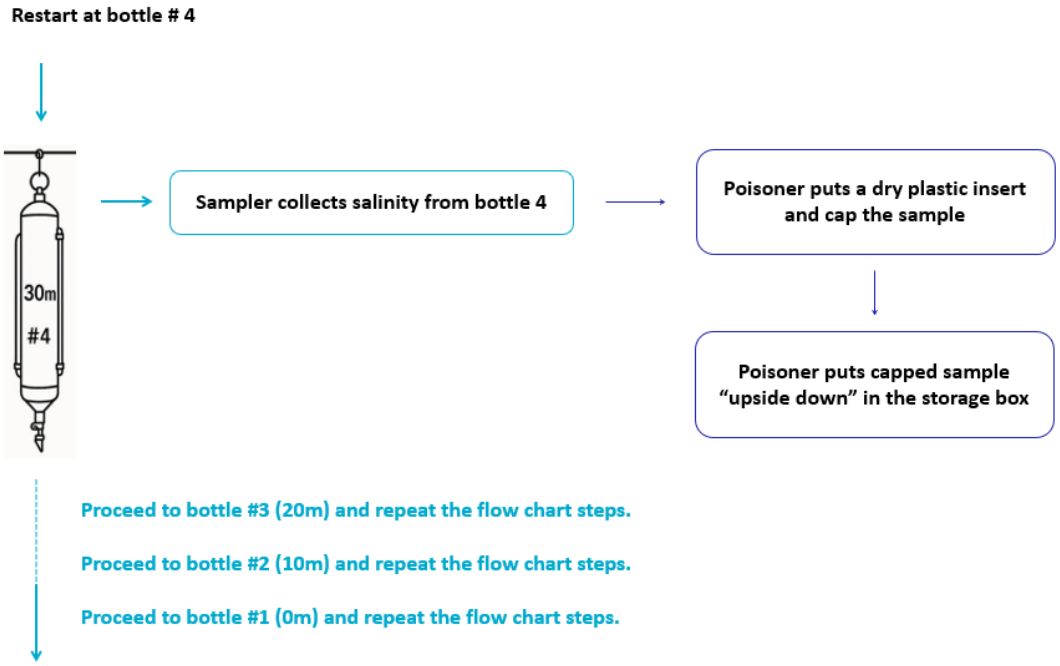
1. DO, DIC, and TA – collect these samples together on every Niskin bottle before collecting nutrients



2. Nutrients – collect nutrients on every Niskin bottle before collecting salinity



3. Salinity – collect salinity from every Niskin bottle



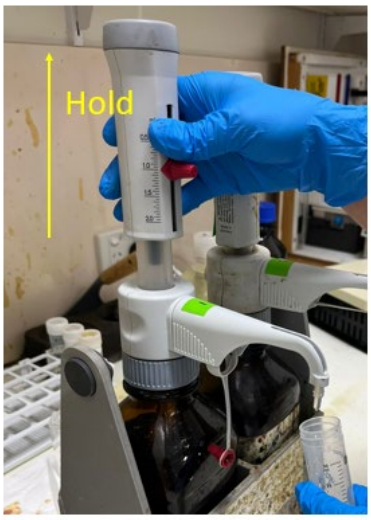
5.5.3 Dissolved Oxygen

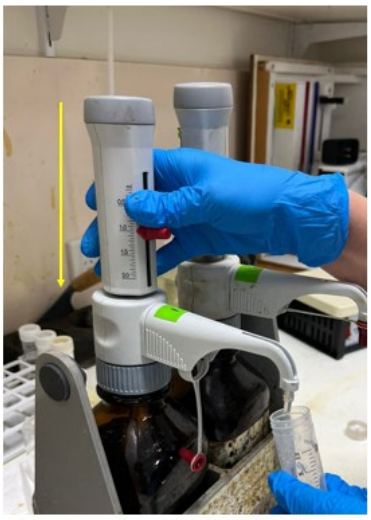
General information

Dissolved oxygen samples must be collected first. Pickling reagents should also be located nearby, ready to use prior to collecting samples. Each DO flask is labelled with flask number, stopper number, and collection depth. Please ensure the numbers on the flask and the stopper are matching. The collection depth is recorded as a label on the field sheet for DO samples.

Priming reagents

Priming DO reagent ensures no bubbles are caught in the dispenser and introduced into the collected samples. Nitrile gloves are recommended when using DO reagents at any time. To prime the DO reagents, pump each reagent dispenser by pulling the dispenser all the way up and hold (do not let go of the dispenser as this may cause spills), then push the dispenser down gently into waste containers. Make sure no bubbles are left in the dispensing tip. The priming process only has to be done once for each trip.





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.

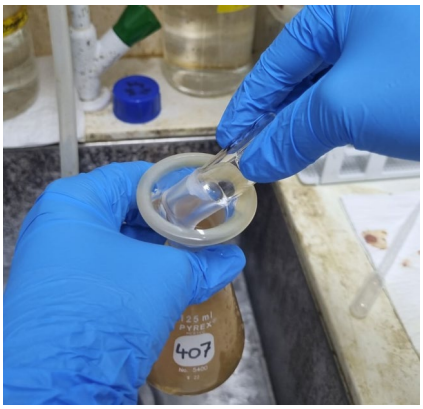
- Open the spigot by lining up pin and pushing inward.
- Repeatedly pinch the tubing to remove the bubbles. Rinse the sample bottle and stopper 3 times, pinching the tube to stop flow between rinses to conserve water.

- 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

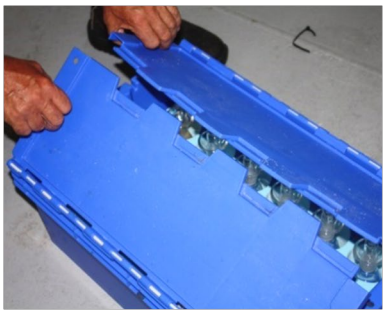
- Pull up and hold the dispenser to fill. Insert tip below water level and dispense the reagent 1 followed by reagent 2. Again, making sure no bubbles are dispensed.

- After both reagents have been added carefully insert the correct flask lid. The insertion at an angle 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.



5.5.4 Carbon

Two types of samples are required for carbon dioxide measurements:

- Total dissolved inorganic carbon (DIC) – square bottles with black lids
- Total alkalinity (TA) round bottles with blue lids

Bottles are supplied pre-labelled (except for date) in strong boxes for safe transport.

5.5.4.1 Sampling Total Dissolved Inorganic Carbon (DIC)

- Fit the flexible end of the sampling tube over the Niskin spigot.

- Start the flow. Put the end of the tube into the bottle. Open the valve at the top of the Niskin to start the flow out of the spigot. If there are air bubbles in the sampling tube pinch and release the tube a few times to remove them.
- Rinse. Fill the bottle to 1/3 full. Pinch the flexible part of the tube to stop the water flow. Swirl and invert the bottle to pour out the rinse water. Rinse three times.
- Fill. Insert the tube to the bottom of the sample bottle and SLOWLY release the pinch on the tube to allow water to flow without bubbles/turbulence. Allow the bottle to fill and overflow for about half the volume of the bottle. While overflowing turn the bottle to rinse the rim of the bottle and rinse the bottle cap in the overflow three times. Pinch the tube to stop flow and withdraw it carefully from the bottle. The level of sample in the DIC bottle should be as shown in this picture. If it is too full, pour out a little water.

- Poison the sample. Pipette 100µL of saturated mercuric chloride solution into the bottle. Keep the pipette tip just above the sample surface. Pipette slowly to prevent bubbles. You can reuse the pipette tip if you do not touch the sample surface. Screw the cap on tight and invert the bottle 3 times to distribute the mercuric chloride. 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.
- Store samples at room temperature in the box provided and retighten the lids after an hour or so.

5.5.4.2 Sampling Total Alkalinity (TA)

TA is sampled immediately after DIC.

- Use the same sampling technique as for DIC. After withdrawing the sample tube tip out some water to get the correct level.

- Using the pipette provided add 100µL of saturated mercuric chloride solution to the bottle. Keep the pipette tip just above the sample surface. Screw the cap on tightly and invert the bottle 3 times to distribute the mercuric chloride. 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.
- Store samples at room temperature in the box provided and retighten the lids after an hour or so.

All carbon samples must be returned to Hobart in the boxes provided.

5.5.5 Nutrients

General information

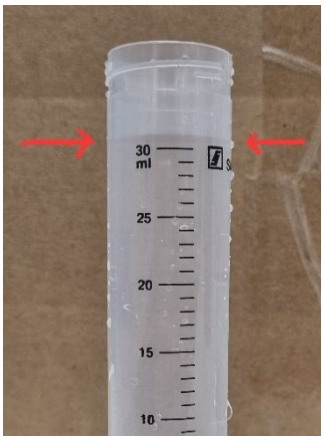
Nutrient samples at NRS sites are not filtered during collection as filtering my introduce contamination and particle loads are generally low in oceanic waters. For consistency across the NRS program, nutrient samples at coastal stations are also unfiltered, though particle loads may be significant at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

Duplicate samples are collected in single use 30 mL polypropylene tubes with screw caps. Collection tubes should be labelled with a depth (e.g., 0m, 10m) as well as the site code (e.g., MAI) and a trip date. The collection depth must be recorded as a label on the field sheet for nutrient samples.

Nutrient contamination occurs readily from sample contact with skin, sunscreen, sweat, etc. Do not touch the sample spigot and ensure your fingers do not contact the spigot outlet or the inside of the collection tubes or the caps. Powder-free vinyl gloves are highly recommended for nutrients collection. Please do not use nitrile gloves any time when nutrients are collected as they are one source of contaminants.

Sample Collection

- Nutrient is collected without the sampling tube. Half fill the collection tubes with sample water, loosely cap the collection tubes and shake in order to rinse the inside of the collection 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 collection tubes for sample expansion upon freezing. The water line should be roughly level with the 30 mL marking.



- Place the collection tube in the esky, top up with ice as soon as all samples are collected. The samples can be kept cool in an esky with ice or freezer packs for a few hours before freezing.
- Freeze the samples in an upright position as soon as possible and store frozen until analysis.

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.

5.5.6 Salinity

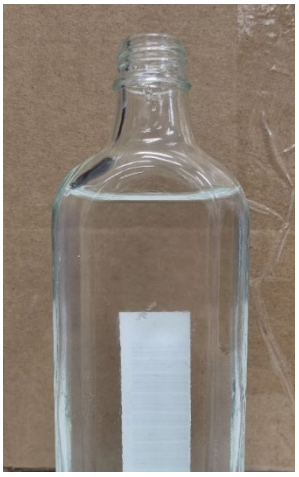
General information

The last parameter to be taken from the Niskin bottle is salinity. The collection depth must be recorded as a label on the field sheet for nutrient samples.

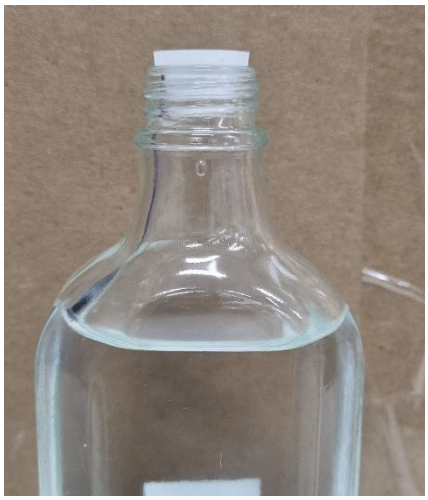
Sample Collection

- Remove the cap and empty the old sample from the bottle (if applicable), the removed cap is regarded as “dirty” cap. If the bottle and cap are clean to begin with for the trip, please make sure to remove the cap before collecting sample
- Attach sampling tube to the spigot, rinse the bottle with sample from the Niskin bottle by filling about 1/3 volume and emptying 3 times. Make sure that the bottle thread is also rinsed to remove any salt crystals. Pinch the tube where necessary to preserve water.

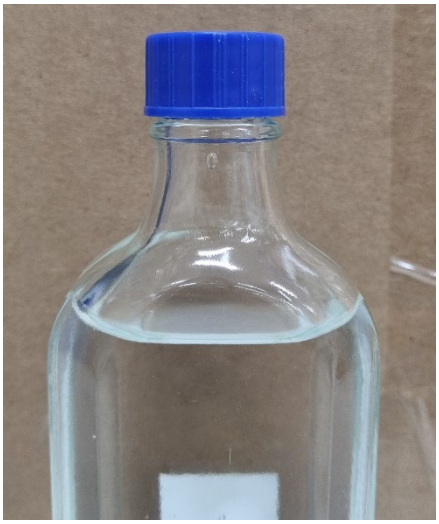
- Insert the tube until it touches the bottom of the bottle, slowly release the tube allowing water to flow into the bottle.
- Fill the bottle until it is overflowing, make sure there is no bubble during the collection.
- Pour out the sample until the water level is at the level shown in the image below. DO NOT OVERFILL.



- Place a clean dry plastic insert (this is recommended to be done by a person with dry hand) from the container supplied in the carry case, press down the insert tightly. If inserts are wet, rinse with the sample before using.
- If possible, turn the bottle (with plastic insert) upside down, rinse the outside of the thread and insert with MQ water, pad dry with paper towel.



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



- Place the bottle in the carry case upside down

5.5.7 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.

5.5.8 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.

5.5.9 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.

5.5.10 Marine Microbiome Initiative microbial and picoplankton sampling

Note – the Australian Marine Microbial Biodiversity Initiative (AMMBI) was the precursor to the IMOS Marine Microbiome Initiative Facility.

Equipment:

- 2-4 (depending on the number of depths to be sampled) x seawater containers (plastic; dark or covered ~4 L capacity)
- 1 x Funnel (plastic; compatible with seawater containers)
- large esky to carry 4 L plastic containers (unless processing directly after sampling)

On station:

- Conduct Niskin bottle cast(s) to pre-determined depths (x2-4 depending on NRS). Make cast as close to last activity on station as possible.

Note as of 1st January 2025 there has been a reduction in the number of depths that water samples are collected for microbial and flow cytometry sample processing, and the inclusion of a field control which is a filtration blank (MilliQ).

Sample collection depths are:

NRS	Collection Depth			
DAR	0	17		
YON	0	20	26	
ROT	0	20		
NSI	0	20		
MAI	0	20	50	85
VBM	0	20	50	85
KAI	0	20	50	100
PHB	0	25	50	100

- For each depth, rinse funnel and container (labelled with relevant depth) with retrieved seawater (~0.5 L). Discard.
- Fill container with a minimum of 3 L seawater and store in esky. Repeat for all depths.
- Surround containers with ice and if possible, keep esky/containers out of direct sunshine to minimise sample temperature shifts prior to sample processing.

5.6 Ichthyoplankton

Ichthyoplankton samples are no longer sampled through this program. For sampling details please see previous versions of the manual.

This section has been removed as of version 4.0.

5.7 Preservation and storage of samples

Care should be taken for the following procedures:

- Preserving zooplankton samples with formaldehyde 37%.
- Preserving the carbon samples with mercuric chloride
- 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.

6 Post-sampling activities

There are videos of all these procedures available at:
https://www.youtube.com/channel/UCXp5vxj2TaCatkura7vMYOA

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.

6.1 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
Zooplankton bulk biomass					yes
Microbiome	yes				

6.2 Pooled Depth Sample

If the Lugol's 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.

6.3 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.

6.4 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

6.5 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 glutaraldehyde. Store the glutaraldehyde in the fridge.
- Using the cryopen, label each vial with the site, date, depth and PHYCYT

Label example: **MAI100215 0m 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.

Reminder – as of 1st January 2025 there has been a reduction in the number of depths that water samples are collected for microbial and flow cytometry. Please refer to Section 5.5.10.

6.6 Preparation of zooplankton sample for genomics

- Equipment:
- 5 mL Cryovial x 1
- Fine mesh screen
- Sterile metal spatula (cleaned with 10% bleach prior to use and rinsed with either distilled or site water)
- Squirt bottle to be filled with seawater.

The seawater used should be from site water collected during your activities on station, e.g., it can be taken from the remaining water in microbiome sample containers after filtration has been completed.

- Cryo marker pen (withstand ultra-cold temperatures, e.g., -80 °C, LN2 dewar)
- Laboratory single-use gloves (e.g., latex)

Sample processing:

- Wearing gloves at all times during handling of the zoogen sample, fine mesh and cryovials.
- Use a cryopen to label according to the coding described in the sample coding section, above.
- Once back on land, pre-wet the mesh screen with seawater and pour the “cool blackened jar” sample through the fine mesh screen. Concentrate sample to one side using a squirt bottle of seawater.
- Next rinse lightly with seawater, 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.
- Place as much of the collected zooplankton sample into one clearly labelled 5 mL cryovial. However, if there is a lot of material, do not fill more than two thirds of tube. As long as the sample has been well mixed/homogenised, a sub-sample of the whole will still be representative of the community present.

- Store the cryovials at -80 °C prior to transport to Hobart.

6.7 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 and Bronwyn Holmes in September 2025.

Remember to collect water for the picoplankton samples from this water (Section 6.5 above).

Reminder – as of 1st January 2025 there has been a reduction in the number of depths that water samples are collected for microbial and flow cytometry. Please refer to Section 5.5.10.

From 1st January 2025 we have also included a field control which is a filtration blank (2L of MilliQ) for critical QA/QC processes.

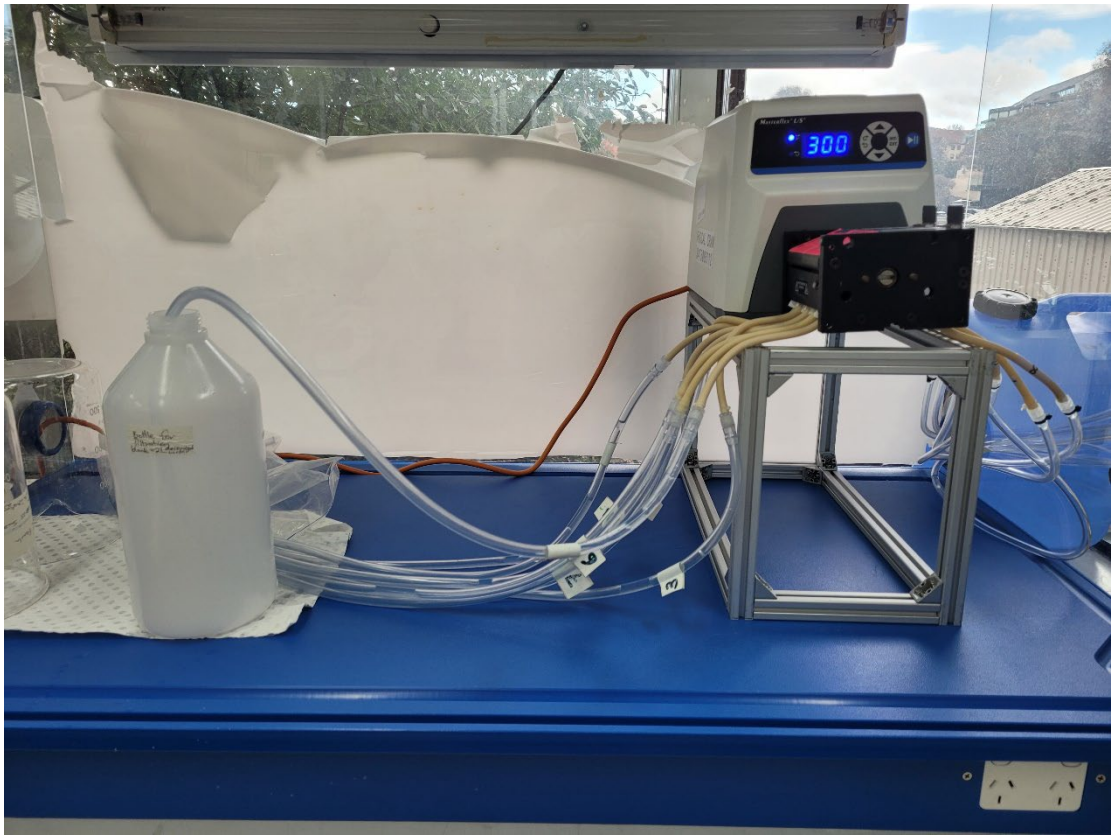
Equipment:

- Peristaltic pump, Pump head & tubing with Sterivex luer attachment
- Millipore Sterivex GP 0.22 µm filters (Cat. # SVGPL10RC) – one per depth plus one for MilliQ water blank
- ≥2 (depending on the number of depths to be sampled) x 2 L plastic volumetric cylinders or bottles (ideally 1 per depth to be filtered)
- MilliQ or deionised water for filtration blank in 2L volumetric cylinder or bottle
- Large snap-lock bags big enough to hold up to 4 Sterivex, labelled with date and station
    - *IF you have run out of Sterivex inlet/outlet caps then you will need small snap-lock bags for individual Sterivex to avoid contamination; labelled with date/station/depth/volume filtered. We would prefer to cut down on soft plastic waste so only use when no caps available.*
- Cryo marker pen (withstand ultra-cold temperatures, e.g., -80 °C, LN2 dewar)
- Esky containing ice
- Laboratory single-use gloves (e.g., latex)
- 5-10% bleach
- 70% EtOH

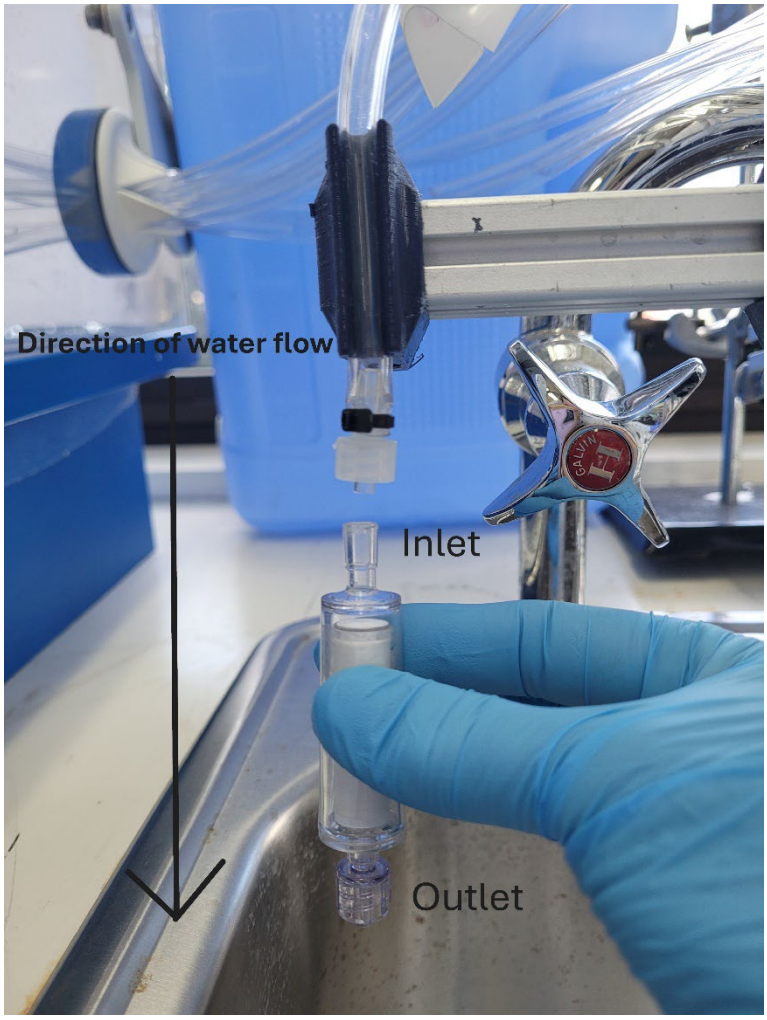
Sample processing:

- Always wearing gloves during filtration and handling of Sterivex filters, fill 2 L volumetric cylinders with seawater from each of the depths and label the cylinders accordingly. Prepare another cylinder with 2L of MilliQ water for the blank.
- 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 Sterivex filter attached).

- Run ~200 mL seawater for collected samples (or MilliQ water for the blank) through tubing with flow adjusted to approx. 200 mL/min on the old style pumps, or 300 RPM on the new style pumps (see image below).



- Remove the tubing from the cylinders, holding the tubing in the air (thereby not contaminating it by placing it on surrounding surfaces) and allow the remaining water to be pumped through the tubing. This will completely empty the tubing prior beginning the filtration of water samples.
- Replace the now empty tubing into the cylinders and refill volumetric cylinders to exactly 2 L of seawater for each depth (making sure that the correct depth seawater is being used), refill the MilliQ container to 2L
- Label each sterivex filter with station, depth and date e.g. MAI_0M_16052024
- Attach Sterivex filter to *fitting (luer lock), being careful not to touch lock end (refer to photo for the correct orientation of the filter)*



- 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 the actual volume that was filtered into the field log sheet and on the corresponding Sterivex filter. Please see below for further information on blocked Sterivex filters.
- After 2 L has passed through filter, let the pump continue for ~1 min to assist in drying filter. Emptying all remaining water within the filter housing is critical as it can affect down-stream DNA extraction processes.
- Disconnect each filter from tubing, giving each filter several "flicks" to remove as much residual water from inside the filter housing as possible.
- 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.
- Put capped filters into one larger snap-lock bag (labelled "NRS samples"; MAI; Date) and place on ice in the dark. Store all samples at -80 °C as soon as possible after processing.
- To clean the pump tubing after use, run 200 mL 5-10% bleach solution through tubing (allow bleach to sit for 10 minutes in tubing prior to emptying it through), followed by 3 rinses of 200 mL milliQ or distilled water per line, and lastly 200 mL 70% EtOH (to dry the tubing and prevent biofilm growth between uses). Make sure that all of the ethanol has passed through the tubing.

Blocked Sterivex filters - Sometimes this is unavoidable, particularly when the site water has an increased level of particulate matter (non-biological and biological) but a Sterivex housing full of water can affect down-stream DNA extraction processes. Here are some suggestions that may help avoid this situation

- Watch the outlet end of the Sterivex filter. If the flow rate changes from a steady stream to a slow drip then it is likely that your filter is starting to block.
- Remove the end of the intake line from the 2L cylinder so the excess water can drain out of the filter.
- If it appears that no more water will drain away you can try reducing the flow rate on the pump. **DO NOT increase the flow rate as this can lead to breakage of the filter or the housing of the Sterivex. Stervix filters have a pressure limit that we do not want to exceed.**

Note – each year we will provide new tubing for peristaltic pumps to each station. In addition, if you notice that your tubing is degrading/unclean and unable to be cleaned properly, please request new tubing at other times. Please direct any enquiries about peristaltic pumps (pump heads, batteries), including faults and/or replacements to the Marine Microbiome team (see contact details).

6.8 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

6.9 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.

6.9.1 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.

6.9.2 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 the contact details at Transport Contact Details NRS Sampling

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

6.10 Transport of NRS samples for analysis

This section has been replaced by a document available at Transport Contact Details NRS Sampling

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.

7 Sampling equipment maintenance

7.1 Zooplankton 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.

7.2 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.

7.3 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

7.4 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.

8 Data handling, Archival and Retrieval

8.1 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 and / or IMOS BGC CTD Processing Utility User Manual V2_1 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 if any previously published file needs to be replaced by an updated version.

The CTD is to be sent to the calibration facility at CSIRO in Hobart every 12 months for calibrations and service. To ensure the CTD is off site for the minimum time, samplers should coordinate the service with the Calibration Facility before it is sent. The Calibration Facility will upload the new calibration factors to the CTD and, if requested, will send an .xml file to the stations. The FLNTU and DO calibrations will need ot be entered manually as these are not part of the CTD firmware.

8.2 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 uploaded to the Teams fileshare 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.

Please notify AODN if any previously published file needs to be replaced by an updated version

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.

8.3 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 to 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

8.4 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

9 Analytical Methods

9.1 Zooplankton

Laboratory processing at CSIRO Brisbane.

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

9.2 Phytoplankton

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.).

9.3 Pigments

HPLC pigments from the “water column” and for WQM Calibration.

Phytoplankton pigments - Samples will be analysed by HPLC at CSIRO Hobart 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 $\approx$ 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^{o}C for $\approx$ 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.
- Values below detection limits will be displayed as 0's in the data. Detection limits vary according to volume filtered and are therefore difficult to report on a sample by sample basis. This information can be made available on request

9.4 Flow cytometry

Picoplankton analysis by flow cytometry

Photosynthetic picoplankton (Prochlorococcus, Synechococcus and picoeukaryotes) are enumerated by flow cytometry. Using discreet depth seawater samples collected at depths that match samples taken for genomics (molecular) analysis, subsamples of 1 ml are fixed with EM grade glutaraldehyde (0.25 % final concentration) for 15 min in the dark and frozen at –80 °C until analysis (Marie et al. 1999). Samples are thawed at 37 °C and 1 µm fluorescent beads (Molecular Probes) added as an internal standard. Samples are analysed using a suitable 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 are discriminated using scatter plots, including red and orange autofluorescence of chlorophyll against the accessory pigment phycoerythrin, and side-scatter against red fluorescence (Marie et al. 1999).

Total heterotrophic bacteria analysis by flow cytometry

Total bacteria are enumerated following adapted methods described by Marie et al. (2000), as follows:

- Stock solutions prepared beforehand:
    - SYBR Green I DNA stain – From primary stocks of 10,000x SYBR Green I stain, prepare separate aliquots of 10x SYBR Green I secondary stocks. All stains (primary and secondary) are stored at -20°C.
    - Prepare aliquots of 0.2 µm-filtered TE buffer. Extra aliquots can be stored at 4 °C and warmed to room temperature before use.
    - Dilute (500:1) 1 µm fluorescent beads (Molecular Probes) stored at 4 °C.

- Thaw an aliquot of 10x SYBR Green I secondary stock and mix immediately prior to use. Keep this 10x stock in the dark where possible during use and return to -20°C after use.
- After thawing each 1 mL replicate taken from IMOS NRS's for flow cytometry analysis, transfer a 100 µL aliquot to a separate microcentrifuge tube.
- To the 100 µL aliquot, add 10µL of 10x SYBR Green I secondary stock, mix, and incubate in the dark at room temperature for 15 minutes.
- Remove samples from incubation. Add 890 µL TE buffer and 10 µL of diluted 1 µm fluorescent beads, mix and load in appropriate format in readiness for flow cytometry measurement.
- Measure samples in low light using an appropriate flow cytometer fitted with a 488 nm laser and green fluorescence detector at a flow rate of 60 µL/min for 2 min. Total bacteria are discriminated using a side-scatter versus green fluorescence scatter plot.

9.5 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 analysing 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 $\mu molkg^{-1}$
- 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.

9.6 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:
Ausmicrobiome Scientific Manual - Ausmicrobiome Scientific Manual - Confluence (csiro.au)

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

9.7 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°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°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°C. After the furnace has reached this temperature, wait 3 hours before programming the temperature of the furnace to 20°C. When the furnace has reached 20°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.

9.8 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 Rees *et al*. 2018 and the analysis of ammonia is a based on the 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 µM

- Nitrate +Nitrite (NOx): 0.1 µM
- Phosphate: 0.05 µM
- Ammonia: 0.05 µM

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.

9.9 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

9.10 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 $\mu molL^{-1}$.

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.

Brown, M, Van De Kamp, J, Ostrowski, M, Seymour, J, Ingleton, T, Messer, L, et al. (2018) Systematic, continental scale temporal monitoring of marine pelagic microbiota by the Australian Marine Microbial Biodiversity Initiative. Nature Scientific Data. 5(180130):1-10.

Bucklin, A., (2000) Methods for population genetic analysis of zooplankton. In Zooplankton Methodology Manual. Edited by Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M. pp. 533-570

Carpenter, J.H. (2003) The accuracy of the Winkler method for dissolved oxygen analysis. *Limnology and Oceanography*, 10.

CSIRO Marine Laboratories Report 236, (1999) Rebecca Cowley, Gary Critchley, Ruth Eriksen, Val Latham, Ron Plaschke, Mark Rayner and David Terhell.

Culberson, C. H., G. Knapp, M. C. Stalcup, R. T. Williams and F. Zemlyak. (1991) A comparison of methods for the determination of dissolved oxygen in seawater. WHPO Publication 91-2.

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

Heron, A.C., (1982) A vertical free fall plankton net with no mouth obstructions. Limnology &Oceanography: 380-383

Hood, E.M., Sabine, C.L., & Sloyan, B.M. (Eds.). (2010). The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines, Version 1. IOCCP Report 14, ICPO Publication Series 134.

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

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.

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

Marie, D., Simon, N., Guillou, L., Partensky, F., & Vaulot, D. (2000) Flow cytometry analysis of marine picoplankton. In R.A. Diamond & S. DeMaggio (Eds.), *In living color: Protocols in flow cytometry and cell sorting* (pp. 421-454). New York, NY: Springer-Verlag, Berlin Heidelberg.

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

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.

Swift, J. H. (2010). The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines. IOCCP Report No. 14, ICPO Publication Series No. 134. UCSD Scripps Institution of Oceanography.

World Ocean Circulation Experiment – Operations Manual, Volume3; WHP Office Report WHPO 91-1 – WOCE Report No. 68/91, Revision1.

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**