Great Barrier Reef
MARINE MONITORING PROGRAM

Annual Report
QUALITY ASSURANCE AND
QUALITY CONTROL MANUAL
2019-20

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Front cover image: A submersible light logger exposed at low tide, Shellley Cove/Pallarenda in 2013, © Dieter Tracey.

The Great Barrier Reef Marine Park Authority acknowledges the continuing Sea Country management and custodianship of the Great Barrier Reef by Aboriginal and Torres Strait Island Traditional Owners whose rich cultures, heritage values, enduring connections and shared efforts protect the Reef for future generations.

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List of acronyms

AIMS................. Australian Institute of Marine Science
Authority........... Great Barrier Reef Marine Park Authority
ANZECC………. Australian and New Zealand Environment and Conservation Council
ARMCANZ……. Agriculture and Resource Management Council of Australia and New Zealand
CYWMP…………Cape York Water Monitoring Partnership
CDOM................ Coloured dissolved organic matter
Chl-*a*................. Chlorophyll *a*
CSIRO .... Commonwealth Scientific and Industrial Research Organisation
CTD .... Conductivity Temperature Depth profiler
DIN .... Dissolved inorganic nitrogen
DOC…………….Dissolved organic carbon
DON.................. Dissolved organic nitrogen
DOP.................. Dissolved organic phosphorus
ED..................... Empore disk
GC .... Gas chromatography
GPC.................. Gel permeation chromatography
HCl .... Hydrochloric acid
HPLC................. High-performance liquid chromatography
JCU .... James Cook University
LC-MS .... Liquid chromatography-mass spectrometry
MMP .... **Great Barrier Reef** Marine Monitoring Program
MODIS.............. Moderate-resolution imaging spectroradiometer
MS .... Mass spectrometry
MTSRF .... Marine and Tropical Sciences Research Facility
NASA .... National Aeronautics and Space Administration
NATA................. National Association of Testing Authorities
NH_4^+ .... Ammonium
NH_3 .... Ammonia
NO_2 .... Nitrite
NO_3 .... Nitrate
NO_x .... Nitrate/nitrite (NO_3 + NO_2)
NRM .... Natural resource management
PAH .... Polyaromatic hydrocarbons
PDMS .... Polydimethylsiloxane
PN..................... Particulate nitrogen
PO_4 .... Dissolved inorganic phosphorus(phosphate)
POC…………….Particulate organic carbon
PP..................... Particulate phosphorus
PRC .... Performance reference compounds
QA/QC.............. Quality assurance/quality control
QAEHS............. Queensland Alliance for Environmental Health Sciences
QHFSS .... Queensland Health Forensic & Scientific Service
$Si(OH)_4$ .... Silicate
SOPs .... Standard operating procedures

SPMD ....Semipermeable membrane devices
TDN ....Total dissolved nitrogen
TDP ....Total dissolved phosphorus
TN......................Total nitrogen
TP......................Total phosphorus
TropWATER .....Centre for Tropical Water and Aquatic Ecosystem Research
TSS....................Total suspended solids
UQ ....University of Queensland

1   Introduction

This manual describes the quality assurance and quality control (QA/QC) processes undertaken as part of the Great Barrier Reef Marine Monitoring Program (MMP) activities associated with the annual technical reports for the 2019–20 monitoring year. The MMP is a collaborative effort that relies on effective partnerships between governments, industry, community, scientists and managers. The Great Barrier Reef Marine Park Authority (the Authority) is responsible for the management of the MMP in partnership with monitoring partners including:

- Australian Institute of Marine Science (AIMS)
- James Cook University (JCU)
- University of Queensland (UQ)
- Howley Environmental Consulting/Cape York Water Monitoring Partnership (CYWMP)

We work together to deliver the sub-programs of the MMP which monitor the inshore marine environment including water quality, seagrass and coral. The broad objectives of this monitoring include:

- **Inshore marine water quality monitoring:** To assess temporal and spatial trends in marine water quality in inshore areas of the Reef lagoon in both ambient and wet season flood events.
- **Inshore seagrass monitoring:** To quantify temporal and spatial variation in the status of intertidal and subtidal seagrass meadows in relation to local water quality changes.
- **Inshore coral reef monitoring:** To quantify temporal and spatial variation in the status of inshore coral reef communities in relation to local water quality changes.

Each monitoring provider has a different responsibility in the delivery of the sub-programs that make up the monitoring sub-programs of the MMP (Table 1-1) including a description of the process for calculating Reef 2050 Water Quality Report Card scores.

Table 1-1. Current monitoring themes, sub-programs and monitoring providers

Monitoring sub-program	Component project(s)	Monitoring provider
Inshore marine water quality	Ambient water quality monitoring	AIMS, JCU and CYWMP
	Pesticide monitoring	UQ
	Wet season monitoring	JCU and CYWMP
	eReefs biogeochemical model and satellite images	Not managed by the Authority
Inshore seagrass condition	Inshore seagrass monitoring	JCU
Inshore coral reef condition	Inshore coral monitoring	AIMS

1.1   Inshore marine water quality monitoring

Long-term *in situ* monitoring of spatial and temporal trends in the inshore water quality of the Reef lagoon is essential to assess improvements in regional water quality that are expected to occur as a result of reductions in nutrient and sediment loads from adjacent catchments. In addition, understanding what is delivered by flood waters is essential to quantify the exposure of inshore ecosystems to these loads.

The MMP water quality design was reviewed in 2014. A conceptual model (Haynes *et al.* 2007) and the Driver, Pressure, State, Impact, and Response (DPSIR) framework were used to identify indicators linking water quality and ecosystem health, which were refined in consultation with monitoring providers and independent experts. Program design recommendations were based on a statistical review (Kuhnert *et al.* 2015). The new sampling design for the inshore water quality monitoring program was implemented in 2015, to increase the detection of links between end-of-catchment loads and marine water quality through higher frequency sampling at a larger number of sites in the focus area. The design focused on four areas — the Russell-Mulgrave, Tully, Burdekin Rivers, and the Mackay Whitsunday region. Extra reporting for the Barron-Daintree sub-region of the Wet Tropics was also included due to the continued collection of data along the long-term 'Cairns transect' where sampling started in 1989.

In 2016–17, four transects were added in the Cape York region relevant to the Pascoe, Normanby-Kennedy, Annan-Endeavour and Stewart Rivers. These transects are monitored by the Cape York Water Monitoring Partnership coordinated by Howley Environmental Consulting.

The sites in each focus area are located to capture water masses along cross-shelf and alongshore gradients. The site selection in the focus areas was informed by the plume frequency model (Petus *et al.* 2014a; Devlin *et al.* 2013) and the river tracer model (Brinkman *et al.* 2014).

Monitoring includes measurement of dissolved and particulate nutrients (nitrogen and phosphorus) and carbon, total suspended solids (TSS), chlorophyll *a* (Chl-*a*), silica [$Si(OH)_4$], salinity, turbidity, coloured dissolved organic matter (CDOM) and temperature. Additionally, samples for pesticides are collected in the wet season.

Techniques used are a combination of:

- continuous measurement of salinity and temperature at seven stations
- continuous measurement of Chl-*a* and turbidity at 15 stations
- 58 ambient stations sampled during the year with more frequent sampling during the wet season (85 sites in total)
- 27 event-based stations sampled during flood conditions.

Wet season water quality conditions and the movement of flood plumes is assessed using images from remote sensing (moderate-resolution imaging spectroradiometer, MODIS) imagery (see Chapter 3).

The revised design supports closer integration between MMP components, leading to outputs that are expected to meet stakeholder needs, including:

- a robust data foundation and continuous improvement of all reporting metrics (those in the Reef 2050 Water Quality Report Card reports)
- improved reporting of pressure indicators via models of exposure that link marine water quality to end-of-catchment loads (*water quality as a state*)
- a robust data foundation for detecting, attributing and interpreting relationships between water quality and coral reef and seagrass condition (*water quality as a pressure*)
- ongoing validation of the eReefs model to allow for more confident predictions of water quality in areas that are not monitored.

1.2   Remote sensing of water quality

The use of remotely-sensed data in combination with *in situ* water quality measurements provides a powerful source of data in the assessment of water quality across the Reef. Remote sensing studies using derived water quality level-2 products and quasi-true colour (hereafter true colour) satellite images have been used to map and characterise the spatial and temporal distribution of river plumes and understand the impact of these river plumes on Reef ecosystems.

To define and map wet season conditions, and the movement, composition and frequency of occurrence of wet season water quality conditions across the Reef, current remote sensing methods based on the extraction and analysis of MODIS true colour data have been tested and are described more fully in Chapter 3.

To illustrate wet season marine conditions and identify where seagrass and coral reefs may be at risk, several satellite-derived products from MODIS satellite imagery are produced. These include weekly panel maps of environmental and marine wet season conditions, frequency maps of occurrence of wet season water types and exposure maps, as well as the extraction of the area (km^2) and percentage (%) of coral reefs and seagrass meadows affected by different categories of exposure (or potential risk).

A more detailed description of methodologies is provided in Chapter 3.

1.3   Pesticide monitoring

The off-site transport of pesticides from land-based applications has been considered a potential risk to the Reef. Of particular concern is the potential for compounding effects that these chemicals have on the health of the inshore reef ecosystem, especially when delivered with other water quality pollutants during flood events (this project is also linked to flood plume monitoring and the collection of water samples directly from research vessels, Section 2).

Passive samplers are used to measure the concentration of pesticides in the water column integrated over time by accumulating chemicals via passive diffusion. The monitoring of specific pesticides during flood events and throughout the wet season is essential to

evaluate long-term trends in pesticide concentrations along inshore waters of the Reef. Key points include:

- pesticide concentrations are measured with passive samplers at selected sites (sampling occurred at 5 sites in 2019-20) at monthly intervals during the wet season.
- pesticide concentrations are assessed against relevant Guidelines (Australian and New Zealand Governments 2018; Great Barrier Reef Marine Park Authority 2010). Note that new pesticide guideline values are also reported as categories of sub-lethal stress defined by the published literature and taking into account mixtures of herbicides that affect photosynthesis.
- the continual refinement of techniques that allow a more sensitive, time-integrated and relevant approach for monitoring pollutant concentrations in the lagoon and assessment of potential effects that these pollutants may have on key biota.

1.4   Inshore coral monitoring

Coral reefs in inshore areas of the Reef are frequently exposed to runoff (Furnas and Brodie 1996). Monitoring temporal and spatial variation on the status of inshore coral reef communities in relation to changes in local water quality is essential in evaluating long-term ecosystem health.

Monitoring covers a comprehensive set of community attributes including the assessment of hard and soft coral cover, macroalgal cover, density of juvenile hard coral colonies, hard coral community composition and the rate of change in coral cover as an indication of the recovery potential of the reef following a disturbance (Thompson *et al.* 2020). In addition, the incidence of ongoing coral mortality is recorded and, where possible, attributed to the causative agent. Comprehensive water quality measurements are also collected at many of the coral reef sites (this project is linked to inshore water quality monitoring, Chapter 2). Key points include:

- reefs are monitored biennially at 31 inshore coral reefs in the Wet Tropics, Burdekin, Mackay Whitsunday and Fitzroy regions along gradients of exposure to run-off from regionally important rivers
- for reefs not scheduled for monitoring in a given year contingency sampling is undertaken when a disturbance was likely to have impacted reef since it was last monitored
- at each reef, two sites are monitored at two depths (two metres and five metres) across five replicate transects
- in addition to the monitoring of benthic community attributes, the measurement of sea temperature as an indicator of thermal stress is included.

1.5   Inshore seagrass monitoring

Seagrasses are an important component of the marine ecosystem of the Reef. They form highly productive habitats that provide nursery grounds for many marine and estuarine species. Monitoring temporal and spatial variation in the status of inshore seagrass meadows in relation to changes in local water quality is essential in evaluating long-term

ecosystem health. The seagrass monitoring project is closely linked to the global Seagrass-Watch monitoring program (http://www.seagrasswatch.org).

Monitoring includes seagrass abundance (% cover) and species composition, macroalgal cover, epiphyte cover, canopy height, mapping of the meadow landscape (extent) and assessment of seagrass reproductive effort, which provide an indication of the capacity for meadows to regenerate following disturbances and changed environmental conditions. Leaf tissue nutrient composition is assessed in the laboratory as an indicator of potential nutrient enrichment. Key points include:

- monitoring occurs at 47 sites across 22 locations, including 13 nearshore (estuarine, coastal and fringing reef) and nine offshore reef locations.
- monitoring is conducted during the late-dry season and post-wet season; extra sampling is conducted at more accessible locations in the dry and monsoon seasons
- monitoring includes *in situ* within canopy temperature and light levels
- data is also collected annually from an additional 26 sites across 14 locations by Seagrass-Watch (intertidal) and Queensland Parks and Wildlife Service (QPWS; subtidal drop-camera) during the late-dry season. (Please note: 5 of the additional sites occur within 3 locations monitored by MMP)

1.6 Marine Monitoring Program quality assurance and quality control methods and procedures

Appropriate QA/QC procedures are an integral component of all aspects of sample collection and analysis. These QA/QC procedures have been approved by an expert panel convened by the Authority. The Authority set the following guidelines for implementation by MMP program leaders:

- Appropriate methods must be in place to make sure of consistency of in-field procedures to produce robust, repeatable and comparable results, including consideration of sampling locations, replication and frequency.
- All methods used must be fit-for-purpose and suited to a range of conditions.
- Appropriate accreditation of participating laboratories or provision of standard laboratory protocols to demonstrate that appropriate laboratory QA/QC procedures are in place for sample handling and analysis.
- Participation in inter-laboratory performance testing trials and regular exchange of replicate samples between laboratories.
- Rigorous procedures to guarantee 'chain of custody' and tracking of samples.
- Appropriate standards and procedures for data management and storage.

In addition to the QA/QC procedures outlined above, the MMP employs a proactive approach to monitoring through the continual development of new methods and the refinement of existing methods, such as the:

- operation and validation of autonomous environmental loggers
- validation of algorithms used for the remote sensing of water quality
- improvement of passive sampling techniques for pesticides

- introduction of extra monitoring sub-programs to evaluate the condition of inshore reefs, specifically coral recruitment.

The monitoring providers for the MMP have a long-standing culture of QA/QC in their monitoring activities. Common elements across the providers include:

- ongoing training of staff (and other sampling providers) in relevant procedures
- standard operating procedures (SOPs), both for field sampling and analytical procedures
- use of standard methods (or development of modifications)
- publishing of methods and results in peer-reviewed publications
- maintenance of equipment
- calibration procedures including participation in regular inter-laboratory comparisons
- established sample custody procedures
- QC checks for individual sampling regimes and analytical protocols
- procedures for data entry, storage, validation and reporting.

This manual summarises the monitoring methods and procedures for each project. Detailed sampling manuals, SOPs, analytical procedures and other details are provided as appendices.

2   Inshore marine water quality monitoring

Jane Waterhouse[1], Renee Gruber[2], Stephen Lewis[1], Caroline Petus[1], Christina Howley[3], Michele Skuza[2], Dieter Tracey[1], Irena Zagorskis[2], and Murray Logan[2]

[1]James Cook University, [2]Australian Institute of Marine Science, [3]Howley Environmental Consulting

2.1   Introduction

The aim of water quality monitoring is to assess the concentrations and transport of terrestrially-derived components, with a focus on the movement of TSS, Chl-*a* and particulate and dissolved nutrients into the Reef lagoon. Current sampling methods include discrete water profile sampling combined with analysis of remote sensing imagery (MODIS) as a tool for qualitatively assessing flood plume extent within the Reef. The MMP inshore water quality monitoring program informs the Paddock to Reef program by:

- Describing spatial patterns and temporal trends in inshore concentrations of sediment, Chl-*a*, nutrients, and pesticides as assessed against the Guidelines (or other water quality guidelines if appropriate).
- Monitoring local water quality using autonomous instruments for high-frequency measurements at selected inshore locations.
- Determining fine scale water quality depth profiles by deploying continuous monitoring equipment along transects in key areas (note, only from 2015–16).
- Determining three dimensional extent and duration of flood plumes and linking concentrations of suspended sediment, nutrients, and pesticides to end-of-catchment loads.
- Calculating the Water Quality Index and site-specific metrics for nutrients, turbidity, and suspended solids.
- Establishing the extent, frequency and intensity of impacts on Reef inshore seagrass meadows and coral reefs from flood plumes and the link to end-of-catchment loads.

2.2   Methods

2.2.1   Sampling locations and frequency

The current design of the joint AIMS, TropWATER, and Cape York sampling program comprises 58 routine sampling sites visited throughout the year. There is more frequent sampling during the wet season which includes 27 additional stations that are supplementary to the routine sites. The sampling sites are located across focus areas in four NRM regions: Cape York —Pascoe, Stewart, Normanby-Kennedy, and Annan-Endeavour Rivers; Wet Tropics — Barron-Daintree (Cairns transect), Russell-Mulgrave and Tully; Burdekin; and Mackay Whitsunday. These include the original 14 'core' sites of the inshore coral reef monitoring program and key sites under the wet season monitoring (Table 2-1 and Figure 2-1). At these sites, detailed manual and instrumental water sampling is undertaken.

Table 2-1 Description of the water quality sites sampled by AIMS, JCU and CYWMP during 2019–20. Sites in bold font were part of the ambient monitoring design from 2005 to 2015. The proposed number of visits is shown in black text, while the actual number of visits is shown in brackets in red text. * Not event samples but taken during routine sampling for Russell Mulgrave before that was ceased.

Site Location	Logger Deployment		Ambient sampling at fixed sites (proposed and actual)		Event-based sampling
NRM region	**Turbidity and chlorophyll**	**Salinity**	**Number of times site is visited/year by AIMS**	**Number of times site is visited/year by JCU/ CYWMP**	**Additional surface-sampling/year by JCU/ CYWMP**
Cape York					
Normanby-Kennedy transect					* (specific sites TBD)
Kennedy mouth				4 (Sampling 2 depths) (4)	1
Kennedy inshore				4 (Sampling 2 depths) (4)	
Cliff Islands				4 (Sampling 2 depths) (5)	1
Bizant River mouth				4 (Sampling 1 depth) (4)	
Normanby River mouth				4 (Sampling 2 depths) (4)	1
Normanby inshore				4 (Sampling 2 depths) (4)	
NR-03				4 (Sampling 2 depths) (4)	
NR-04				4 (Sampling 2 depths) (4)	
NR-05				4 (Sampling 2 depths) (4)	
Corbett Reef				4 (Sampling 2 depths) (2)	
Additional sites/ event samples					3
Pascoe transect					* (specific sites TBD)
Pascoe mouth north				5 (Sampling 1 depth) (5)	
Pascoe mouth south				5 (Sampling 1 depth) (5)	
PR-N2				5 (Sampling 2 depths) (5)	
PR-N3				5 (Sampling 2 depths) (5)	
PR-N5				5 (Sampling 2 depths) (5)	
PR-N6					1
PR-S2.5				5 (Sampling 2 depths) (5)	
Middle Reef				5 (Sampling 2 depths) (5)	
PR-S5				5 (Sampling 2 depths) (5)	
Additional sites/ event samples					2
Annan and Endeavour transect					* (specific sites TBD)

Site Location	Logger Deployment		Ambient sampling at fixed sites (proposed and actual)		Event-based sampling
NRM region	Turbidity and chlorophyll	Salinity	Number of times site is visited/year by AIMS	Number of times site is visited/year by JCU/ CYWMP	Additional surface-sampling/year by JCU/ CYWMP
Annan mouth				5 (Sampling 1 depth) (5)	(3)
Walker Bay				5 (Sampling 1 depth) (5)	(2)
Dawson Reef	√			5 (Sampling 2 depths) (5)	(1)
Endeavour mouth				5 (Sampling 1 depth) (6)	(3)
Endeavour north shore				5 (Sampling 1 depth) (6)	(3)
Endeavour offshore				5 (Sampling 1 depth) (6)	(2)
Egret and Boulder Reef				5 (Sampling 2 depths) (5)	(2)
Additional sites/ event samples					(10)
Stewart transect					* (specific sites TBD)
Stewart mouth				5 (Sampling 1 depth) (5)	1
SR-02				5 (Sampling 2 depths) (5)	
SR-03				5 (Sampling 2 depths) (5)	
SR-04				5 (Sampling 2 depths) (5)	
Hannah Island				5 (Sampling 2 depths) (5)	
Additional site					2
Wet Tropics					
Cairns Long-term transect					
Cape Tribulation			3 (Sampling 2 depths) (3)		
Port Douglas			3 (Sampling 2 depths) (3)		
Double Island			3 (Sampling 2 depths) (3)		
Yorkey's Knob			3 (Sampling 2 depths) (3)		
Fairlead Buoy			3 (Sampling 2 depths) (3)		
Green Island			3 (Sampling 2 depths) (3)		
Russell-Mulgrave Focus Area					
Fitzroy Island West	√		5 (Sampling 2 depths) (5)		
RM2					** (Surface sampling only) (2)*
RM3			5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
RM4					** (Surface sampling only) (1)*
High Island East					** (Surface sampling only) (2)
Normanby Island					** (Surface sampling only) (2)

Site Location	Logger Deployment		Ambient sampling at fixed sites (proposed and actual)		Event-based sampling
NRM region	Turbidity and chlorophyll	Salinity	Number of times site is visited/year by AIMS	Number of times site is visited/year by JCU/ CYWMP	Additional surface-sampling/year by JCU/ CYWMP
Frankland Group West (Russell Island)	√		5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
High Island West	√	√	5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Palmer Point					** (Surface sampling only) (1)
Russell-Mulgrave River mouth mooring	√	√	5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Russell-Mulgrave River mouth					** (Surface sampling only) (2)
Russell-Mulgrave junction [River]					** (Surface sampling only) (1)
Tully Focus Area					
King Reef					** (Surface sampling only)
East Clump Point			5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Dunk Island North	√	√	5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
South Mission Beach					** (Surface sampling only) (2)
Dunk Island South East			5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Between Tam O'Shanter and Timana			5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Hull River mouth					** (Surface sampling only) (2)
Bedarra Island			5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Triplets					** (Surface sampling only) (2)
Tully River mouth mooring	√	√	5 (Sampling 2 depths) (5)	5 (Sampling 2 depths) (5)	
Tully River					** (Surface sampling only) (1)
Burdekin					
Burdekin Focus Area					
Pelorus and Orpheus Island West	√		4 (Sampling 2 depths) (4)	5 (Sampling 2 depths) (5)	
Pandora Reef	√		4 (Sampling 2 depths) (4)	5 (Sampling 2 depths) (5)	
Cordelia Rocks					** (Surface sampling only)
Magnetic Island (Geoffrey Bay)	√		4 (Sampling 2 depths) (4)	5 (Sampling 2 depths) (5)	
Inner Cleveland Bay					** (Surface sampling only)
Cape Cleveland					** (Surface sampling only)
Haughton 2			4 (Sampling 2 depths) (4)	5 (Sampling 2 depths) (5)	

Site Location	Logger Deployment		Ambient sampling at fixed sites (proposed and actual)		Event-based sampling
NRM region	Turbidity and chlorophyll	Salinity	Number of times site is visited/year by AIMS	Number of times site is visited/year by JCU/ CYWMP	Additional surface-sampling/year by JCU/ CYWMP
Haughton River mouth					** (Surface sampling only)
Barratta Creek					** (Surface sampling only)
Yongala IMOS NRS	√	√	12 (Sampling 2 depths) (12)		
Cape Bowling Green					** (Surface sampling only)
Plantation Creek					** (Surface sampling only)
Burdekin River mouth mooring	√	√	4 (Sampling 2 depths) (4)	5 (Sampling 2 depths) (5)	
Burdekin Mouth 2					** (Surface sampling only)
Burdekin Mouth 3					** (Surface sampling only)
Mackay-Whitsunday					
Whitsunday focus area					
Double Cone Island	√		5 (Sampling 2 depths) (5)		
Hook Island W					** (Surface sampling only)
North Molle Island					** (Surface sampling only)
Pine Island	√		5 (Sampling 2 depths) (5)		
Seaforth Island	√		5 (Sampling 2 depths) (5)		
OConnell River mouth			5 (Sampling 2 depths) (5)		
Repulse Islands dive mooring	√	√	5 (Sampling 2 depths) (5)		
Rabbit Island NE					** (Surface sampling only)
Brampton Island					** (Surface sampling only)
Sand Bay					** (Surface sampling only)
Pioneer River mouth					** (Surface sampling only)
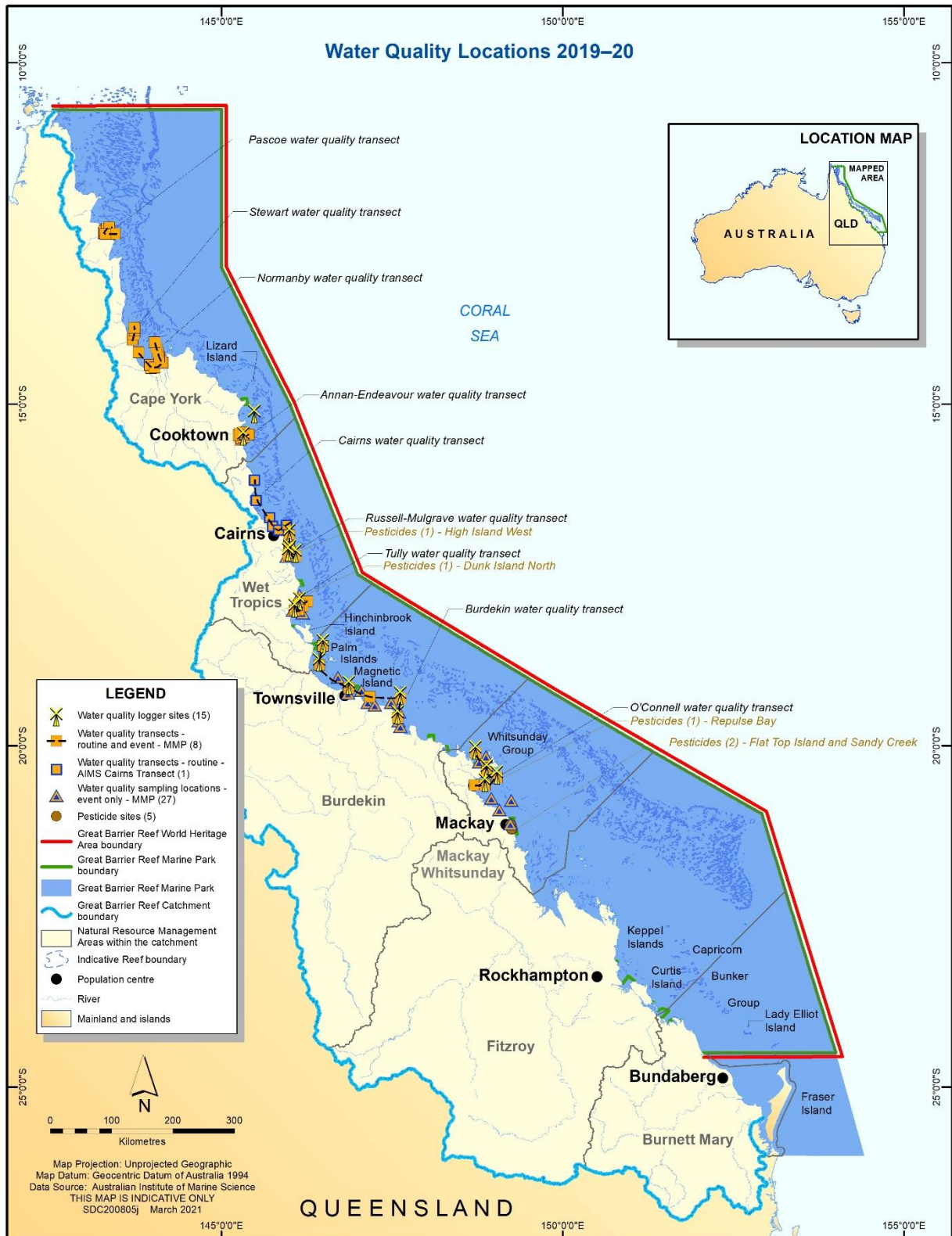
Figure 2-1 Sampling locations of the water quality monitoring sampled from 2015 onwards. Note that the Cape York transects were added in 2017.

2.2.2   Sample collection, preparation and analysis (JCU)

The guidelines for water quality sampling listed in this document are based on the protocols required by the TropWATER laboratory for the collection and storage of samples.

Staff must always be accompanied by at least one other person. Staff must have conducted a risk assessment of the sampling area incorporating current weather conditions and have an up-to-date emergency plan. Staff must be aware of the operational capabilities of the vessel and work through the safety protocols with the ship master. The following must also be observed:

- PVC disposable gloves must be worn by staff at all times during sample collection and manipulation. Before sampling, staff must clean their hands thoroughly with fresh water. Grease, oils, soap, fertilisers, sunscreen, hand creams and smoking can all contribute to contamination.
- The sampling container and boat bilge pump and rose must be well rinsed before sampling. Bottles must be rinsed if required by the TropWATER laboratory.
- The filling instructions (contained in the following sections) must be adhered to when filling containers.
- On each sampling run, the date, time and unique sample identification must be recorded on the field datasheet. Each sampling kit for each site contains sets of sampling bottles and vials with a unique ID number (convention is MMP-<sample number>).
- Any significant change of sampling conditions (i.e. weather and other factors which may influence the ability to collect samples) must be noted in the comments section of the record sheet.
- If possible, a few photographs at each sampling site should be taken.

At each sampling station in the Wet Tropics, Burdekin and Mackay Whitsunday regions, vertical profiles of water depth, temperature, salinity, dissolved oxygen and light are taken with a SeaBird SBE-19Plus Conductivity Temperature Depth profiler (CTD). In Cape York, a CastAway CTD is used to collect vertical profiles of water temperature, salinity, and depth. In all cases, the CTD must be deployed on the sunny side of the boat to avoid interference with light data from the boat shadow. The CTD must be held at the surface for three minutes before performing the downcast to allow for the stabilisation of the sensors. Secchi depth is determined at each station and is deployed on the shady side of the boat by a person not wearing sunglasses.

Immediately following the CTD cast, water samples are collected up to 0.5 metres below the surface and one to two metres from the bottom for laboratory analyses. Surface samples are collected with a rinsed clean 10 L sampling container. Depth samples are collected using a rinsed 5 L Niskin bottle; the 'bottom' is determined by the depth sounder on the boat. The sample is then transferred to a rinsed 10 L container where sub samples are taken for different water quality parameters (see Appendix B1). For the Cape York transects, surface

samples are collected using an extended sampling pole and clean 500 mL bottle while depth samples are collected using a rinsed 5 L Niskin sampler. The water quality parameters collected for all sites include:

- Concentrations of nitrate/nitrite (NO_x), ammonium (NH_4^+), filterable reactive phosphorus (FRP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total nitrogen (TN), total phosphorus (TP)
- Concentrations of chlorophyll *a* (Chl-*a*)
- Concentrations of total suspended solids (TSS)
- Coloured dissolved organic matter (CDOM)
- Salinity
- Silica

A summary of the field processing and storage requirements are listed in Table 2-2 and explained in further detail in Appendix B. A summary is provided below.

Dissolved nutrient, silica and CDOM samples are collected from the container using sterile 60 mL syringes. Water in the 10 L container is rigorously stirred before being collected by the syringe to ensure it is well mixed before transferred to the sampling tubes. For dissolved nutrients, a 0.45 µm disposable membrane filter is fitted to the syringe and 60 mL of sample is filtered into six 10 mL sampling tubes. For silica, a 0.45 µm disposable membrane filter is fitted to the syringe and filtered into a 60 mL tube. All sampling tubes are placed in a clean plastic bag and stored on ice in an insulated container on the sampling vessel. Samples are then stored either at 4° C or frozen (see Table 2.2) prior to transport to the laboratory for analysis. The analysis methods are described in Appendix B5 to B9.

PN and PP samples are collected in sterile 500 mL containers and stored on ice before being filtered on Whatman GF/F filter papers on return to the laboratory. The target filter volumes for PN and PP are 500 mL and 250 mL, respectively. The filter papers are frozen and transported to the AIMS laboratory for analysis.

CDOM is collected by passing 100 mL of water through a 0.2 µm disposable membrane filter into 120 mL amber glass bottle. Samples are refrigerated prior to transport to the laboratory for analysis which must be completed within 7 days of collection. The analysis methods are described in Appendix B12.

Table 2-2 Summary of sampling protocols with identification of post-sampling procedures, laboratory containers, and storage techniques.

Water quality parameter	Field processing	Post field processing	Laboratory container	Storage	Laboratory method
Oxidised nitrogen (nitrate + nitrite: NO_x)	Filtered sample (0.45 µm)	n/a	10 mL plastic tube	Frozen	Appendix B7
Ammonium (NH_4^+)	Filtered sample (0.45 µm)	n/a	10 mL plastic tube	Frozen	Appendix B5
Total dissolved nitrogen (TDN)	Filtered sample (0.45 µm)	n/a	10 mL plastic tube	Frozen	Appendix B8
Particulate nitrogen (PN)	n/a	Filtered on Whatman GF/F papers on return to lab	500 ml container	n/a	Analysed by AIMS laboratory
Particulate phosphorus (PP)	n/a	Filtered on Whatman GF/F papers on return to lab	500 ml container	n/a	Analysed by AIMS laboratory
Filterable reactive phosphorus (PO_4)	Filtered sample (0.45 µm)	n/a	10 mL plastic tube	Frozen	Appendix B6
Total dissolved phosphorus (TDP)	Filtered sample (0.45 µm)	n/a	10 mL plastic tube	Frozen	Appendix B9
Chlorophyll-*a* (Chl-*a)*	Filtered 100 ml sample (0.7 µm GF/F paper)	n/a	GF/F filter paper wrapped in aluminium foil	Frozen	Appendix B10
TSS	Unfiltered sample (1000 mL) in clear bottle	Filtered onto polycarbonate (0.4 µm) 47 mm paper	1000 mL clear bottle	Stored at 4°C	Appendix B11
Salinity	Unfiltered sample (60 mL) in a clean dry vial	n/a	60 mL plastic vial	Stored at 4°C	Appendix B13
Coloured Dissolved Organic Matter (CDOM)	Filtered sample (0.2 µm)	n/a	120 mL dark bottle	Stored at 4°C	Appendix B12
Silica	Filtered sample (0.45 µm)	n/a	60 ml plastic tube	Stored at 4°C	Aquakem Discrete Analyser using APHA 4500-SiO_2 D Heteropoly Blue Method.

TSS samples are collected in pre-rinsed 1000 mL plastic containers using a well mixed 10 L container. Each container is rinsed at least once with the sample water, taking care to avoid any contact with the sample (gloves are worn at all times). Samples are stored on ice on the sampling vessel. Once the vessel has returned to shore, the TSS samples are refrigerated and must be filtered within 7 days of collection. The analysis methods are described in Appendix B11. For salinity samples, unfiltered water is stored in 60 mL bottles and stored on ice on the sampling vessel (see Appendix B13 for analysis methods). Chl-*a* samples are collected with a 60 mL syringe from a well mixed 10 L container. The sample is filtered through a 25 mm diameter GF/F filter paper held with a plastic syringe filter holder attached to the filter. 100 mL of sample is filtered and then air is passed through the filter holder (using the syringe) to remove any excess water from the filter paper. The filter paper sample is then folded, wrapped in aluminium foil, labelled with the sample number and frozen. The analysis method for the Chl-*a* samples is provided in Appendix B10.

TropWATER laboratory takes part in an inter-calibration program with AIMS. All processed data are stored in a Microsoft Access database.

2.2.3   Sample collection, preparation and analysis (AIMS)

Vertical profiles of the water column are conducted using a Conductivity Temperature Depth (CTD) profiler. AIMS uses several Sea-Bird Electronics CTD profilers (SBE 19plus), which are fitted with additional sensors including fluorometers, transmissometers and Photosynthetically Active Radiation (PAR) sensors. Annual calibrations of the profiler instrumentation are carried out in Sea-Bird Electronics laboratories in the USA. These calibration values are included within the SBE configuration files. Pre-trip CTD checks are carried out at AIMS. These include checking the physical status of the sensors and cables.

Prior to deployment of the CTD profiler on board the boat, the CTD is secured to the hydrographic wire or line. Tygon tubing is removed from the CTD to allow flush water to drain from the conductivity-temperature cell and any protective caps are removed from the other sensors. To activate logging, the magnetic switch is moved to the "on" position and the CTD cage is lowered into the water sitting ~0.2 m below the surface. A three minute soak of the CTD begins, to allow sensors to equilibrate and air bubbles to be flushed by the pump.

The profile is commenced at a rate no greater than 0.5 m s^{-1} to achieve a minimum sensor scan rate of 8 scans m^{-1} vertically. The CTD is sent to near-bottom, ensuring it does not touch the seafloor, and retrieved to the surface where the switch is turned off. See Appendix A1 for details of data post-processing and storage.

Immediately following the CTD cast, discrete water samples are collected from two depths through the water column (~0.5 m below the water surface and ~1 m above the seabed) with Niskin bottles. Duplicate sub-samples are taken from the Niskin bottles for analysis of dissolved inorganic nutrients [NH_3, NO_2, NO_3, PO_4, $Si(OH)_4$], total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved organic carbon (DOC), CDOM, particulate nutrients (PN and PP), particulate organic carbon (POC), TSS, and Chl-*a*. Samples are also taken for laboratory salinity measurements using a high-precision salinometer (Appendix

A2). Temperatures at each sampling depth are measured with reversing thermometers attached to the Niskins.

In addition to the ship-based sampling, water samples are collected by diver-operated Niskin bottles for validation of turbidity and fluorescence measurements by moored loggers. Samples for lab analysis of Chl-*a* and TSS are collected close to the deployed loggers and are processed for Chl-*a* concentration, TSS and salinity, using the same methods as those for the ship-based samples.

Samples for dissolved nutrient analysis are immediately filtered through a 0.45 μm filter cartridge (Sartorius Minisart N) into acid-washed, screw-cap plastic test tubes, which are pre-rinsed twice with filtered site water. Dissolved inorganic nutrient (NH_3, NO_2, NO_3, PO_4) and total dissolved nutrient (TDN and TDP) samples are then stored frozen (-18°C) until subsequent analysis ashore. Samples for DOC analysis are filtered, acidified with 100 μL of AR-grade HCl, and stored refrigerated (4°C) until analysis. Samples for $Si(OH)_4$ are filtered and stored refrigerated (4°C) until analysis. Samples for CDOM analysis are filtered through a 0.2 μm filter cartridge (Pall-Acropak supor membrane) into acid-washed, amber glass bottles and stored refrigerated (4°C) until analysis (within 7 days of collection).

Inorganic dissolved nutrient [NH_3, NO_2, NO_3, PO_4, $Si(OH)_4$] concentrations are determined by standard wet chemical methods (Ryle *et al.* 1981) implemented on a segmented flow analyser after return to the AIMS laboratory (Appendix A3). Analyses of total dissolved nutrients (TDN and TDP) are performed using persulfate digestion of water samples (Valderrama 1981) (Appendix A3), which are then analysed for inorganic nutrient concentrations, as above.

To avoid potential contamination during transport and storage, analysis of ammonium (NH_4^+) concentrations in triplicate subsamples per Niskin bottle are immediately performed during the Cairns transect sampling aboard *RV Cape Ferguson* using a fluorometric method based on the reaction of ortho-phthal-dialdehyde with ammonium (Holmes *et al.* 1999). These samples are analysed on fresh unfiltered seawater samples using specially cleaned glassware (Appendix A4). This analysis can only be done on board a vessel with a laboratory, and so is not done during most MMP sampling, which is based from small vessels conducting day trips.

DOC concentrations are measured by high temperature combustion (720°C) using a Shimadzu TOC-L carbon analyser. Before analysis, CO_2 remaining in the sample water is removed by sparging with O_2 carrier gas (Appendix A5).

CDOM samples are measured on a Shimadzu UV Spectrophotometer (UV-1900) equipped with 10 centimetre cells using Milli-Q water as a blank. Before analysis, samples are allowed to warm to room temperature (~25° C) (Appendix A6).

Samples for POC, PN, PP and Chl-*a* are collected on pre-combusted (450°C for 4 h) 25 mm diameter glass fibre filters (Whatman GF/F, pore size ~0.7 μm). Filters are wrapped in pre-combusted aluminium foil envelopes and stored frozen (-18°C) until analysis.

Concentration of PP is determined spectrophotometrically as inorganic phosphorus (PO_4) (Parsons *et al.* 1984) after digesting the particulate matter in five per cent potassium persulfate (Appendix A7). The method is standardised using orthophosphoric acid and dissolved sugar phosphates as the primary standards.

The POC content of material collected on filters is determined by high temperature combustion (950°C) using a Shimadzu TOC-V carbon analyser fitted with a SSM-5000A solid sample module (Appendix A8). Filters containing sampled material are placed in pre-combusted (950°C) ceramic sample boats. Inorganic C on the filters (for example $CaCO_3$) is removed by acidification of the sample with 2 M hydrochloric acid. The filter is then introduced into the sample oven (950°C), purged of atmospheric CO_2 and the remaining organic carbon is combusted in an oxygen stream and quantified by infrared gas analysis. The analyses are standardised using certified reference materials (for example NCS DC73047).

PN is determined using a Shimadzu TN unit (model TNM-1) fitted in series to the aforementioned carbon analyser. After the carrier gas stream moves from the carbon detector it enters an ozone saturated reaction chamber where NO_2 reacts with ozone. This reaction generates chemiluminescence, which is then measured using a chemiluminescence detector (Appendix A8). The analyses are standardised using certified reference materials (for example NCS DC73047).

Chl-*a* concentrations are measured fluorometrically using a Turner Designs 10AU fluorometer after grinding the filters in 90% acetone (Appendix A9) (Parsons, Maita, and Lalli 1984). The fluorometer is calibrated against Chl-*a* extracts from log-phase diatom cultures (Chl-*a* and *c*). The extract Chl-*a* concentrations are determined spectrophotometrically using the wavelengths and equation specified in (Jeffrey and Humphrey 1975). The solutions are then diluted and used to produce a standard curve matching the sample concentration range and of suitable concentration for use in the fluorometer.

Samples for TSS are collected on pre-weighed 0.4 µm polycarbonate filters (47 mm diameter, GE Water & Process Technologies), which are triple rinsed with Milli-Q water following sample filtration to remove any salts from the filter. TSS concentrations are determined gravimetrically from the difference in weight between loaded and unloaded filters after the filters have been dried overnight at 60° C (Appendix A10).

2.2.4   Autonomous environmental water quality loggers (AIMS)

In situ autonomous water quality monitoring is undertaken using WET Labs Environmental Characterisation Optics (ECO) FLNTUSB Combination Fluorometer and Turbidity Sensors. The ECO FLNTUSB instruments perform simultaneous *in situ* measurements of chlorophyll fluorescence, turbidity, and temperature (Appendix A11).

Chlorophyll fluorescence measured *in situ* is dominated by the concentration of the Chl-*a* pigment, but also includes accessory chlorophyll pigments and some degradation products. Measurement of Chl-*a* from filtered water samples (described above) specifically measures the Chl-*a* from phytoplankton. To clarify the difference between measurements from filtered

water samples versus instrumental data, the *in situ* fluorescence measurements from loggers are referred to as 'chlorophyll' to distinguish them from direct water sampling measurements of 'Chl-*a*'.

The instruments are used in 'logging' mode and record a data point every 10 minutes for each of the three parameters; each data point is calculated as the mean of 50 instantaneous burst readings (Appendix A11). Pre- and post-deployment checks of each instrument include measurements of the maximum fluorescence response and the dark count (instrument response with no external fluorescence, essentially the 'zero' point). Factory servicing and calibration checks are performed at the WET Labs facility in the USA after 12-18 months of in-water deployment time (Appendix A11).

After retrieval from the field locations, the instruments are cleaned and data downloaded and converted from raw instrumental records into measurement units (μg L^{-1} for chlorophyll fluorescence, NTU for turbidity, and ^{o}C for temperature) according to the standard procedures of the manufacturer. Deployment information and all raw and converted instrumental records are stored in an Oracle-based data management system developed by AIMS. Records are quality-checked using time-series data editing software (WISKI$^{©}$-TV, Kisters) and unreliable data caused by instrument problems removed (see Appendix A11 and A12).

Instrument data are validated against concurrently-collected water samples. The relationship between optically measured turbidity and TSS analysed on filters is relatively good (Figure 2-2), and the linear equation [TSS (mg L^{-1})] = 1.3 x FLNTUSB Turbidity (NTU)] has been used for conversion between these two variables. Though these relationships are valid it should be remembered that the two variables are measures of two different things that do not necessarily co-vary. Using this equation, the TSS trigger value in the Water Quality Guidelines of 2.0 mg L^{-1} (Great Barrier Reef Marine Park Authority 2010) translates into a turbidity trigger value of 1.5 NTU.

SS = 1.3 x Turbidity
R² = 0.83
N=1750
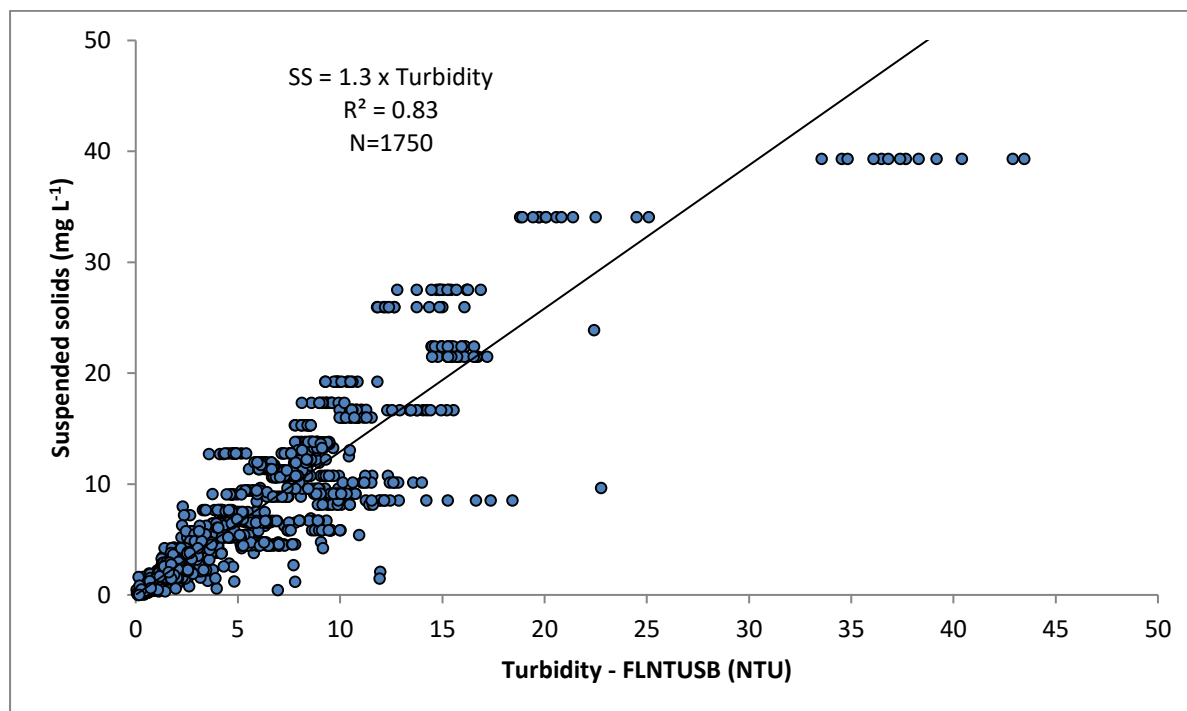
Figure 2-2 Correlation between instrument readings of turbidity (NTU) from field deployments of WET Labs Eco FLNTUSB loggers with values from concurrently-collected total suspended solids (TSS) samples.

2.2.5   Sample collection, preparation and analysis: wet season — event response (JCU)

Event response sampling is conducted in addition to routine wet season sampling. Samples are collected at the fixed routine sites and at the reactive flood plume sampling sites and the parameters measured and sample collection procedures are identical to those described for the routine program. The event sampling is triggered when the downstream river flow gauge levels approach or exceed the 'moderate flood level' and if weather sea state conditions are suitable for sampling. The river flood levels are regularly monitored on the Bureau of Meteorology website (http://www.bom.gov.au/qld/flood/rain_river.shtml) and include the gauges 'Burdekin R at Inkerman Br' (Burdekin River), 'Tully R at Euramo' (Tully River), 'Mulgrave R and Peets Bridge' and 'Russell R at Bucklands' (Russell-Mulgrave River), 'O'Connell R at Stafford Crossing' (Proserpine/O'Connell Rivers) and 'Pioneer R at Dumbleton TW' (Pioneer River) (Table 2-1).

Extra samples may also be collected depending on the extent of the plume. The majority of extra samples are collected inside the visible area of the plume, although some samples are taken outside the edge of the plume for comparison purposes.

Reactive flood sampling is conducted in the focus areas but may also be extended to other regions in large events, such as the Fitzroy River in 2016–17 with the high river flow associated with cyclone Debbie.

The timing of sampling is dependent on the type of event and how quickly boats are mobilised. Sampling in flood plumes requires rapid response sampling protocols as a detailed pre-planned schedule is not possible due to the unpredictability of the river flood events. The need for a responsive, event-driven sampling strategy to sample plumes from flowing rivers is essential to capture the high flow conditions associated with these rivers (Wheatcroft and Borgeld 2000).

2.3 Data management

Data management practices are a major contributor to the overall quality of the data collected; inappropriate data management can lead to errors and lost data and can reduce the value of the MMP data. Several specific data systems have been developed for the MMP water quality monitoring to improve data management procedures and reduce the likelihood of human error (details on these are in Appendix A12). Data from the AIMS MMP inshore water quality monitoring are stored in a custom-designed data management system in Oracle 11g databases to allow cross-referencing and access to related data. Once data are uploaded into the Oracle databases after the QA and validation processes, they are consolidated via Oracle views (see Appendix A12).

It is AIMS policy that all datasets collected have an associated metadata record. The metadata record is created using a Metadata Entry System (also known as the AIMS Data Catalogue) where the metadata is in the form of ISO19139.MCP XML. This is the chosen format for many agencies across Australia and the international community that deal with spatial scientific data. You can visit the AIMS Data Explorer at: https://apps.aims.gov.au/metadata/search

Specific metadata records exist for AIMS' MMP datasets and are linked below:

- Biogeochemical database (containing all dissolved and particulate nutrient data, including data from JCU and CYWMP): https://apps.aims.gov.au/metadata/view/09163cdc-decc-40ec-b5a8-9a07dabf7fb8
- FLNTU logger database (containing all fluorescence/turbidity measurements from AIMS' ECO FLNTUSB loggers): https://apps.aims.gov.au/metadata/view/8a698de1-3fbf-48a5-b068-358b07aad35c
- Salinity/temperature time-series database (containing salinity/temperature data from AIMS' SBE37 loggers at MMP sites): https://apps.aims.gov.au/metadata/view/351d44f5-6888-45fc-ac46-44ddbd623bf1
- CTD database (containing all AIMS' vertical cast data from CTD instruments): https://apps.aims.gov.au/metadata/view/9080e85b-d9a6-4001-b196-e21547d31653

For JCU samples, station description and details (for example geographical position, date, time, sampling depth, local depth, weather, surface water temperature and comments) are

recorded on weatherproof field datasheets (Appendix B2) and transferred into Microsoft® Excel spreadsheets. All Excel spreadsheets are collated and inputted into the TropWATER Flood Plume Monitoring database (Microsoft® Access database, see Appendix B3 for metadata details). The field sheets are also scanned and stored at JCU. All water samples and filters are labelled with unique sample identifiers. The TropWATER laboratory organises a flood sampling kit for each site that has the unique identifier for all dissolved and total nutrients, silica, salinity, Chl-*a*, TSS, phytoplankton and CDOM bottles.

The spreadsheet data are then transferred into the TropWATER Flood Plume Monitoring Microsoft® Access database. Data are also relayed onto the TropWATER laboratory input sheets (Appendix B4). Data are checked before and after transfer for completeness (for example agreement of station and sample numbers, all samples that were collected have been analysed) and correct data entry (i.e. comparison with previous data, cross-checking of data outside typical ranges with archived raw data records, for example, as hard copies or instrument files). Data are independently checked after entry into the database (see Figure 2-3).

For analysis of trends, data are transferred into the AIMS water quality database. Copies of the TropWATER Flood Plume Monitoring database (Microsoft® Access database) are stored in external media and also on Dropbox.
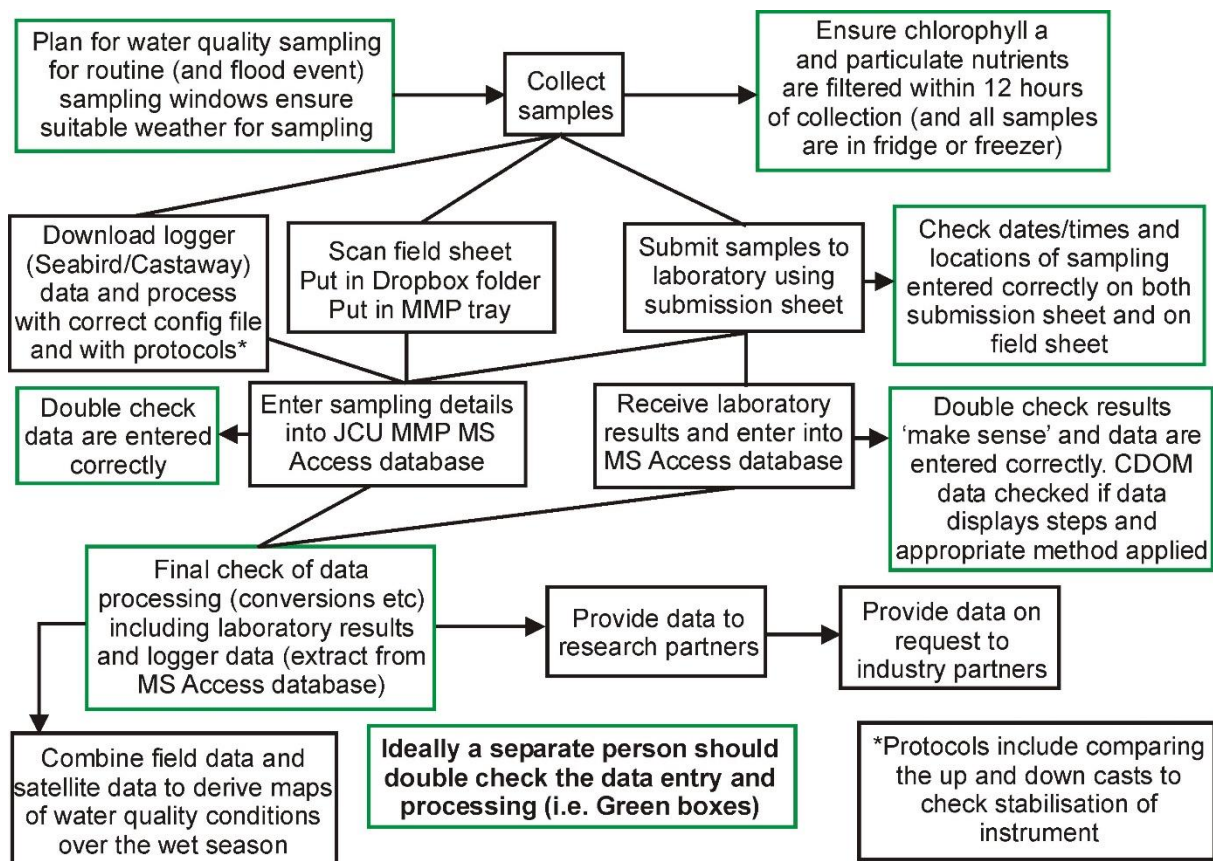
Figure 2-3 Flow chart outlining the JCU procedures and QA/QC measures once samples have been collected in the field.

2.4 Summary of Quality Control measures

- Training of field personnel, including deployment guidelines and records
- Overlap of manual and instrumental sampling
- Unique sample identifiers
- Sample custody
- Analytical QC measures including inclusion of QA/QC samples (replication of sampling and procedural blanks)
- Regular inter-laboratory calibration exercises
- Continual evaluation, method development and improvement of methods
- Periodic servicing of sensors by manufacturer
- Advanced data management and security procedures
- Document control
- Metadata updates

2.5 Inter-laboratory comparison exercises between AIMS and JCU

To test for differences between the results obtained by the AIMS and JCU water quality monitoring programs, a number of inter-laboratory comparison exercises have been conducted since 2015. Two campaigns were conducted in 2016 (Great Barrier Reef Marine Park Authority 2019) following implementation of the new sampling design for the inshore water quality monitoring program in 2015 (see Section 1.1). Sampling campaigns were also conducted in August 2018 and July 2019. These inter-laboratory comparisons are presented in detail in previous MMP QA/QC Reports.

In previous years, there were several notable differences in lab and field methods between the AIMS and JCU programs. However, the JCU team revised their methods over several years to more closely align to the AIMS field and laboratory procedures based on the inter-laboratory comparison exercises. Notable changes included:

- TSS: JCU transitioned from using Whatman GF/C filters to the polycarbonate filters (pore size 0.4 µm) used by AIMS in December 2018.
- Chl-*a*: JCU transitioned to analysis using a fluorometer instead of a spectrophotometer; analyses in 2018–19 were done on both instruments
- PN and PP: JCU began sampling following the filtration method used by AIMS (filtration onto GF/F) and all samples were sent to the AIMS laboratory for analysis. The previous JCU method of calculating PN = TN-TDN or PP = TP-TDP was discontinued in December 2018.

Inter-laboratory comparisons between AIMS and JCU were not conducted during the 2019–20 monitoring year. During the dry season period (when these comparisons are done), AIMS was enforcing strict passenger limits on all vessels as well as restrictions on external visitors boarding vessels due to the COVID-19 pandemic. Because of these limitations, as well as highly increased workloads under COVID-19 restrictions, it was not possible to conduct an inter-laboratory comparison.

3 Remote sensing and modelling of the Reef water quality and river flood plumes

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3.1 Introduction

This component of the MMP provides remotely sensed information on river flood plume areas and resuspension events during the wet seasons (December to April). It assesses frequencies and composition of Reef surface water body types and provides spatial information on the exposure and potential risk of coral reefs and seagrass meadows to river plumes, sediment resuspension and land-sourced pollutants.

There have been a number of different methods within the flood plume program to characterise, map and monitor flood events in the Reef over the last 20 years.(Devlin *et al.* 2015) These techniques and their resulting products evolved in complexity with time, from basic aerial photography in combination with *in situ* monitoring, to the application of advanced regional parameterised ocean colour algorithms (e.g. (Brando *et al.* 2010; Brando *et al.* 2012)), to the analyses of true colour products correlated with *in situ* water quality gradients (Petus *et al.* 2019; Devlin *et al.* 2015).

Current work focuses on improving the integration between satellite water colour information and field water quality data sampled in inshore waters during the wet season (including plume waters) (Petus. *et al.* 2014a; Álvarez-Romero *et al.* 2013; Collier *et al.* 2014; Petus *et al.* 2016; Petus *et al.* 2018). Wet season water type maps are produced using MODIS true colour imagery reclassified to six distinct colour classes defined by their colour properties. The wet season colour classes are regrouped into three wet season water types (primary, secondary and tertiary) characterised by different concentrations of optically active components (TSS, CDOM and Chl-*a*), which control the colour of the water and influence the light attenuation (Petus *et al.* 2018), as well as different pollutant concentrations (Petus *et al.* 2019).

The brownish to brownish-green turbid waters (colour classes 1 to 4 or primary water type) are typical for inshore regions experiencing river plumes or nearshore marine areas with high concentrations of resuspended sediments found during the wet season. These water bodies in flood waters typically contain high nutrient and phytoplankton concentrations but are also enriched in sediment and dissolved organic matter resulting in reduced light levels. The greenish-to-greenish-blue turbid waters (colour class 5 or secondary water type) is typical of coastal waters rich in algae (Chl-a) and contain dissolved matter and fine sediment. This water body is found in open coastal waters as well as in the mid-water plumes where relatively high nutrient availability and increased light levels due to sedimentation (Bainbridge *et al.* 2012) favour coastal productivity. Finally, the greenish-blue

waters (colour class 6 or tertiary water type) correspond to waters with above ambient water quality concentrations. This water body is typical for areas towards the open sea or offshore regions of river flood plumes.

Several monitoring products are derived from the water type maps and water quality data. They are summarised in Figure 3-1 and aim to:

- Map Reef water types and water quality gradients during the wet season and assess the extent of river flood plumes during high flow conditions;
- Characterise the composition of the Reef wet season water types (mean long-term TSS, Chl-*a,* CDOM, DIN, DIP, PP and PN concentrations and SDD values);
- Identify where mean long-term concentrations of TSS, Chl-*a,* PP, and PN may be above wet season GVs. Wet season GVs for the whole of the Reef (hereafter Reef-wide GVs) are derived from (De'ath and Fabricius 2008);
- Assess the exposure of coral reefs and seagrass ecosystems to potential risk to river plumes, sediment resuspension and land-sourced pollutants.

These products are used to illustrate wet season conditions for every wet season and to compare seasonal trends with longer-term or reference trends in water composition.

Maps representing the distribution of surface loads also provide further understanding of the movements of pollutants from river discharge. The maps combine eReefs tracer maps and end-of-catchment pollutant loads and are useful for comparing the intensity of exposure to pollutant loads from year to year and for comparison with different scenarios (for example, pre-development loads).

Wet Season Water Types

Colour Class Frequency

Pollutant Surface Loads

Spatial Risk (Exposure)

Wet season water type maps provide a broad scale approach to monitoring plume waters in the GBR marine environment. These weekly maps are are classified into six water types defined by their colour properties, and associated with characteristic combinations of optically active components (TSS, CDOM, and chlorophyll-a) and differing pollutant concentrations. Example maps show turbid waters following heavy rainfall in the Fitzroy River catchment (2016) and Burdekin to Wet Tropics (2011).

Colour class frequency maps illustrate the extent and movement of the different water types over the wet season. The colour class frequency is calculated as the number of weeks within a wet season (Dec – April) that a pixel was exposed to a given colour class divided by the number of observations at that pixel. The maps above report the frequency of primary plus secondary wet season water types (colour classes 1-5) in 2016, and the mean frequency of colour classes 1-5 in the GBR from 2006 to 2013.

Surface load maps allow further understanding of the movements of pollutants within plume waters. They are useful for comparing the intensity of exposure to pollutant loads from year to year and for comparison with alternate scenarios (such as pre- development loads). These maps combine eReefs tracer maps and end-of-catchment pollutant loads. The maps above report the surface DIN mass distribution in 2019 compared to the average for 2003-2019.

Spatial risk (exposure) maps are a preliminary product aiming to evaluate the ecological risk to GBR ecosystems from exposure to wet season water types. These maps display qualitative categories of risk associated with different combinations of water type frequency and mean colour class value. The water type frequency is used to account for the intensity that water types inundate a particular area, and mean colour class accounts for the severity of the inundation as related to pollutant concentrations.

Figure 3-1: Summary description of the wet season water quality products.

3.2   Methods

TropWATER was funded by the Authority to collect information on the movement of flood plumes across inshore waters of the Reef and to quantify the exposure of Reef ecosystems to flood plumes and resuspension events, as well as to land-sourced pollutants carried by the flood plumes, using satellite imagery. To detect, map and characterise river flood plumes, ocean colour remote sensors can use differences in water colour properties between plumes and ambient marine waters. The optical signature and colour signature of a surface water body is related to the optical active constituents of the water, including the presence and combination of Chl-*a*, CDOM and TSS. Coastal water bodies, including resuspended sediments and flood river plumes of the Reef during the wet season are mapped using daily MODIS true colour images and a semi-automated classification technique clustering bodies with homogeneous surface water colour (Álvarez-Romero *et al.* 2013). The term "wet season waters" refers to flood river plumes and sediment resuspension occurring in the GBR during the austral wet season.

3.2.1   MODIS satellite data

The remote sensing component of the MMP uses remotely sensed ocean-colour information from NASA's MODIS instrument (https://modis.gsfc.nasa.gov/). The MODIS instrument is carried by two different satellites, Terra (providing the morning overpass at approximately 10:30 am) and Aqua (providing the afternoon overpass at approximately 1:30 pm). Working in tandem to see the same area of the Earth in the morning and the afternoon, the two satellites help make sure that MODIS and other instruments are measuring accurately by optimising cloud-free remote sensing of the surface and minimising any optical effects — like shadows or glare — that are unique to morning or afternoon sunlight. The remote sensing component of the MMP have used remotely sensed information from NASA's MODIS Aqua satellite from 2002 onwards.

Until 2016, MODIS remote sensing L0 (raw) data were ordered from the NASA Ocean Colour website (http://oceancolor.gsfc.nasa.gov/) and processed in-house by TropWATER. A routine written in R was used to download the data and a set of IDL/SeaDAS (SeaWiFS Data Analysis System) routines was implemented to process MODIS Aqua data from Level-0 to true colour images (Baith *et al.* 2001).

In 2016–17, the processing of true colour images was handed over to the Bureau of Meteorology. MODIS-Aqua L0 data is routinely downloaded from the NASA Ocean Biology Processing Group (OBPG) webpage (https://oceancolor.gsfc.nasa.gov/). Downloaded data is routinely migrated to external drives. Observations within the area of interest that are not found on either the rolling or tape archives are downloaded from the OBPG webpage. The MODIS-Aqua L0 is processed using the SeaWiFS Data Analysis System (SeaDAS, version 7.4, https://seadas.gsfc.nasa.gov/) following several steps:

- L0 data (raw radiance counts — digital numbers) are processed using the SeaDAS modis_L0_to_L1A.py tool to get L1A data at native resolution (250, 500 and 1000 m); where multiple granules are required to cover the region of interest, the SeaDAS pdsmerge tool is used to merge the adjacent granules before being processed to make sure that there are no gaps between granules.
- The L1A file is then used to calculate the geometry (GEO file) using the SeaDAS modis_GEO.py tool.
- The GEO and L1A files are used to compute the calibrated top of atmosphere radiances (L1B data) using the SeaDAS modis_L1B.py tool at native resolution (250, 500 and 1000 m).
- The SeaDAS l1mapgen tool is then used to compute the Rayleigh corrected reflectance for MODIS bands 1 (645 nm at 250 m resolution), 4 (555 nm at 1000 m resolution) and 3 (469 nm at 1000 m resolution). Using Rayleigh corrected reflectance (without an aerosol removal step) assumes that fully accurate atmospheric corrections are less crucial for turbid (case 2) Reef flood waters than it would be for clear (case 1) waters.

The output of the SEADAS l1mapgen tool is a true colour image in GeoTIFF format. Where multiple granules are required to cover the region of interest, the true colour GeoTIFF images are combined using the imagemagick convert tool. During this process, the GeoTIFF

location tags are removed. They are added back to the GeoTIFF image via the use of the GDAL gdalcopyproj.py script. The argument and keywords used are shown in Table 3-1.

True colour images have a spatial resolution of 500 m × 500 m and combine three ocean bands (i.e. red, green and blue, RGB) in the visible spectrum. They have a daily temporal resolution and cover the entire Reef area (extreme coordinates: -10.5, -27.0, 142.3 and 154.0). The true colour images cover the wet season period (i.e. December to April, inclusive) since December 2003 (MODIS mission started in March, 2002).

Table 3-1 Keywords and justification used in SeaDAS 7.4 to process MODIS true colour images of the Great Barrier Reef.

Step	Term	Description
Step 2: modis_GEO.py	terrain correction	Given the area of interest is the ocean, terrain correction is not used (in modis_GEO.py the --enable-dem flag is not used)
Step 3: modis_L1B.py	look up table (LUT)	The arguments "-l 6.1.33.4_OC -d /short/er8/packages/seadas/7.0/ocssw/run/var/modisa/cal/EVAL/" are passed to use the 6.1.33.4_OC L1B look up table.
Step 4: l1mapgen	Calibration table	The msl12_defaults.par was updated to use the xcal_modisa_axc39d cross-calibration files.
	Atmospheric correction	The atmocor keyword was set to "on" to make sure that the Rayleigh correction was applied.
	resolution	The resolution keyword was set to "250" to use band 1 as input.

Processed MODIS data are stored in external media and also at the national Research Data Services, which is part of Research Data Australia (https://researchdata.ands.org.au/).

True-colour images for 2011 (very wet), 2016 and 2017 (dry) years, and from 2018 onward (2018 and 2019) were processed by BOM, while all other years were processed in-house by TropWATER. MODIS-Terra true colour images are also occasionally downloaded from the National Aeronautics and Space Administration (NASA)'s EOSDIS worldview website and processed to daily water type maps. MODIS-Terra are only used when MODIS data are too cloudy or unavailable, and when satellite information are required in near-real-time (rapid response mapping of flood events).

3.2.2   Supervised classification using spectral signatures

A set of spatial analyses (Python, ArcGIS) based on supervised classification of spectrally enhanced true colour images is used to classify 'turbid' waters, including river flood plumes and resuspension events, and 'non-turbid' areas in the Reef, focusing on the wet season period. The true-colour images are spectrally enhanced (from red-green-blue to hue-saturation-intensity colour system) and classified to six colour categories through a supervised classification using spectral signatures from typical wet season water masses types (including river plumes) in the Reef lagoon. The six colour classes are further reclassified into three wet season water types (primary, secondary and tertiary) corresponding to the three wet season water types, as described above and defined

originally by (Devlin and Schaffelke 2009) and Devlin *et al.* 2012. This supervised classification is used to classify daily MODIS images.

3.2.3   Production of weekly wet season water type maps

Weekly wet season water type composites are then created to minimise the image area contaminated by dense cloud cover and intense sun glint (Álvarez-Romero *et al.* 2013). The minimum colour-class value of each cell/week is used to map the colour class with the maximum turbidity per pixel for each week of the wet season (i.e., assuming the colour classes represented a gradient in turbidity i.e., CC1 > CC2 > CC3 > CC4 > CC5 > CC6).

All weekly composites are automatically cleaned. The aim of cleaning is to minimise the image area contaminated by dense cloud cover and intense sun glint, and to remove shallow water interference around reefs. In all cases the effect of these phenomena can be that offshore waters are misclassified as, for example, primary waters (CC 1-4). To minimise these effects an automated process is applied to the rasters that has the effect of sequentially infilling contiguous water-type areas one colour class at a time from CC7 (ambient ocean water) inwards towards the coast. This processing is performed using Python 2.7.3 (Python Software Foundation 2012) and ArcGIS 10.7 (ESRI, 2019). Infilling is achieved using the following steps: 1) Raster to Polygon conversion (not simplified), 2) Union (no gaps) then 3) removal, using Erase, of an external polygon, and 4) Polygon to Raster conversion. This process generates a separate raster mask (values 1 or 0) for each colour class, and the final cleaned raster is created by adding the component raster masks. Whilst this process is effective at removing noise offshore it can occasionally have the effect of removing areas of turbid coastal and plume water if they are not directly connected to the coast. To counter this, a final step is included in the cleaning process whereby waters classified as CC1 and CC2 (i.e. values < 3*) in the cleaned raster are replaced with pixels of CC1 and CC2 in the original raster, using Con (Spatial Analyst). Thus, pixels adjacent to the coast that are classified as highly turbid water are kept and pixels within otherwise contiguous water types offshore are removed. *The script is occasionally re-run using a different value than 3 in cases where moderately turbid inshore waters have evidently being removed during the cleaning process but that was not required this year. Extra cleaning steps can sometimes be applied to weeks with large residual noise (manual cleaning or exclusion of very noisy day images from the database) if needed.

3.2.4   Production of annual, multi-annual and typical Wet and Dry wet season water type maps

Weekly wet season water type composites are thus overlaid in ArcGIS (i.e., presence/absence of one wet season water type) and normalised, to compute each year a seasonal normalised frequency maps of occurrence of wet season water type. Pixel (or cell) values of these maps range from 1 to 22; with a value of 22 meaning that one pixel has been exposed 22 weeks out of 22 weeks of the wet season. Annual frequency maps are normalised (0–1) and overlaid in ArcGIS to create multi-annual normalised frequency composites of occurrence of wet season water types.

Multi-annual composites are calculated over different time frames, including (i) all previous wet seasons (2003–18) and (ii) a typical recovery period for Reef corals (2012–2017). Composite frequency maps are also produced to represent typical wet year and dry year conditions. To account for broad-scale spatial variability in wet season river flows, wet- and dry-year maps are first produced separately by averaging frequency maps from the wettest and driest years in each NRM region. Wet years are defined as those in the top quartile for total catchment discharge in the NRM region; dry years as those in the bottom quartile (Table 3-2).

Table 3-2: Wettest and driest years used to compute the Typical Wet and Typical Dry Composite frequency maps in each NRM region.

Region	Wet years				Dry years				
Cape York	2004	2006	2011	2019	2003	2005	2007	2012	2016
Wet Tropics	2009	2011	2018	2019	2003	2005	2015	2016	2017
Burdekin	2008	2009	2011	2019	2003	2004	2014	2015	2016
Mackay-Whitsunday	2008	2010	2011	2012	2003	2004	2006	2015	2018
Fitzroy	2008	2010	2011	2013	2004	2005	2006	2007	2019
Burnett-Mary	2010	2011	2012	2013	2005	2006	2007	2014	2019

The wet-year maps for each NRM region are combined into a single, composite, Reef-wide map using the maximum value of the input rasters. This method captures wet-year plume conditions across the entire Reef even if the most significant plume events originate outside the NRM (e.g. if Fitzroy plumes are dominant in the Mackay-Whitsunday region the top-quartile discharges from the Fitzroy are already included in the composite raster). Conversely, the dry-year maps are combined into a Reef-wide composite map using the minimum value of the input rasters, which thus represents the least extensive plume from an average of the driest years in each NRM region.

Except for the coral recovery period, reference maps (long-term, Wet and Dry frequency maps) are updated every 4 years (and/or in the case of extremely wet year or specific event patterns) to ensure they remains valid as a representative period and to improve their accuracy as more satellite data are available. Last update was in 2019. The daily, weekly and wet season frequency maps are used to illustrate the wet season conditions for every year and to assess the extent of river flood plumes and resuspension events Reef-wide and in the focus regions. They are used to compare seasonal with long-term trends, as well as trend in water composition during typical dry and wet years.

3.2.5   Surface loading maps

Surface loading maps that model the transport of land-sourced pollutants (DIN, TSS and PN) are now created using the eReefs marine model tracers (Margvelashvili *et al.* 2018; Skerratt *et al.* 2019; Steven *et al.* 2019) for each river plume and dispersion of end of catchment loads. The first step involves generation of tracer maps, which follows the same method described below for the cumulative exposure index. By taking the cumulative sum of each river tracer concentration over the course of a water year (1 October to 30 September), the

cumulative exposure of each map location to water from each river in that year is estimated. A preliminary GIS analysis is then used to generate the loading maps. In this step, the end-of-catchment load for TSS, DIN or PN is dispersed for each river assuming a direct relationship between pollutant and tracer concentration (conservative mixing). Thus, surface load of TSS, DIN or PN per km^2 is calculated as:

$$Surf.load = \frac{\text{tracer}}{pixel} \times \frac{[\text{total load}]}{[\text{sum of tracer}]} \times \frac{pixel}{km^2}$$

The total Reef surface load is calculated by summing the surface load outputs for the 17 rivers for which tracer data is available: Normanby, Daintree, Barron, Russell-Mulgrave, Johnstone, Tully, Herbert, Haughton, Burdekin, Don, O'Connell, Pioneer, Fitzroy, Calliope, Boyne, Burnett and Mary.

The difference between the estimated wet season TSS, DIN, and PN loadings (tonnes km^2) in the Reef lagoon for the 2020 water year (1 October 2019 to 30 September 2020) is calculated and compared to the pre-development loads (which have a degree of uncertainty). This can be interpreted as 'anthropogenic' TSS, DIN or PN loadings, highlighting the areas of greatest change with current land use characteristics.

3.2.6 Mean long-term water quality concentrations across water types and colour classes

Additional information on wet season conditions are reported by characterising the mean long-term water quality concentrations across colour class and water types. Match-ups between sample data and corresponding weekly wet season water type maps are performed at the site location using the PointGeometry and Extract Values to Points tools in ArcGIS 10.7 (ESRI, 2019), automated using PythonWin 2.7.3.Several land-sourced pollutants are investigated through match-ups between *in-situ* data and the six colour class maps, including DIN, PO_4, PP, PN, TSS, Chl-*a*, CDOM and Secchi depth (previously K_D).

Boxplots of water quality parameters across water types and colour classes and the mean long-term water quality concentrations across the three wet season water types in all focus regions are presented (Figure 3-3). Detailed summaries of water quality parameters (mean, standard deviation, minimum, maximum and number of values for each pollutant across colour classes and water types) for the long-term and reporting year are also available in each MMP report. Long-term water quality values are calculated using all surface data (<0.2 m) collected between December and April by JCU (since 2004), AIMS and the CYWMP (since 2016–17). Long-term water quality values are reviewed and updated every 4 years (and/or in the case of extremely wet year or specific event patterns) to ensure the water type characterisation remains valid as a representative period and to improve its accuracy as more field data are collected every wet seasons. Last update was in 2019, using field data collected from 2004 to 2019. Before 2016–17, the mean water quality concentrations were calculated using the JCU dataset only, assuming it was representative of high flow conditions. Long-term mean DIN, PP and PN concentrations are calculated as DIN = NO2+NO3+NH3, PP= TP-TDP and PN = TN-DIN, respectively.
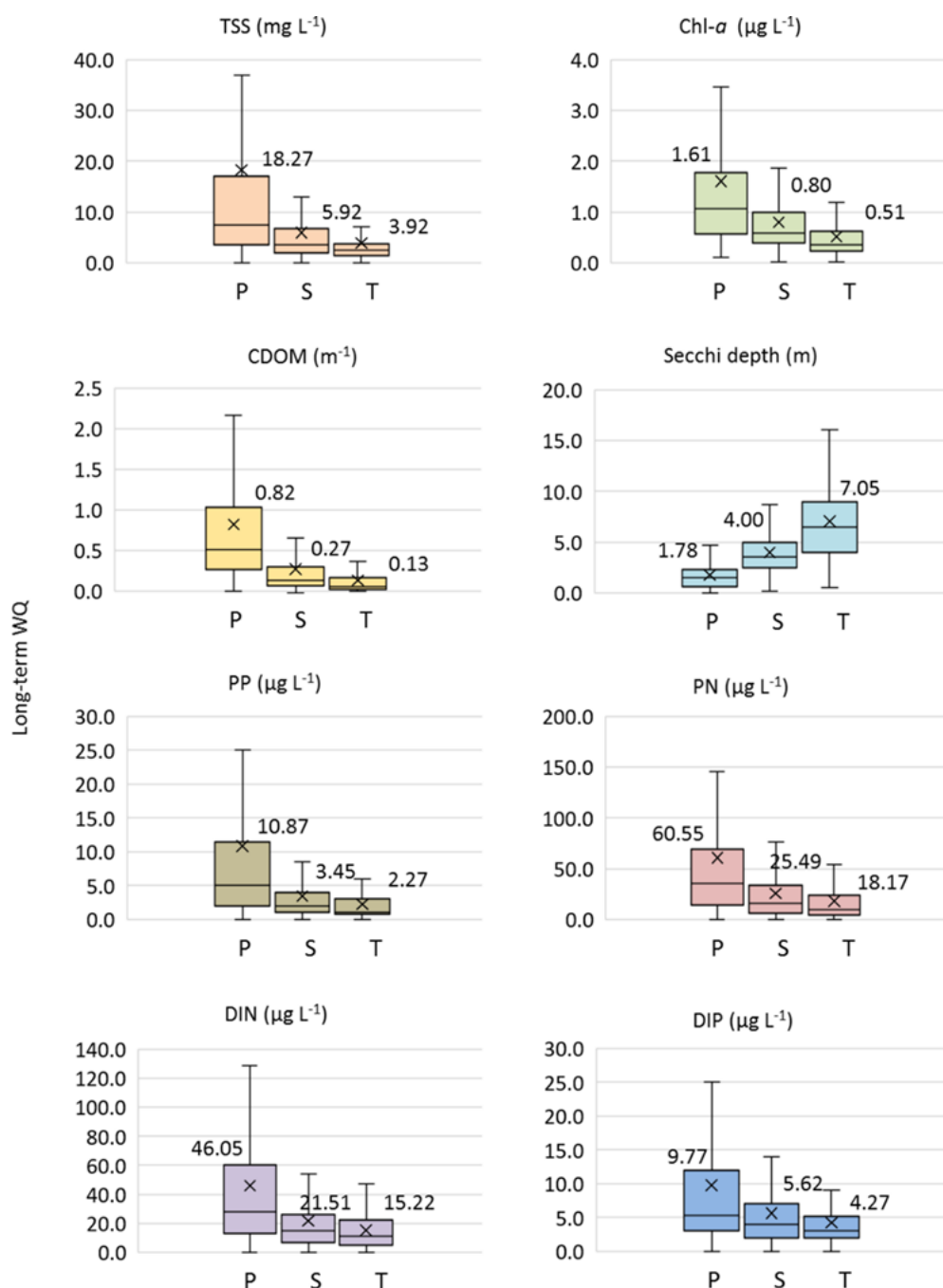
Figure 3-2: Long term (2004 to 2019) water quality (WQ) concentration and Secchi disk depth boxplots for each wet season water type (P = primary, S = secondary, T = tertiary). The mean is plotted as a cross and its numerical value is indicated. The interquartile range is delimited by the box and the median by the line inside the box. Whiskers indicate variability outside the upper and lower quartiles. Data beyond the whiskers range are considered outliers and are not plotted. Long-term WQ values are reviewed and updated every 4 years (and/or in the case of extremely wet year or specific event patterns) to ensure the water type characterisation remains valid as a representative period, and to improve its accuracy as more field data are collected every wet seasons. Last update was in 2019, using all field data available.

3.2.7   Exposure maps and exposure assessment

Information on the long-term pollutant concentrations measured in the wet season colour classes (Figure 3-2) are compared to published water quality guideline values (Table 3.3) and, are used in a *"magnitude x likelihood"* risk management framework to develop surface exposure maps (also referred to as potential risk maps in some Reef studies).
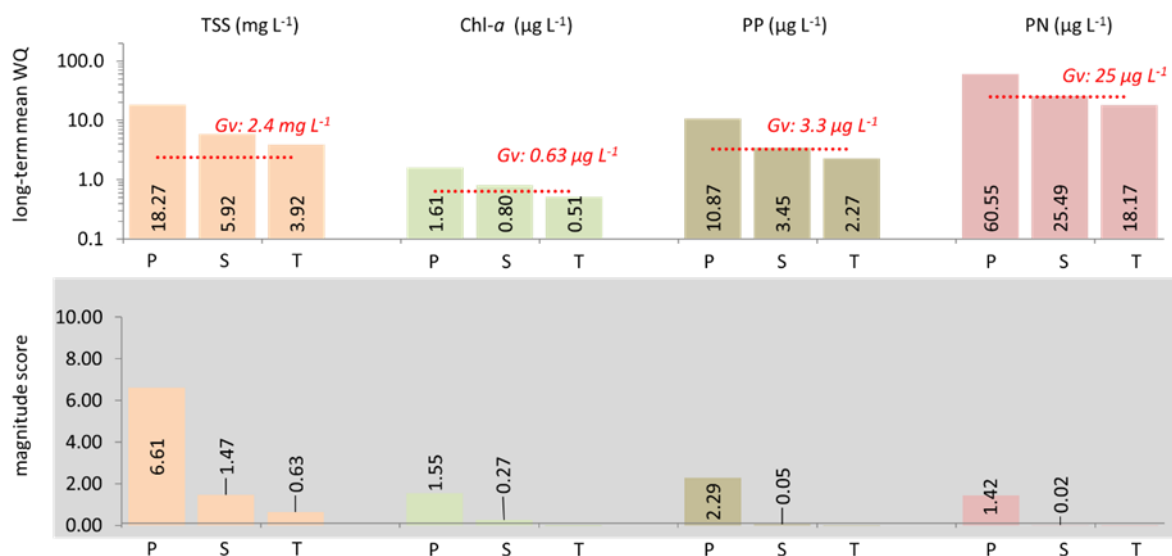
Figure 3-3: (top) Mean long-term water quality concentrations and (bottom) magnitude score across the three wet season water types. Red lines show the Reef-wide wet season GVs (Appendix D). Magnitude scores are calculated as the proportional exceedance of the guideline:
$$magnitude_{water\ type} = ([Poll.]_{water\ type} - GV)/GV$$ **and Poll. = TSS, Chl-a, PP or PN. Negative Magnitude score are scored as zero. Mean long-term water quality concentrations and Magnitude score are re-calculated every 4 years as additional field data is collected (and/or in the case of extremely wet year or specific event patterns). Mean long-term water quality concentrations include samples collected from the enclosed coastal water type where high concentrations are likely to contribute to exceedances of the Reef-wide GVs, particularly for Primary waters.**

Different frameworks have been used to estimate the exposure and potential risk from exposure, (Petus *et al.* 2014b; Petus, C. *et al.* 2016), and used in the MMP reports before 2015–16. In a collaborative effort between the MMP monitoring providers (JCU water quality and seagrass teams and the AIMS coral monitoring team), an updated exposure assessment framework was developed in 2015–16 (modified from (Petus *et al.* 2016), where the 'potential risk' corresponds to an exposure to above guideline concentrations of land-

sourced pollutant during wet season conditions and focuses on the TSS, Chl-*a*, PP and PN concentrations.

In this *magnitude × likelihood* framework, the 'potential risk' corresponds to an exposure to above wet season GV concentrations of land-sourced pollutants during the wet season and focuses on TSS, Chl-*a*, PP and PN concentrations (Gruber *et al.* 2020, Waterhouse *et al.* 2021). The *'magnitude of the exposure'* corresponds to the mean long-term (2004–2019) wet season concentration of pollutants relative to water quality GV (proportional exceedance) mapped through the primary, secondary and tertiary water types (Figure 3-3).

Table 3.3: Reef-wide wet season guideline values used to calculate the exposure score for satellite exposure maps.These guidelines are based on seasonal adjustments to reef-wide annual guidelines (Great Barrier Reef Marine Park Authority, 2010), where wet season guidelines are +20% for TSS, PN, and PP, and +40% for Chl-*a* of annual guidelines (De'ath and Fabricius 2008).

Parameter	Unit	Reef-wide
Chlorophyll *a*	µg L^{-1}	0.63
Particulate nitrogen	µg L^{-1}	25
Particulate phosphorus	µg L^{-1}	3.3
Suspended solids	mg L^{-1}	2.4

These GVs are compared against the mean long-term concentrations to calculate the exposure score in the satellite exposure maps (proportional exceedance of the guideline). Mean long-term water quality concentrations are calculated using all available surface water quality data in all Reef marine regions and water bodies (Table 3-4).

The *'likelihood of the exposure'* is estimated by calculating the frequency of occurrence for each Reef cell of each wet season water type. The exposure for each of the water quality parameters defined is the proportional exceedance of the GV multiplied by the likelihood of exposure in each of the wet season water types.

For each cell (500 m x 500 m) of the Reef and water type:

$$magnitude_{water\ type} = ([Poll.]_{water\ type} - GV)/GV$$

$$likelihood_{water\ type} = frequency_{water\ type}$$

$$Poll\_expo_{water\ type} = magnitude_{water\ type} \times likelihood_{water\ type}$$

where $water\ type$ is the primary, secondary or tertiary wet season water types, $[Poll.]_{water\ type}$ is the mean long-term TSS, Chl-*a*, PN, or PP concentration measured in each respective wet season water types and GV is the Reef-wide wet season guideline value from De'ath and Fabricius (2008) for TSS, Chl-*a*, PP, and PN (Table 3.3).

For each pollutant, the total exposure ($Poll\_expo$) is calculated at the exposure for each of the wet season water types:

$$Poll\_expo = Poll\_expo_{Primary} + Poll\_expo_{Secondary} + Poll\_expo_{Tertiary}$$

The overall exposure score ($Score\_expo$) is calculated as the sum of the total exposure for each of the water quality parameters:

$$Score\_expo = TSS.\exp + Chla.\exp + PP.\exp + PN.exp$$

Table 3-4: Number of collected *in situ* samples used in exposure scoring by region and water type. Samples include all wet season (Dec–April) surface samples since 2004 (from JCU) and since the 2016–17 water year (AIMS and the CYWMP).

Region	Water type	Number of samples								
		Salinity	Secchi depth	TSS	Chl-a	CDOM	DIN	PO4	PP	PN
Cape York	Primary	125	109	125	136	101	138	137	91	135
	Secondary	124	120	124	132	51	131	132	98	131
	Tertiary	61	47	61	61	25	63	63	52	63
	Marine	9	4	9	9	3	9	9	8	9
Wet Tropics	Primary	224	164	375	368	357	321	324	301	309
	Secondary	244	289	482	495	438	475	476	446	447
	Tertiary	109	121	172	172	141	169	169	166	167
	Marine	17	22	29	29	27	28	28	20	20
Burdekin	Primary	100	81	132	131	86	129	131	126	127
	Secondary	104	146	188	187	132	187	187	177	176
	Tertiary	28	35	47	45	37	47	47	43	45
	Marine	14	16	18	21	15	22	22	19	19
Mackay - Whitsunday	Primary	12	9	26	23	25	26	26	24	24
	Secondary	44	34	86	81	53	86	86	77	78
	Tertiary	10	9	18	18	9	17	17	17	17
Fitzroy	Primary	15		76	77	56	77	78	75	76
	Secondary	13		34	48	43	52	54	53	53
	Tertiary	2		2	7	4	7	7	6	7
	Marine			6	6	1	6	6	6	6
Burnett-Mary	Primary	7	7	20	20	11	11	20	20	11
	Secondary	2	5	12	12	5	8	12	11	8
	Tertiary	1		1	1		1	1	1	1
	Marine			3	3	1	3	3	3	3
Reef-wide	Primary	483	370	754	755	636	702	716	637	682
	Secondary	531	594	926	955	722	939	947	862	893
	Tertiary	211	212	301	304	216	304	304	285	300
	Marine	40	42	65	68	47	68	68	56	57

The overall exposure scores are then grouped into four potential classes (I to IV) based on a "Natural Break (or Jenks)" classification. Jenks is a statistical procedure, embedded in ArcGIS that analyses the distribution of values in the data and finds the most evident breaks in it (i.e., the steep or marked breaks; (Jenks and Caspall 1971). The Jenks classification determine the best arrangement of values into different classes by reducing the variance within classes and maximizing the variance between classes.

The exposure classes are defined by applying the Jenks classification to the mean long-term exposure map, because this map presented the highest number of observations. Using the 2003–2018 mean exposure map, categories were defined as [>0–0.9] = cat. I, [0.9–3.2] = cat. II, [3.2–7.5] = cat III and >7.5 = cat IV). Category I and areas mapped as "exposure = 0 (no exposure)", are re-grouped into a unique category I (no or very low exposure). These categories are applied to all exposure composites created (seasonal, coral recovery period, typical wet and dry periods – Reef-wide and for all focus regions).

The methods presented above are slightly different than methods used in the two previous wet season's reports (2016–17 and 2017–18 wet seasons) where (i) seasonal mean water quality concentrations across water types were used to produce the seasonal exposure map, (ii) exposure maps were reclassified using four equally-distributed colour classes and (iii) category I and areas mapped as "exposure = 0 (no exposure)" were not re-grouped into a unique category. Changes in 2019 (using only long-term mean WQ concentrations, a Jenk's classification of the exposure maps, and regrouping cat I and 0) were made in response to: (i) concerns that water quality concentrations collected in a specific wet season would likely get biased toward the sample size and the location and timing of sampling in this particular wet season conditions and (ii) concerns that the equally-distributed categories were not responsive enough to changes in environmental pressures of each year, and (iii) to recognise that cat I is likely to have a low risk of any detrimental ecological effect.

Exposure maps are produced for the whole of the Reef, for all focus regions and over different time frames: for the current reporting wet season, over the long-term (2002–03 to 2018–19: 16 wet seasons), over a documented recovery period for coral reefs (2012–2017 period) and representation of typical wet-year and dry-year conditions. Except for the coral recovery period, reference maps (long-term, Wet and Dry frequency maps) are updated every 4 years (and/or in the case of extremely wet year or specific event patterns) to ensure they remain valid as a representative period and to improve their accuracy as more satellite data are available. Last update was in 2019. The 'potential risk' is influenced by the available MODIS data on cloud-free days, with the likelihood of risk assessment exposure likely underestimated in higher rainfall and cloudy regions like the Wet Tropics and Cape York.

Finally, assessments of ecosystem exposure are made through the calculation of the areas (km^2) and percentages (%) of each region, coral reefs and seagrass meadows affected by different categories of exposure. The area and percentage are calculated as a relative measure between regions and waterbodies. The difference in percentages between 2019

and in the long-term is also calculated. Processed data are stored in external Medias and in Dropbox.

Figure 3-4 presents the marine boundaries used for the Marine Park, each NRM region, the Reef waterbodies and the seagrass and coral reefs ecosystems. We assumed in this study that the shapefile can be used as a representation of the actual seagrass distribution. It is known, however, that absence on the composite map does not definitively equate to absence of seagrass and may also indicate un-surveyed areas.
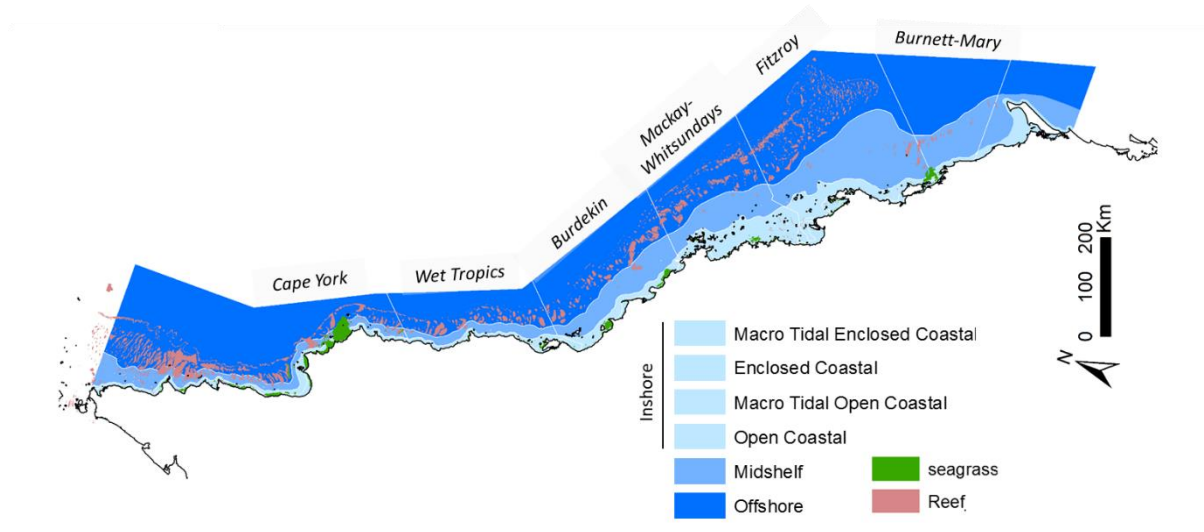
Figure 3-4: Boundaries used for the Marine Park, each NRM region and the coral reefs and seagrass ecosystems. Coral reef and NRM layers derived from the Authority, supplied 2013. Seagrass layer is a composite of surveys conducted by Department of Agriculture and Fisheries, Qld.

4 Pesticide monitoring

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4.1 Introduction

The inshore waters of the Reef are impacted by the water quality of discharges from a vast catchment area, which can include inputs of pesticides (i.e. insecticides, herbicides and fungicides). The need for a long-term monitoring program on the Reef, which provides time-integrated data to assess temporal changes in environmentally relevant pollutant concentrations, was identified as a priority to address the information deficiencies about risks to the ecological integrity of this World Heritage Area in 2000.(Haynes and Michalek-Wagner 2000) The aim of this component of the MMP is to assess spatial and temporal trends in the concentrations of specific organic chemicals using time-integrated passive sampling techniques primarily through routine monitoring at specific sites.

Passive samplers accumulate organic chemicals such as pesticides from water in an initially time-integrated manner until equilibrium is established between the concentration in the water (C_W ng.L^{-1}) and the concentration in the sampler (C_S ng.g^{-1}). The concentration of the chemical in the water can be estimated from the amount of organic chemical accumulated within a given deployment period using calibration data obtained under controlled laboratory conditions.(Booij *et al.* 2007)

Passive sampling techniques offer time-integrated monitoring of both temporal and spatial variation in exposure in the often remote locations encountered on the Reef (Shaw and Mueller 2005) These techniques are particularly suited to large scale studies with frequently recurring pollution events(Schäfer *et al.* 2008) to ensure these events are captured and to allow the assessment of temporal trends in concentrations in systems over the long term.(Muller *et al.* 2011; Kennedy *et al.* 2010)

Different types of organic chemicals must be targeted using different passive sampling phases. The passive sampling system utilized in the MMP include:

- **SDB-RPS Empore^{TM} Disk (ED):** passive samplers for relatively hydrophilic organic chemicals with relatively low octanol-water partition coefficients (K_{ow}) such as the photosystem II (PSII) herbicides (for example atrazine — a triazine herbicide); also referred to as polar organic chemical samplers.
- **Polydimethylsiloxane (PDMS):** passive samplers for organic chemicals that are relatively more hydrophobic (higher K_{ow}) (for example chlorpyrifos — an organophosphate insecticide); also referred to as non-polar organic chemical samplers.

4.2   Methods

4.2.1   Sampling design — Passive sampling for routine monitoring

Before the 2014–15 monitoring year, 12 sites were routinely monitored across the four NRM regions (Wet Tropics, Burdekin, Mackay Whitsunday and Fitzroy) (see previous QA/QC and annual reports). Following a review of the program in 2013 and 2014, there was a consensus to discontinue monitoring at several locations due to poor statistical power in detecting trends in pesticide concentrations, and initiate sampling in new locations that would better link end-of-catchment loads with inshore concentrations of pesticides. In 2019-20, a total of 5 sites in the Wet Tropics (High Island and Dunk Island) and Mackay-Whitsundays (Repulse Bay, Flat Top Island and Sandy Creek) were selected for the monitoring. Samplers were deployed monthly during the wet season.

In addition to the scientific requirements of the project, the selection of passive sampling deployment sites is governed by practicalities, which include safety, security, site access and the availability of a responsible community representative to take responsibility for the maintenance of the site. Site establishment is ultimately determined by the Authority.

4.2.2   Target pesticides in the different passive samplers

The chemicals targeted for analysis in the different passive samplers and the instrument limits of reporting (LOR) are indicated in Table 4-1. This list of target chemicals was derived through consultation with the Authority with the criteria being:

- detected in recent studies
- recognised as a potential risk (through known usage patterns, amounts and existing toxicity data)
- analytical affordability
- within the current analytical capabilities of QAEHS or Queensland Health Forensic and Scientific Services (QHFSS)
- likelihood of accumulation in one of the passive samplers (exist as neutral species in the environment)
- analysed frequently by other complimentary Reef Plan programs such as end-of-catchment loads monitoring.

Table 4-1 Pesticides for analysis in different sampler extracts and water samples together with the approximate instrument limits of detection and limits of reporting for analytes.

| Chemical | Description | Priority or of interest | LC-MS/MS | | GCMS |
			LOD µg L^{-1}	LOR µg L^{-1}	LOR µg L^{-1}
2, 4-D	Phenoxy-carboxylic-acid herbicide	Priority	0.03	0.10	
2,4-DB	Phenoxy-carboxylic-acid herbicide	Of interest	5.0	15	
Aciflurofen*	Herbicide: cell membrane disruptor	Of interest			
Ametryn	PSII herbicide – methylthiotriazine	Priority	0.56	1.69	
Asulam^	Herbicide: inhibition of DHP – carbamate	Of interest	0.01	0.10	
Atrazine	PSII herbicide – chlorotriazine	Priority	0.05	0.15	
Atrazine – desethyl	PSII herbicide breakdown product (also active)	Priority	0.005	0.10	
Atrazine – desisopropyl	PSII herbicide breakdown product (also active)	Priority	0.02	0.10	
Bromacil	PSII herbicide – uracil	Of interest	0.02	0.10	
Chlorothalonil*	Organochlorine fungicide	Priority			
Chlorpyrifos	Organophosphate insecticide	Priority			0.5
Diazinon*	Insecticide: inhibits acetylcholinesterase	Of interest			
Diuron	PSII herbicide – pheynylurea	Priority	0.02	0.10	
Ethametsulphuron methyl*	Herbicide: acetolactate synthase (ALS) inhibition	Of interest			
Fipronil*	Phenylpyrazole insecticide	Priority			
Fluazifop^	Herbicide: inhibition of acetyl CoA carboxylase	Of interest	0.02	0.10	

Chemical	Description	Priority or of interest	LC-MS/MS		GCMS
			LOD µg L^{-1}	LOR µg L^{-1}	LOR µg L^{-1}
Fluometuron	PSII herbicide – urea	Of interest	0.01	0.10	
Fluroxypyr	Pyridine carboxylic acid herbicide	Priority	0.02	0.10	
Glyphosate*	Broad-spectrum systemic herbicide	Priority	Glyphosate-specific sampler required		
Haloxyfop	Aryloxyphenoxy-propionate herbicide	Priority	0.04	0.13	
Hexazinone	PSII herbicide – triazinone	Priority	0.01	0.10	
Imazapic	Imidazolinone herbicide	Priority	0.02	0.10	
Imidacloprid	Neonicotinoid insecticide	Priority	0.01	0.10	
Isoxaflutole and DKN*	Isoxazole herbicide	Priority	0.20	0.61	
MCPA	Phenoxy-carboxylic-acid herbicide	Priority	0.05	0.14	
Mesosulphuron methyl*	Herbicide: acetolactate synthase (ALS) inhibition	Of interest			
Metolachlor	Chloracetanilide herbicide	Priority	0.03	0.10	
Metribuzin	PSII herbicide – triazinone	Priority	0.03	0.10	
Metsulphuron methyl	Sulfonylurea herbicide	Priority	0.01	0.10	
MSMA*	Herbicide: inhibition of cell division	Of interest			
Paraquat*	Herbicide: photosystem-I-electron diversion	Of interest			
Pendimethalin	Dinitroaniline herbicide	Priority			1.0
Prometryn	PSII herbicide – methylthiotriazine	Priority	0.54	1.61	
Propazine	PSII herbicide – chlorotriazine	Priority	0.06	0.18	
Propiconazole	Conazole fungicide	Priority			2.0
Prothiophos*	Insecticide: inhibits acetylcholinesterase	Of interest			

| Chemical | Description | Priority or of interest | LC-MS/MS | | GCMS |
			LOD µg L^{-1}	LOR µg L^{-1}	LOR µg L^{-1}
Simazine	PSII herbicide – chlorotriazine	Priority	0.08	0.24	
Tebuconazole	Conazole fungicide	Priority	0.10	0.31	
Tebuthiuron	PSII herbicide – thiadazolurea	Priority	0.01	0.10	
Terbuthylazine*	PSII herbicide – triazine	Priority			
Terbutryn	PSII herbicide – triazine	Of interest	0.55	1.7	
Triclopyr*	Pyridine carboxylic acid herbicide	Priority			
Trifloxysulphuron*	Herbicide: inhibition of ALS – sulfonyl urea	Of interest			
Trifluralin	Herbicide – dintiroaniline	Priority			0.2

* Not currently analysed by QAEHS

^ Currently analysed in grab samples, but ED passive sampler uptake is not adequate to quantify time-averaged water concentrations

Shaded chemicals are included as part of the Paddock to Reef Integrated Monitoring, Modelling and Reporting Program

Red text indicates that the sampling rate (Rs) of atrazine has been assumed

Pesticides are identified as 'priority' (proposed by Pesticide Working Group 18 August 2015) or 'of interest' to the program (feedback from the Paddock to Reef program), and include pesticides that are not currently analysed by QAEHS and are therefore not included in the MMP.

Table 4-1 lists the proposed priority pesticides that have been reported since 2014. Analytes for which analytical method development is required are noted. Note that this list includes priority pesticides and herbicides that may not accumulate well in passive samplers due to their polarity, and may be detectable in grab samples only.

4.2.3   Chemical analysis

From the 2014–2015 monitoring year onwards, the analysis of ED extracts at QAEHS was transferred to the AB Sciex QTRAP 6500 (a new LC-MS/MS model of the QTRAP 5500). The added advantage of this instrument is the enhanced sensitivity of some analytes and the ability to analyse in both positive and negative modes in one injection (effectively halving the analysis time required). LORs of the target analytes were not negatively influenced by the change in instrumentation.

Before the 2014–15 monitoring year, PDMS sampler extracts were analysed for pesticides using gas chromatography mass spectrometry (GCMS) by Queensland Health. QAEHS has developed a pesticide GCMS method that includes five priority pesticides (pendimethalin, propazine, propiconazole, trifluralin and chlorpyrifos), identified by the Pesticide Working Group (Aug 2015). Analysis is conducted on a Thermo Scientific TSQ Quantum XLS Triple Quadrupole GCMS/MS. The mass spectrometer is operated in positive ion, multiple reactions monitoring mode, using argon as the collision gas. Before introduction into the mass spectrometer, compounds are separated on an Agilent J & W DB5-MS (25 m; 0.25 mm i.d.; 0.25 μm film thickness) column. A quantitative and qualitative ion transition is monitored for each compound.

The LOD and LOR for the LC-MS/MS and GCMS instrument data have been defined as follows: a very low level amount of analyte is added to a range of matrices (which include saline (sea) water, Milli-Q water and bore water) and injected 6–7 times into the analytical instrument. The standard deviation of the resultant signals is obtained and a multiplication factor of three is applied to determine the LOD and a factor of nine is applied to get the LOR. A further criterion for the LOR is that the analyte value should exceed three times the mass detected in the blank. It should be noted that the LODs and LORs included in Table 4-1 are for the analytical instrument and do not represent the reporting limits for the water concentrations determined using the passive samplers. The LORs relevant to a particular sample will vary according to the site's sampling rates (i.e. sampler deployment duration and flow rate at the site).

Positive results at QAEHS are confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Samples were reported as positive if the two transitions were present (with peaks having a signal to noise ratio greater than three), retention time was within 0.15 minutes of the standard and the relative intensity of the confirmation transition was within 20 per cent of the expected value. The value reported was that for the quantitation transition.

4.2.4   Passive sampling techniques

SDB-RPS Empore discs

- 3M™ Empore™ Extraction Disks (SDB-RPS) – Phenomenex.
- Deployed in a Teflon "Chemcatcher" housing (Kingston *et al.* 2000) (Figure 4-1).

Routine time integrated monitoring:

- Deployed with a diffusion limiting 47 mm, 0.45 µm polyether sulfone membrane for either one month or two months. From January 2012 onwards, Phenomenex membranes of the same specifications have been used.
- Deployed in a two disk configuration to extend the time-integrated monitoring period when deployed for two months.

Preparation:
- Condition in methanol for no more than five minutes (HPLC grade, Merck).
- Condition in Milli-Q water for a minimum of five minutes.
- Load into acetone rinsed Teflon Chemcatcher housing.
- Cover with membrane and solvent rinsed wire mesh.
- Fill housing with Milli-Q water.
- Seal for transport.
- Store in fridge and transport with ice packs.

Extraction:
- Remove membrane and wipe surface of disk with Kimwipe to remove excess water.
- Spike disk with labelled internal standard.
- Extract disk using acetone and methanol in a solvent rinsed 15 mL centrifuge tube in an ultrasonic bath.
- Filter (0.22 µm PFTE) and concentrate to < 0.5 mL using evaporation under purified N_2.
- Add ultra-pure water to a final volume of 0.5 mL existing in 20 per cent methanol and 80 per cent water solution.
- Spike sample with labelled recovery standard.
- Analyse using LC-MS/MS
- Convert to concentration in water using compound specific *in situ* sampling rates.

Figure 4-1 An Empore Disk being loaded into the Teflon Chemcatcher housing (LHS) and an assembled housing ready for deployment (RHS)

In situ flow monitoring for Empore Disks sampling rate adjustment

Compound specific sampling rates have been determined for a broad suite of herbicides and are applied to the estimation of concentrations in water. Sampling rates are influenced by *in situ* environmental conditions such as flow. A passive flow monitor (PFM), comprised of dental plaster cast into a plastic holder (Figure 4-2), was developed as a means of flow-adjusting sampling rates using an *in situ* calibration device (O'Brien *et al.* 2009). The elimination rate of dental plaster from the PFM during the deployment is proportional to flow velocity, and the influence of ionic strength (salinity) on this process has been quantified (O'Brien *et al.* 2011). The sampling rates of reference chemicals in the ED, such as atrazine, have subsequently been cross-calibrated to the loss of plaster from the PFM under varying flow conditions (Figure 4-3) (O'Brien *et al.* 2011).

Figure 4-2 Passive flow monitors pre-deployment (left) and post-deployment (right)
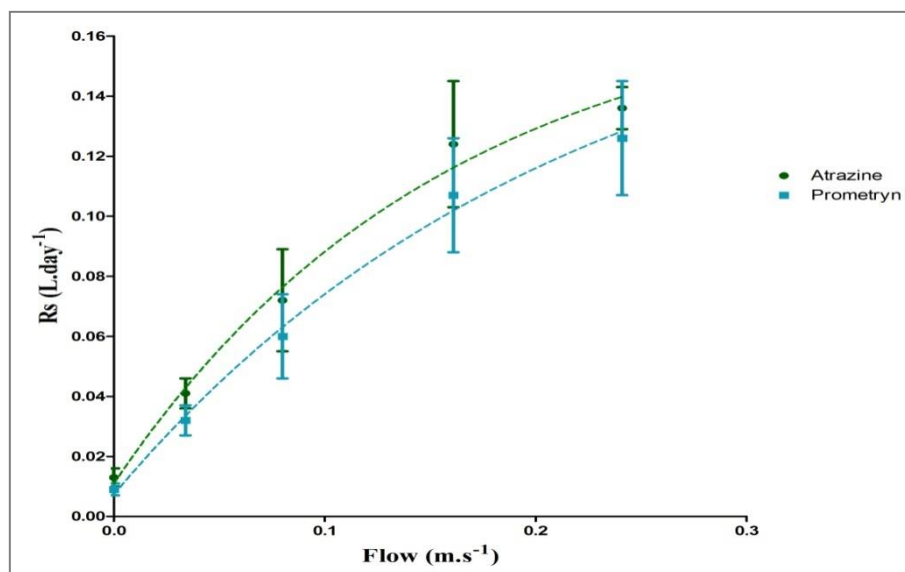
Figure 4-3 Relationship between flow and sampling rates of specific herbicides indicating a shift from aqueous boundary layer control to diffusion limiting membrane control under higher flow conditions

The *in situ* calibration procedure of EDs employed at QAEHS is:

- PFMs are co-deployed alongside EDs.
- Deployment in:
  - Wet season (approx. one month, weather dependent).
- The loss rate of plaster is determined while accounting for the influence of ionic strength.
- The sampling rates of atrazine and prometryn are directly predicted from the PFM loss rate using models.

The sampling rates of other individual herbicides are predicted based on the average ratio of the R_s of atrazine to the individual herbicide R_S across multiple calibration studies (O'Brien *et al.* 2011; Vermeirssen *et al.* 2009; Stephens *et al.* 2009; Shaw and Mueller 2009). For newly included target herbicides where there is no calibration data available, QAEHS may adopt the sampling rate of atrazine (and report this consistently throughout the monitoring program to allow inter-site and inter-year comparisons) or present the data as ng accumulated per sampler per day. Chemicals for which the R_s of atrazine are assumed are highlighted red in Table 4.2. An in-field calibration would be beneficial in determining R_s for the newly included chemicals.

Polydimethylsiloxane (PDMS) samplers

- Silicone rubber 92 cm × 2.5 cm × 410 µm strips.
- Deployed in a marine grade stainless steel deployment cage (Figure 4-4).

- Routine time-integrated (and equilibrium) monitoring:
    - Deployed each month during the wet season at two sites in the Wet Tropics.
- Preparation:
    - Dialysis with acetone (2 × 24 hours) and then hexane (2 × 24 hours) in solvent rinsed glass jars in batches on a shaker.
    - Stored in solvent rinsed glass jars, with Teflon-lined lids, under purified N_2.
    - Individual strips are wound around stainless-steel spikes within the deployment cage (acetone rinsed) in a standard configuration.
    - The cage is assembled and sealed inside a metal can, stored at 4°C and transported with ice packs.
- Extraction and purification:
    - Biofouling is removed from each strip by scrubbing with water.
    - Each strip is then dried with Kimwipes and spiked with a surrogate standard.
    - Each strip is dialysed with 200 mL of a 1:1 ratio of acetone and hexane (2 – 24 hours) (tests in 2015–16 indicated that recoveries using a 1:1 mixture of acetone and hexane resulted in improved recoveries, 92–120 per cent across the five compounds compared to hexame alone (3–106 per cent).
    - Sample extracts are rotary evaporated, further evaporated under purified N_2, dried using Na_2SO_4 columns and filtered (0.45 µm PTFE) and reduced to 1 mL final volume.
    - Up until 2016–17, samples were made up to 10 mL using dichloromethane and subjected to gel permeation chromatography (GPC). Due to the relocation of the QAEHS laboratory in 2018, the use of GPC will no longer be available. Tests conducted comparing samples cleaned up by GPC versus those that were not have shown no appreciable differences.
- Chemical analysis – GCMS.

Figure 4-4 PDMS passive samplers loaded onto stainless steel sampler supports that sits within the deployment cage and is sealed in place with wing nuts

In situ calibration sampling rate adjustment of PDMS passive samplers using PFMs

Previous work has demonstrated the usefulness of the PFM for the *in situ* calibration of herbicides in the ED (O'Brien *et al.* 2011; O'Brien *et al.* 2009). Furthermore, the loss of plaster from the PFM can be applied to predict changes in R_s dependant on flow and turbulence, when deploying PDMS samplers (O'Brien *et al.* 2012).

The uptake of bifenthrin, dieldrin, oxadiazon, pendimethalin, permethrin, prothiophos and trifluralin were investigated as a function of water velocity (determined from r_{PFM}) at flows between 0 and 24 cm s^{-1} (Figure 4-5). A one phase association describing this relationship between R_s and flow for each chemical is shown in Equation 2.

Equation 2

$$R_s = R_{s(0\,cm/s)} + (R_{s(max)} - R_{s(0\,cm/s)}) \times (1 - \exp(-Kr_{PFM} \times r_{PFM}))$$

Where:

$R_{s(0\,cm/s)}$ is the R_s of the chemical of interest when exposed to still waters

$R_{s(max)}$ is the maximum R_s for the chemical of interest

Kr_{PFM} is a rate constant expressed in reciprocal of the units of r_{PFM}

r_{PFM} is the loss rate of the PFM in g/day.
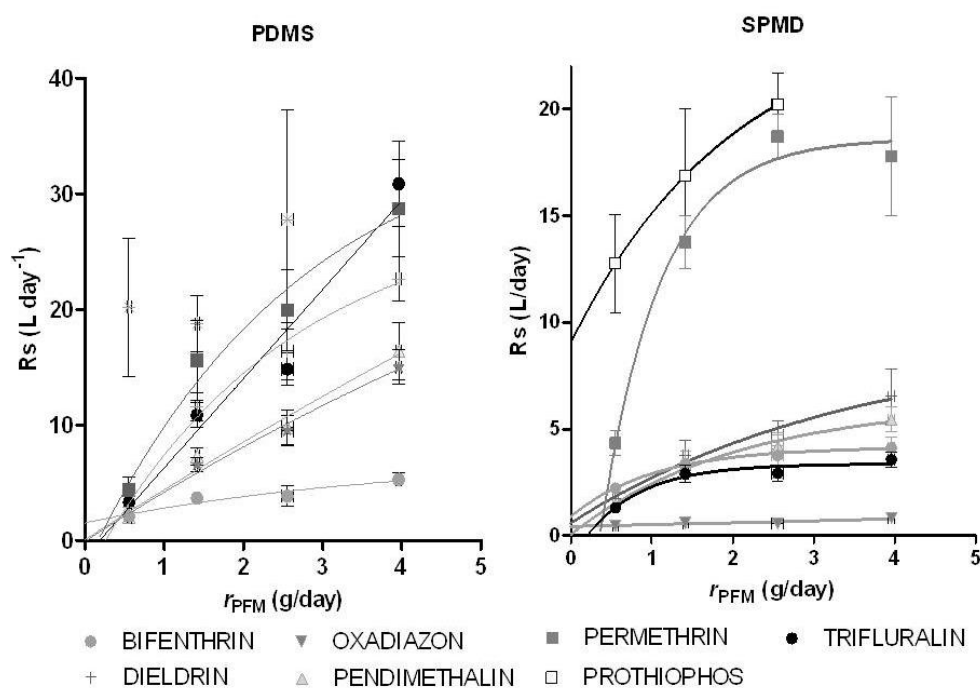
Figure 4-5 PDMS and semipermeable membrane devices sampling rates (Rs) as a function of water velocity (rPFM)

The *in situ* calibration procedure of PDMS using PFMs employed at QAEHS is:

- PFMs are co-deployed alongside marine cages containing PDMS (Figure 4-6).
    - Deployment monthly in the wet season (for one month) – previously with no flow limiting cap. From the 2015–16 monitoring year onwards, a flow limiting cap (reduces plaster loss rate by 15 per cent) has been used to limit the number of PFMs that return to QAEHS empty (and provides an estimate of flow used) due to high flow rates.
- The loss rate of plaster is determined while accounting for the influence of ionic strength.
- Chemical analysis (GCMS) of samplers.
- R_s of 'reference' chemicals — bifenthrin, dieldin, oxadizon, pendimethalin and permethrin (prothiophos and trifluralin) were excluded — are calculated for each site at their specific r_{PFM} using Equation 4.
- Log K_{ow} of the seven reference chemicals are plotted against their R_s.
- The R_s of accumulated chemicals is predicted using the relationship between Log K_{ow} and R_s of the seven reference chemicals.
- Using R_s, estimate C_w using Equation 1.
- For accumulated chemicals with Log $K_{ow} < 4$.
    - Equilibrium phase sampling is assumed.
    - Measured Log K_{sw} (from unpublished collaborative experiment with DES in 2010) is used to estimate a C_w using Equation 2.
    - If no measured log K_{sw} value is available, the Log K_{sw} is predicted from the relationship between Log K_{ow} and Log K_{sw} and the Cw estimated using Equation 2.
- For accumulated chemicals with Log $K_{ow} > 4$, unless otherwise specified, PFM-adjusted R_s is used to estimate C_w.

The PFM method to predict the R_s of chemicals accumulated by PDMS samplers is now routinely used.
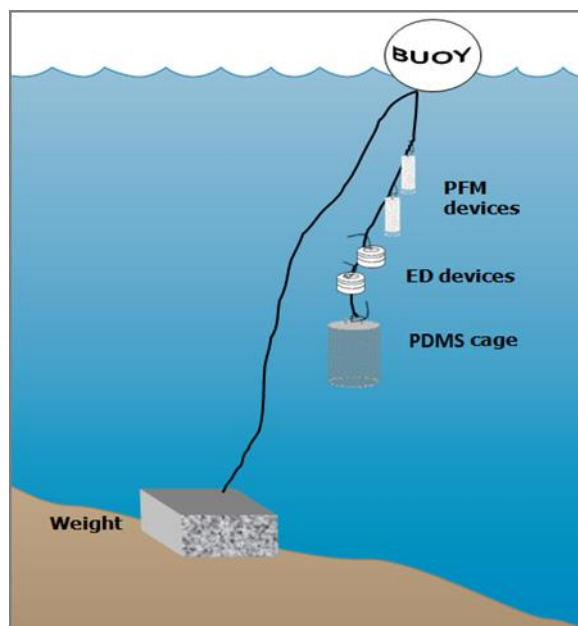
Figure 4-6 A schematic for the deployment of passive samplers (Empore disc in Chemcatcher housings, and Polydimethylsiloxane cages) together with the passive flow monitors for *in situ* calibration of flow effects in the field.

4.2.5   Risk assessment metrics:

Multisubstance-potentially affected fraction

Despite being widely used and simple to calculate, a limitation of the PSII-HEq approach is that matched datasets should ideally be used to derive the potencies relative to a reference chemical (i.e. for each species tested, all PSII herbicides should be included in the same study to assess their toxicity relative to diuron). However, this seldom occurs and, typically, datasets will have data for only a few select compounds. This requirement for matched datasets dramatically reduces the amount of data suitable to derive relative potencies.

The multisubstance-potentially affected fraction ms-PAF method (Traas *et al.* 2002) has been proposed as a more relevant approach to quantify the overall ecological risk of mixtures of pollutants for ecological communities. This approach is based on species sensitivity distributions (SSDs) for all chemicals in a mixture, and thus aligns more closely with the revised methods for proposed individual water quality guideline value derivation, as well as the risk-based approach adopted by the Paddock to Reef program. The PAF of species, i.e. percent of species in an ecosystem that will theoretically be affected at a given mixture environmental concentration, is considered an ecologically relevant assessment end point, which better suits the goals of the Reef Plan.

The ms-PAF can account for both additive and non-additive interactions, i.e. it can determine a cumulative toxicity for a mixture of chemicals with the same toxic mode of action (for

example, for PSII inhibition, effects are assumed additive for all PSII-inhibiting herbicides in a mixture), but also for a mixture of chemicals with different modes of action (non-additive model). Non-additive interactions are an important consideration given the apparent shift towards the use of alternative pesticides with different modes of action in the Reef catchments. At present, however, only the additive model is being implemented (for PSII inhibiting herbicides and SSDs for the full suite of priority chemicals are currently under development). The benefits and rationale of adopting this method include:

- More data can be used to generate more robust estimates of risk.
- The use of SSDs is consistent with the Australian and New Zealand Guidelines for Fresh and Marine Water Marine Quality (ANZECC & ARMCANZ 2000) and with the risk-based approach of the Paddock to Reef Program.
- The risk is quantified in easy to understand terms of a percentage of species that will theoretically be effected (i.e. protecting 95 per cent of species is better than protecting 75 per cent) and again is consistent with the Australian and New Zealand Guidelines for Fresh and Marine Water Marine Quality (ANZECC & ARMCANZ 2000).
- It allows for aggregating risks of compounds in a mixture.
- The toxic effect of mixtures on multiple species can be determined.
- It can be used as a measure of ecological risk, i.e. a certain fraction of species expected to be (potentially) affected above its no-effect level at a given environmental concentration and allows comparisons between substances, species groups, sites and regions.
- Any consistent set of toxicity endpoints can be used to generate SSDs, e.g. no observed effect concentration (NOEC) and half maximal effective concentration (EC_{50}).

To determine the ms-PAF of a field sample that contains a mixture of PSII herbicides, a multisubstance SSD for the global mix, SSD_G, which represents the percentage of species affected by a mixture of pesticides, is determined. To determine the SSD_G for a PSII herbicide mix, individual SSDs for each herbicide are normalised to a common dimensionless scale based on hazard units (HU) so that each re-scaled SSD has a PC50 (concentration at which 50 per cent of species are affected) of one HU (Figure 4-7). To achieve this for each data point in an individual SSD, the concentration is divided by its PC50 value (also referred to as its α (alpha) value, which is the mid-point of the logistic cumulative frequency distribution (CFD) fitted to the SSD) and replotted. An 'averaged' logistic CFD is then fitted to the individual HU-scaled SSDs (Figure 4-7, right graph) to determine a single SSD_G for the mixture defined by an average midpoint of the global distribution (α_G) and an average slope (β_G). To determine the ms-PAF for a field sample using the SSD_G, the concentration of each PSII herbicide in the sample is converted to HUs (by dividing each concentration by the herbicide's SSD α value (PC50)) and then summed for all PSII herbicides. This gives the total HUs for all PSII herbicides in the sample, $HU_{ms} = \Sigma_i HU_i$ for each herbicide, i. The ms-PAF can then be determined from:

$$ms - PAF\ (\%) = \frac{1}{1 + \left(\frac{\sum HU}{\alpha_G}\right)^{-\beta_G}} \qquad\qquad Equation\ (1)$$

The ms-PAF method evaluated in the MPP report has three modifications from the method (Traas *et al.* 2002): (1) a logistic CFD, rather than a log-logistic CFD, is fitted to the ecotoxicity data of individual herbicides to generate the individual SSDs; (2) the midpoint of the curve (α) when PAF equals 50 per cent is used rather than the median to calculate the HUs; and (3) the parameters, α_G and β_G, are determined by fitting a global CFD using SigmaPlot 13.0 (Systat) rather than assuming $\alpha_G = 1$ and then determining β_G as the average of the β's from the individual SSDs.
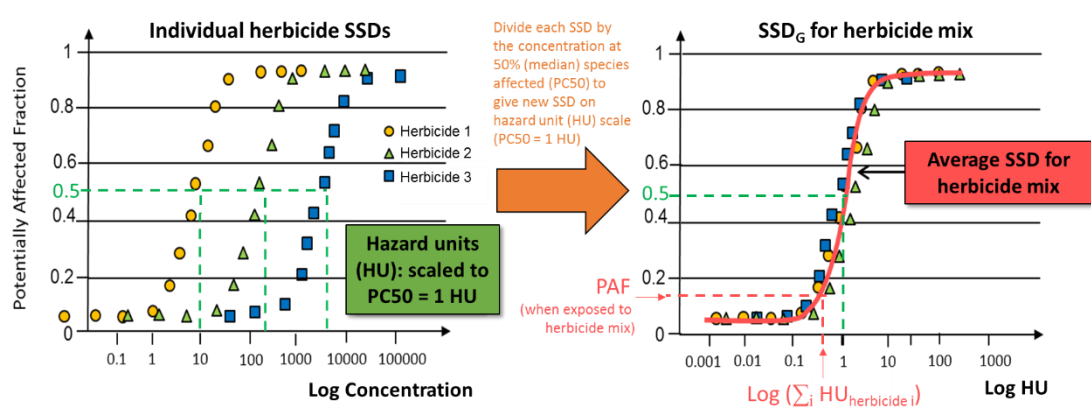
Figure 4-7 Overview of approach to determine a multi-substance species sensitivity distribution for a mixture of herbicides with the same mode of action (concentration additive model).

Phototrophs are highly relevant for ecological risk assessments of Reef ecosystems because corals, which contain photosynthetic zooxanthellae, and seagrasses, which are phototrophs, are keystone species. Additionally, phototrophs (in the form of micro-algae) form the basis of many food chains. Chemicals that have a specific mode of action (such as PSII herbicides), have a bimodal SSD. That is, a more sensitive distribution for the target organisms (phototrophs for PSII herbicides) and another less sensitive distribution for the non-target organisms (all non-phototrophs for PSII herbicides). In such cases, not all the toxicity data can be used, rather only the data from the most sensitive group of organisms are used to calculate the SSD (Australian and New Zealand Environment and Conservation Council (ANZECC and ARMCANZ 2000). Therefore, it is appropriate to only use toxicity for phototrophs to assess the potential impact of PSII herbicides on the Reef ecosystem.

Data (NOEC and EC$_{50}$ data collated from the literature) used in the SSDs should be selected based on the following considerations:

- relevance of species to the Reef region (i.e. phototrophs such as photosynthetic zooxanthellae, seagrasses, and microalgae; keystone species of the Reef)
- appropriateness of the exposure duration (toxicity data that includes both acute and chronic exposure to mimic the extensive periods of low concentrations in the dry season, and pulses in the wet season)
- relevance of the toxicity endpoints (i.e. those related to the long-term survival of populations, communities and ecosystems and include death, immobilisation, growth (individual or population), abundance and reproductive impairment)
- relevance of the measures of toxicity (toxicity data indicative of both a minor and a major risk are used for both short-term (acute) and long-term (chronic) exposure calculations).

In cases where the minimum data requirements to generate a reliable marine SSD are not able to be met, freshwater and estuarine species can also be used. The inclusion of freshwater and estuarine species in the development of SSDs suggests that the risk classes may at least in part be influenced by species irrelevant to the Reef and this should be considered, including in recommendations as to which marine species and pesticides should be further investigated to develop more robust SSDs.

Implementing new risk classes for the ms-PAF method is a current task of the Pesticide Working Group. Five interim risk classes were implemented for the 2014–15 and 2015–16 reporting years (Table 4-2); however, it is important to note that the risk classes need to be validated against Reef species to determine whether these represent meaningful tipping points. Note that these risk classes are for herbicides only and that different classes need to be developed for neurotoxic pesticides that cause mortality.

Table 4-2 Risk classes used for 2019-20 reporting year for the mixture method.

Risk categories (% species affected)	% species protected	Risk category	Risk Level	Pesticides assessment
≤1.0%	≥99%	5	Very low risk	Very good
>1 – 5%	95 – <99%	4	Low risk	Good
>5 – 10%	90 – <95%	3	Moderate risk	Moderate
>10 – 20%	80 – <90%	2	High risk	Poor
>20.0%	<80%	1	Very high risk	Very poor

The ms-PAF approach was first evaluated in the context of quantifying the risk associated with pesticide exposure to the Reef ecosystem in 2014–15. The overall model, as well as the number of PSII herbicides for which reliable SSDs are available, has subsequently been further developed (Spilsbury *et al.* 2020). The MMP has now transitioned to the ms-PAF to assess risk to the Reef ecosystem. Calculations are performed by DES's Catchment Loads Monitoring Program.

4.2.6   QA/QC procedures in the pesticide monitoring program

The development, calibration, field application and validation of passive sampling for monitoring water has been a research focus of QAEHS over many years. The methods described above have been developed as a result of this work in collaboration with analytical method development by QHFSS. These methods are formalized as SOPs, which describe the preparation, extraction and analysis of each type of passive sampler used in the MMP. Considering the number of new target pesticides and herbicides included as priorities under the MMP, an in-field calibration study would be beneficial to determine chemical uptake kinetics.

QA/QC procedures routinely employed by QAEHS in the MMP include:

- SOPs for the preparation, deployment, extraction and analysis of passive samplers
- staff training in these SOPs (laboratory) and a record of this training is maintained
- deployment guides for the training of field staff and volunteers
- generation of a unique alphanumeric identifier code for each passive sampler
- preparation, extraction, storage (4°C or -20°C) and subsequent analysis of procedural blank passive samplers with each batch of exposed passive samplers
- use of labelled internal standards or other surrogate standards to evaluate or correct for recovery or instrument sensitivity throughout the extraction and within the analysis process respectively
- exposure of replicate samplers during each deployment that are extracted and archived in QAEHS specimen bank at -80°C.

QHFSS laboratories are accredited by the National Association of Standards Testing. Details of QHFSS accreditation can be found on the National Association of Testing Authorities (NATA) website (http://www.nata.asn.au/). Sample receipting, handling, analysis and data reporting at QHFSS are based on NATA certified methods. The NATA accreditation held by the QHFSS includes a wide variety of QA/QC procedures covering the registration and identification of samples with specific codes and the regular calibration of all quantitative laboratory equipment required for the analysis.

4.3   Data management and security

The data management protocols for QAEHS are outlined below and include documentation of all steps within the sampling program: passive sampler identification, transport, deployment, transfer of samples to QHFSS for chemical analysis, analytical results, data manipulation, storage and access. This protocol may be summarised as:

- The unique alphanumeric identifier code attached to each passive sampler is applied to all subsequent daughter samples and results, ensuring a reliable link with the original sample.
- Deployment records are sent with the sampling devices, and include information on the unique sampling device identifier, deployment identifier, name of the staff/volunteer who performed the operation, storage location, destination site,

important dates, details of sample treatment and any problems that may have occurred. When returned, the information is entered into Excel spreadsheets and stored on the QAEHS main server with a back-up on one local hard drive.

- Detailed chain of custody records are kept with the samplers at all times. Devices are couriered directly to the tourism operators/community member and monitored via a tracking system. Delivery records are maintained by QAEHS to guarantee traceability of samples.

- Hard copy records are maintained of all sample submission forms provided to QHFSS for analysis.

- Results files provided by QHFSS along with a unique identifier code are transferred from the instrumentation computer to the Entox server and archived on the QHFSS network using an established data management system.

- Excel spreadsheets used for data manipulation and a summary results file (concentration in water estimates) are stored on the QAEHS server. Access to the QAEHS server is restricted to authorised personnel only via a password protection system. Provision of data to a third party only occurs at the consent or request of the program manager.

4.4   Summary

In summary, the following QA/QC measures are employed:

- unique sample identifiers
- comprehensive records and chain of custody paperwork across all components
- training of field personnel, including deployment guidelines and records
- analytical quality control measures
- procedural QA/QC for the preparation, extraction and analysis of passive samplers including SOPs
- inclusion of QA/QC samples (replication of sampling and procedural blanks)
- continual evaluation, method development and improvement of methods for sampler processing and estimation of concentration in water.

In addition to these measures, QAEHS has undertaken an independent and comprehensive internal review of its processes, with the objective being to guarantee and improve consistency and quality of work. This review (conducted in February 2016) consisted of tracking a sample/sample batch from receipt to reporting (i.e. entire lifecycle from receipt, storage, processing, extraction, analysis, data interpretation/validation and reporting). While the review did not centre around the MMP specifically, the recommendations from the review could be directly transferrable and helped to streamline and improve aspects of this program including updating documentation of training and SOPs, improved record keeping of equipment calibration and maintenance, automation of spreadsheets (through the use of macros and hyperlinks) to avoid human error involved in excessive copy and pasting, and simplification of sample labelling. Using the reviewer's input, QAEHS seeks to implement many of their recommendations throughout the current monitoring year.

5   Inshore coral reef monitoring

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5.1   Introduction

This component of the MMP aims to accurately quantify temporal and spatial variation in inshore coral reef community status in relation to variations in local reef water quality. Steep gradients in water quality across the inshore region of the Reef are reflected in differences in benthic community composition (Uthicke *et al.* 2010). Changes in these communities may be due to acute disturbances such as cyclonic winds, bleaching and crown-of-thorns starfish as well as more chronic pressures related to run-off (for example increased sediment and nutrient loads) that may disrupt processes of recovery from such disturbances or lead to long-term changes in communities due to altered selective pressures .

Salient attributes of a healthy ecological community are that it should be self-perpetuating and 'resilient', that is, able to recover from, or resist, disturbance. While the spatial sampling design of the program explicitly attempts include reefs across gradients in environmental conditions, the temporal design and variables measured focus on the resilience of these communities to both acute and chronic environmental pressures (Thompson *et al.* 2020).

To quantify inshore coral reef community status and resilience in relation to variations in local reef water quality, this section of the project has several key objectives:

- Identify the trends in the condition and composition of benthic communities for Great Barrier Reef inshore coral reefs against desired outcomes along identified or expected gradients in water quality.
- Assess the extent, frequency and intensity of acute and chronic impacts on the condition of inshore coral reefs associated with sediments and nutrients transported by run-off.
- Identify the trajectories of recovery for inshore coral reef communities following impacts resulting from exposure to flood plumes (and associated sediments and nutrients), cyclones and thermal bleaching events.
- Identify the key drivers of coral mortality and the trends in coral reef resilience indicators on inshore reefs.
- Provide information about sea temperature as a potential driver of environmental conditions at inshore reefs.
- Provide an integrated assessment of coral community condition for the inshore reefs monitored to serve as a report card against which changes in condition can be tracked.
- Maintain a local database of all sample data and associated metadata and data summaries, with relevant excerpts provided for storage in the Spatial and Scientific Information Management for Reef database.

5.2 Methods

5.2.1 Sampling design

The sampling design was selected for the detection of change in benthic communities on inshore reefs in response to improvements in water quality parameters relevant to specific catchments, or groups of catchments (Region), and to disturbance events. Within each Region, reefs are selected along a gradient of exposure to run-off, largely determined as increasing distance from a river mouth in a northerly direction. To account for spatial heterogeneity of benthic communities within reefs, two sites were selected at each reef (Figure 5-1).

Observations on a number of inshore reefs undertaken by AIMS in 2004 during the pilot study to the current monitoring program (Sweatman *et al.* 2007) highlighted marked differences in community structure and exposure to perturbations with depth; hence sampling within sites is stratified by depth. Within each site and depth, fine scale spatial variability is accounted for by the use of five replicate transects.
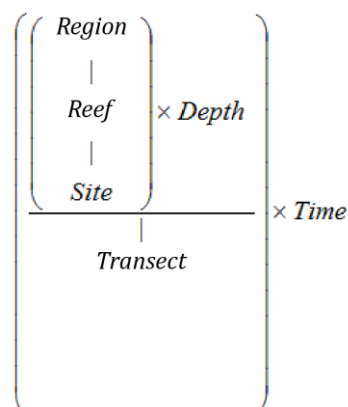
Figure 5-1 Sampling design for coral reef benthic community monitoring. Terms within brackets are nested within the term appearing

5.2.2 Site selection

The reefs monitored were selected by the Authority, using advice from expert working groups. The selection of reefs was based upon two primary considerations:

- To make sure that the sampling locations in each catchment of interest were spread along a perceived gradient of influence from river output.
- Evidence (in the form of carbonate-based substrate) that coral reef communities had been viable (net positive accretion of a carbonate substrate) in the past.

Where well-developed reefs existed on more than one aspect of an island, two reefs were included in the design because, although position relative to run-off exposure would be similar, often quite different communities exist on windward compared to leeward reefs.

Over time, there have been some adjustments made to the sampling design (Table 5-1). For the first two years of the project (2005 and 2006), 35 reefs were surveyed each year. In 2007 fringing reefs along the Cape Tribulation coast were removed from the program due to concerns over crocodile attack. In addition, the sampling frequency changed so that only a subset of "core" reefs were surveyed annually with the remaining "cycle" reefs surveyed every other year (Table 5-1). From 2015, the sampling changed again with King Reef replaced by Bedarra Island in the Tully Catchment and all reefs scheduled for sampling biennially, half surveyed in 'odd-years' and half surveyed in 'even-years'. Importantly, when an acute disturbance is suspected of having impacted survey reefs during the preceding summer, the design allows for contingency sampling of up to six reefs not scheduled for survey so as to improve estimates of impact and book-end the start of the recovery period. In 2020 all reefs were monitored to document the impact of high water temperatures in early 2020. In addition to these adjustments, as of 2015 data from inshore reef sites surveyed under the Long-term Monitoring Program (LTMP) were included in the analysis and reporting of inshore coral reef condition (Table 5-1). A map of sites included in 2020 is presented in Figure 5-2.

Figure 5-2 Coral locations that exist as of 2020. However, not all locations were assessed/sampled in 2020

Table 5-1 Scheduled coral monitoring sampling design for the Marine Monitoring. Site names in italics are monitored by the AIMS Long-term Monitoring Program and included in MMP reporting. Waypoints for each site and depth monitored can be found in Appendix C1.

NRM Region	Catchment	Monitoring location	2005-2006	2007–2014	2015–2020
	Barron Daintree	*Low Isles*	O	O	O
		Snapper North	A	A	O
		Snapper South	A	A	E
	Russell / Mulgrave Johnstone	*Green Island*	O	O	O
		Fitzroy East	A	E	E
		Fitzroy West	A	A	O
		Fitzroy West	O	O	O
		Frankland East	A	O	O
		Frankland West	A	A	E
		High East	A	O	O
		High West	A	A	E
	Herbert Tully	Barnards	A	O	O
		Bedarra			A
		Dunk North	A	A	E
		Dunk South	A	E	E
Burdekin	Burdekin	Havannah Island	A		O
		Havannah Island (North)	O	O	O
		Lady Elliot	A		E
		Magnetic	A	A	O
		Middle Reef	O	O	O
		Palms East	A		
		Palms West	A	A	
		Pandora	A	A	
		Pandora (North)	O	O	O
Mackay Whitsunday	Proserpine	*Border*	O	O	O
		Daydream	A	A	E
		Dent	A	E	E
		Double Cone	A	A	E

NRM Region	Catchment	Monitoring location	2005-2006	2007–2014	2015 –2020
		Hayman	O	O	O
		Hook	A	E	E
		Langford	O	O	O
		Pine	A	A	O
		Seaforth	A	O	O
		Shute	A	O	O
Fitzroy	Fitzroy	Barren	A	A	O
		Keppels South	A	A	E
		Middle	A	E	E
		North Keppel	A	O	O
		Peak	A	A	O
		Pelican	A	E	E

A, Reefs designated as 'core reefs' and sampled annually, prior to 2015
O, Reefs scheduled for sampling sampled in odd numbered years
E, Reefs scheduled for sampling in even numbered years
Italics identify reefs surveyed by the AIMS long-term monitoring program

Table 5-2 Distribution of sampling effort

Survey Method	Information provided	Transect coverage	Spatial coverage
Photo point intercept	Percentage cover of the substrate for major benthic habitat components	Approximately 25 cm belt along upslope side of transect from which 160 points are sampled	Full sampling design
Juvenile counts *	2005-2006 Size structure of coral communities, recorded into: 0-2cm, >2-5cm, >5-10cm,>10-20cm, >20-50cm, >50-100cm and >100cm categories	A 34 cm belt along the upslope side of each transect	First 10m of each transect
	2007-2018, density of juvenile corals in size classes: : 0-2cm, >2-5cm, >5-10cm	A 34 cm belt along the upslope side of each transect	Full sampling design
	2019 on, density of juvenile corals in size classes: : 0-2cm, >2-5cm	A 34 cm belt along the upslope side of each transect	Full sampling design
Scuba search	Incidence of factors causing coral mortality	Two-metre belt centred on transect	Full sampling design

5.2.3   Depth selection

From observations of a number of inshore reefs undertaken by AIMS in 2004, marked differences in community structure and exposure to perturbations with depth were noted (Sweatman *et al.* 2007). The lower limit for the inshore coral surveys was selected at five metres below datum because coral communities rapidly diminish below this depth at many reefs; two metres below datum was selected as the shallow depth. Shallower depths were considered but discounted for logistical reasons, including the inability to use the photo technique in very shallow water, site markers creating a danger to navigation and difficulty in locating a depth contour on very shallow sloping substrata typical of reef flats. Sites surveyed under the LTMP are generally in the range of 5-7metres below datum and reported along with the 5m depths of the MMP.

5.2.4   Field survey methods

Site marking

Each selected reef site is permanently marked with steel fence posts at the beginning of each 20-metre transect and smaller (10 mm diameter) steel rods at the 10-metre mark and end of each transect. Compass bearings coupled with distance along transects record the transect path between these permanent markers. Transects were set initially by running two 60-metre fibreglass tape measures out along the desired five- or two-metre depth contours. Digital depth gauges are used along with tide heights from the closest location included in 'Seafarer Tides' electronic tide charts produced by the Australian Hydrographic Service. There are five-metre gaps between each consecutive 20-metre transect. The position of the first picket of each site is recorded with a Global Positioning System (GPS).

Sampling methods

Three separate sampling methodologies are used to describe the benthic communities of inshore coral reefs (Table 5-2). These are each conducted along the fixed transects identified in the sampling design, although there are subtle differences in width or length of transects as listed in Table 5-1.

Photo point intercept method

This method is used to gain estimates of the per cent cover of benthic community components. The method follows closely SOP Number 10 of the AIMS LTMP (Jonker et al., 2008). In short, digital photographs are taken at 50-centimetre intervals along each 20-metre transect. Estimation of cover of benthic community components is derived from the identification of the benthos lying beneath points overlaid onto these images. For the majority of hard and soft corals, at least genus level identification is achieved. The categories used for identification of benthos are listed in (Jonker *et al.* 2008), although additional genus level codes for some algae have since been added.

The primary difference in the application of the method in this project from that described in (Jonker *et al.* 2008) is in the sampling design. Sampling for this project is based on 20-metre

transects, rather than 50-metre transects. To compensate for transects being shorter than in the standard method, the density of frames per unit area of transect is doubled (images captured at 0.5 metres rather than at one-metre intervals). This alteration to the standard technique was adopted owing to the limited size of some of the reefs sampled. This modification in methodology of course does not apply to the sites monitored under the LTMP, which use the 50-metre transects and one image per meter.

Juvenile coral surveys

These surveys aim to provide an estimate of the number of hard and soft coral colonies that were successfully recruiting and surviving early post-settlement pressures. In the first year of sampling under this program, these juvenile coral colonies were counted as part of a demographic survey that counted the number of individuals falling into a broader range of size classes (Table 5.1). As the focus narrowed to just juvenile colonies, the number of size classes reduced allowing an increase in the spatial coverage of sampling.

From 2006 to 2017, coral colonies less than 10 centimetres in diameter were counted within a belt 34-centimetres wide (data slate length) along the upslope side of each 20-metre transect. Each colony was identified to genus and assigned to a size class of 0–2 cm, >2–5 cm or >5–10 cm. Importantly, this method aims to estimate the number of juvenile colonies that result from the settlement and subsequent survival and growth of coral larvae rather than small coral colonies resulting from fragmentation or partial mortality of larger colonies. With the exception of the transect dimension and the size classes used, this method is consistent with SOP Number 10 of the AIMS LTMP, Part 2, in which further detail relating to juvenile/fragment differentiation can be found (Jonker *et al.* 2008). Data on juvenile density provided by the LTMP for the relevant sites listed in Table 5-1 are collected according to (Jonker *et al.* 2008) with no modification.

Since 2018 only the 0-2 cm and >2-5 cm size classes were recorded as these are the only size classes that are reported in the Reef report card, and provides a more consistent method to that used by the LTMP.

Scuba search transects

Scuba search transects document the incidence of agents causing coral mortality or disease. Tracking of these mortality agents is important because declines due to these agents must be carefully considered as covariates for possible trends associated with response to outcomes. The method used follows closely SOP Number 9 of the AIMS LTMP, Part 2 (Miller *et al.* 2009). In short, a search is made of a two-metre wide belt (one metre either side of the transect midline) for any recent scars, bleaching, disease or damage to coral colonies. An extra category not included in the standard procedure is physical damage. This is recorded on the same five-point scale as coral bleaching and describes the proportion of the coral community that has been physically damaged, as indicated by toppled or broken colonies. This category may include anchor as well as storm damage. Scuba search data provided by the LTMP for the relevant sites listed in Table 5-1 is collected with strict adherence to SOP Number 9 of the AIMS LTMP, Part 2 (Miller *et al.* 2009).

5.2.5   Observer training

The AIMS personnel collecting data in association with this project are without exception highly experienced in the collection of benthic monitoring data. Each observer was employed specifically for their skills in benthic monitoring and benthic organism identification.

Ongoing standardisation of observers is achieved by photo-based comparisons that, for the most, mitigate inconsistencies in identification. As a final step in reducing bias in sampling, all photo-transect identifications are double checked by a single observer.

In the event that new observers enter the team, training in each sampling method is by direct tuition with an experienced observer. New observers must meet the standards listed in Table 5-3 before collecting data for the project.

Table 5-3 Observer training methods and quality measures

Monitoring method	Training method	Quality measure
Photo point intercept	In-field identification of benthic components. On screen classification of photo points. In-field tuition on photographic protocol.	All identifications double checked.
Juvenile counts	In-field identification of corals to genus level and application of technique with experienced observer supervision.	No greater than 10 per cent of colonies misidentified, overlooked or misclassified in size during supervised demographic surveys of two sites.
Scuba search	In-field tuition in the classification of coral scars and damage.	Observation of at least 90 per cent of damaged colonies and their correct classification during supervised surveys of two sites of damaged colonies.

Classification to genus level underwater is augmented by the use of a small digital camera to take images for post-dive scrutiny of difficult to identify colonies. However, some small juvenile corals are difficult to differentiate in the field and, while they are identified to genus level, they are typically merged with similar genera for analysis and reporting.

Sea Research is responsible for surveys in the Daintree catchment. The Sea Research observer, Tony Ayling, is the most experienced individual in Australia in surveying the benthic communities of near-shore coral reefs. He has 30+years' experience surveying the sites in this catchment, amongst many others. His taxonomic skills are undoubted at genus level and, as such, observer standardisation for demography and scuba search surveys are limited to detailed discussion of methodologies with AIMS observers and explicit following of

the protocols listed here. Sea Research also use the same pre-printed datasheets. Analysis of transect images collected by Sea Research is undertaken by AIMS.

5.2.6   Temperature monitoring

Temperature loggers are deployed at, or in close proximity to, all locations at both two-metre and five-metre depths and routinely exchanged either at the time of the coral surveys (i.e. every 12-24 months) or every four months at sites where FLNTU loggers are co-located (Table 2.1). Three types of temperature loggers have been used for the sea surface temperature logger program. The first type was the Odyssey temperature loggers (http://www.odysseydatarecording.com/), which were superseded by the Sensus Ultra Temperature logger (http://reefnet.ca/products/sensus/). In 2015, Vemco minilog temperature loggers (http://vemco.com/products/minilog-ii-t/) began to replace aging Sensus loggers.

The Odyssey loggers were set to take readings every 30 minutes. The Sensus and Vemco loggers were set to take readings every 10 minutes. Loggers were calibrated against a certified reference thermometer after each deployment and generally accurate to ± 0.2°C.

Detailed data download, quality checks and data management methods are described in Appendix A12.

5.3   Data management

Data management practices are a major contributor to the overall quality of the data collected; poor data management can lead to errors and lost data and can reduce the value of the Reef Plan MMP data. Data from the AIMS MMP inshore coral reef monitoring are stored in a custom-designed MMP data management system in Oracle databases to allow cross-referencing and access to related data (see Appendix A12 for details about general AIMS in-house procedures for data security, data quality checking and backup).

It is AIMS policy that all data collected have a metadata record created for it. The metadata record is created using a Metadata Entry System where the metadata is in the form of ISO19139 XML. This is the chosen format for many agencies across Australia and the international community that deal with spatial scientific data.

Metadata is available here:

https://apps.aims.gov.au/metadata/view/c30cfb2d-46be-4837-9733-9bb60489b65b

All coral monitoring field data is recorded on pre-printed datasheets. The use of standard datasheets aids in ensuring standard recording of attributes and makes sure that required data are collected.

On return from the field, all data is entered on the same day into a SQLite database using a custom written data entry program. Each field on the data entry program forms mirror those on the pre-printed datasheets and includes lookup fields to make sure that data entered is of

the appropriate structure or within predetermined limits. For example, entry of genera to the demography data table must match a pre-determined list of coral genera.

On return to the office, the data is uploaded to an Oracle Database using a custom written synchronisation process. All keyed data is printed and checked against field datasheets before final logical checking (ensuring all expected fields are included and tally with the number of surveys). Photo images are also stored on a server that is included in a routine automatic backup schedule. Photo images are copied to an external hard drive before analysis as a second backup.

Image analysis of reef monitoring photos is performed within the AIMS monitoring data entry package "reefmon". This software contains logical checks to all keyed data and is directly linked to a database to guarantee data integrity. The directory path to transect images is recorded in the database. This functionality allows the checking of benthic category identification. All photo transect data is checked by a second experienced observer before data analysis and reporting of results.

5.4   Summary of Quality Control measures

Quality control is achieved by:

- Use of published SOPs.
- Data collection staff are trained and assessed by experienced observers before commencing field work to make sure that their identification skills are consistent with the resolution required.
- Data entry via database forms that include logical checking on format and content of entered fields, and confirmation of data by second observer.
- Continual evaluation, method development and improvement of methods.
- Advanced data management and security procedures.

6   Inshore seagrass monitoring

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6.1   Introduction

Approximately 3464 square kilometres of inshore seagrass meadows have been mapped in Great Barrier Reef World Heritage Area waters shallower than 15 metres, relatively close to the coast, and in locations that can potentially be influenced by adjacent land use practices. Monitoring of the major marine ecosystem types most at risk from land-based sources of pollutants is being conducted to make sure that any change in their status is identified. Seagrass monitoring sites are associated with the river mouth and inshore marine water quality monitoring tasks in the MMP to enable correlation with concurrently collected water quality information.

The key aims of the inshore seagrass monitoring under the MMP are to:

- Monitor, assess and report the condition and trend of Great Barrier Reef inshore seagrass meadows against desired outcomes along identified or expected gradients in water quality.
- Monitor, assess and report the extent, frequency and intensity of acute and chronic impacts on the condition of Great Barrier Reef inshore seagrass meadows from sediments, nutrients and pesticides.
- Monitor, assess and report recovery of Great Barrier Reef inshore seagrass meadows from exposure to flood plumes, sediments, nutrients and pesticides.

6.2   Methods

6.2.1   Sampling design

The sampling design was selected to detect changes in inshore seagrass meadows in response to improvements in water quality associated with specific catchments or groups of catchments (NRM region) and to disturbance events. Within each region, a relatively homogenous section of a representative seagrass meadow is selected to represent each of the seagrass habitats present (estuarine, coastal, reef). Meadow selection was informed using mapping surveys across the regions before site establishment and by the Authority, using advice from expert working groups. To account for spatial heterogeneity, two sites are selected within each location (Site [Habitat (Region)]). Subtidal sites are not replicated within some locations. Within each site, finer scale variability is accounted for by assessing 33 quadrats. The final constraint on site selection is that the minimum detectable relative difference (MDRD) must be below 20 per cent (at the 5 per cent level of significance with 80

per cent power). An intertidal site is defined as a 5.5 hectare area and in the centre of each site a 50 m × 50 m area is examined using 33 quadrats (50 cm × 50 cm), placed every five metres along each of the 50 metre transects, placed 25 metres apart. A pilot assessment is conducted prior to establishing long-term monitoring to ensure no spatial autocorrelation within or between transects. The sampling strategy for subtidal sites is modified depending on whether assessments were conducted using SCUBA or drop-camera. Owing to logistical problems with SCUBA diving in often poor visibility, sampling is conducted along 50-metre transects two–three metres apart (aligned along the depth contour). Drop-camera sampling is conducted from a surface vessel using a real-time underwater GoPro® camera mounted to a frame with a 0.25 m² quadrat in the field of view. The drop-camera is deployed within a 50 metre radius of permanent waypoints, using vertical drops and the footage recorded for post-field analysis. A van Veen grab is used in conjunction with the visual assessment to confirm seagrass taxonomy and sediment type. At each site, monitoring is conducted during the late-monsoon (April) and late-dry (October) periods each year; extra sampling is conducted at more accessible locations in the dry (July) and monsoon (January) periods.

6.2.2   Field survey methods — Inshore seagrass meadow abundance, community structure and reproductive health

Site marking

The sampling locations for this program are shown in Figure 6-1 and listed in Table 6-1. Where possible, each selected inshore seagrass site is permanently marked with a plastic star picket at the start of the centre transect. Labels identifying the sites and contact details for the program are attached to these pickets. Positions of zero metre and 50 metre points for all transects at a site are also noted using GPS (accuracy ±3 m). This guarantees that the same site is monitored at each event.

Seagrass cover and species composition

Survey methodology follows standardised protocols (weblink https://www.seagrasswatch.org/seagrass-monitoring/). A site is defined as an area within a relatively homogenous section of a representative seagrass community/meadow (McKenzie *et al.* 2000).

Monitoring at the 47 sites identified for the MMP long-term inshore monitoring in late-monsoon (April) and late-dry season (October) of each year is conducted by qualified and trained scientists who have demonstrated competency in the methods (see Sub-section 6.2.3). Monitoring conducted outside these periods is also conducted by a trained scientist and, at two locations (Magnetic Island and Townsville), is assisted by volunteers.

Figure 6-1 Inshore seagrass locations that exist as of 2019-20. However, not all locations were assessed/sampled in 2019-20.

Table 6-1 Inshore seagrass long-term monitoring sites surveyed in 2019-20

NRM region from www.nrm.gov.au. * = intertidal, ^=subtidal.

	NRM region	Basin	Monitoring location		Site	Longitude	Latitude	Seagrass community type
Far Northern	Cape York	Jacky Jacky / Olive-Pascoe	Shelburne Bay *coastal*	SR1*	Shelburne Bay	142.914	-11.887	*H. ovalis* with *H. uninervis/T. hemprichii*
				SR2*	Shelburne Bay	142.916	-11.888	*H. ovalis* with *H. uninervis/T. hemprichii*
			Piper Reef *reef*	FR1*	Farmer Is.	143.234	-12.256	*T. hemprichii* with *C. rotundata/H. ovalis*
				FR2*	Farmer Is.	143.236	-12.257	*T. hemprichii* with *C. rotundata/H. ovalis*
		Normanby / Jeannie	Flinders Group *reef*	ST1*	Stanley Island	144.245	-14.143	*H. ovalis/H. uninervis* with *T. hemprichii/C. rotundata*
				ST2*	Stanley Island	144.243	-14.142	*H. ovalis/H. uninervis* with *T. hemprichii/C. rotundata*
			Bathurst Bay *coastal*	BY1*	Bathurst Bay	144.233	-14.268	*H. uninervis* with *H. ovalis/T. hemprichii/C. rotundata*
				BY2*	Bathurst Bay	144.232	-14.268	*H. uninervis* with *H. ovalis/T. hemprichii/C. rotundata*
Northern	Wet Tropics	Daintree	Low Isles *reef*	LI1*	Low Isles	145.565	-16.385	*H. ovalis/H. uninervis*
				LI2^	Low Isles	145.564	-16.383	*H. ovalis/H. uninervis*
		Mossman / Barron / Mulgrave-Russell / Johnstone	Yule Point *coastal*	YP1*	Yule Point	145.512	-16.569	*H. uninervis* with *H. ovalis*
				YP2*	Yule Point	145.509	-16.564	*H. uninervis* with *H. ovalis*
			Green Island *reef*	GI1*	Green Island	145.973	-16.762	*C. rotundata/T. hemprichii* with *H. uninervis/H. ovalis*
				GI2*	Green Island	145.976	-16.761	*C. rotundata/T. hemprichii* with *H. uninervis/H. ovalis*
				GI3^	Green Island	145.973	-16.755	*C. rotundata/ H. uninervis/C. serrulata/S. isoetifolium*
		Tully / Murray / Herbert	Mission Beach *coastal*	LB1*	Lugger Bay	146.093	-17.961	*H. uninervis*
				LB2*	Lugger Bay	146.094	-17.961	*H. uninervis*
			Dunk Island *reef*	DI1*	Dunk Island	146.141	-17.944	*H. uninervis* with *T. hemprichii/ C. rotundata*
				DI2*	Dunk Island	146.141	-17.946	*H. uninervis* with *T. hemprichii/ C. rotundata*
				DI3^	Dunk Island	146.140	-17.932	*H. uninervis / H. ovalis/H. decipiens/C. serrulate*
Central	Burdekin	Ross / Burdekin	Magnetic Island *reef*	MI1*	Picnic Bay	146.841	-19.179	*H. uninervis* with *H. ovalis* & *Zostera/T. hemprichii*
				MI2*	Cockle Bay	146.829	-19.177	*C. serrulata/ H. uninervis* with *T. hemprichii/H. ovalis*
				MI3^	Picnic Bay	146.841	-19.179	*H. uninervis* with *H. ovalis* & *Zostera/T. hemprichii*
			Townsville *coastal*	SB1*	Shelley Beach	146.771	-19.186	*H. uninervis* with *H. ovalis*
				BB1*	Bushland Beach	146.683	-19.184	*H. uninervis* with *H. ovalis*
			Bowling Green Bay *coastal*	JR1*	Jerona (Barratta CK)	147.241	-19.423	*H. uninervis* with *Zostera/H. ovalis*
				JR2*	Jerona (Barratta CK)	147.240	-19.421	*H. uninervis* with *Zostera/H. ovalis*
	Mackay-Whitsunday	Proserpine / O'Connell	Lindeman Is. *reef*	LN1^	Lindeman Is.	149.028	-20.438	*H. uninervis* with *H. ovalis*
				LN2^	Lindeman Is.	149.032	-20.434	*H. uninervis* with *H. ovalis*
			Repulse Bay *coastal*	MP2*	Midge Point	148.702	-20.635	*Zostera* with *H. uninervis/H. ovalis*
				MP3*	Midge Point	148.705	-20.635	*Zostera* with *H. uninervis/H. ovalis*
			Hamilton Island	HM1*	Hamilton Island	148.957	-20.344	*H. uninervis* with *H. ovalis*

	NRM region	Basin	Monitoring location	Site		Longitude	Latitude	Seagrass community type
			reef	HM2*	Hamilton Island	148.971	-20.347	*Z. muelleri* with *H. ovalis/H. uninervis*
		Plane	Sarina Inlet *estuarine*	SI1*	Sarina Inlet	149.304	-21.396	*Z. muelleri* with *H. ovalis (H. uninervis)*
				SI2*	Sarina Inlet	149.305	-21.395	*Z. muelleri* with *H. ovalis (H. uninervis)*
Southern	Fitzroy	Shoalwater / Fitzroy	Shoalwater Bay *coastal*	RC1*	Ross Creek	150.213	-22.382	*Zostera muelleri* with *H. ovalis*
				WH1*	Wheelans Hut	150.275	-22.397	*Zostera muelleri* with *H. ovalis*
			Keppel Islands *reef*	GK1*	Great Keppel Is.	150.939	-23.196	*H. uninervis* with *H. ovalis*
				GK2*	Great Keppel Is.	150.940	-23.194	*H. uninervis* with *H. ovalis*
		Calliope / Boyne	Gladstone Harbour *estuarine*	GH1*	Gladstone Hbr	151.301	-23.767	*Zostera muelleri* with *H. ovalis*
				GH2*	Gladstone Hbr	151.304	-23.765	*Zostera muelleri* with *H. ovalis*
	Burnett-Mary	Baffle	Rodds Bay *estuarine*	RD1*	Rodds Bay	151.655	-24.058	*Zostera muelleri* with *H. ovalis*
				RD3*	Rodds Bay	151.589	-24.038	*Zostera muelleri* with *H. ovalis*
		Burrum	Burrum Heads *coastal*	BH1*	Burrum Heads	152.626	-25.188	*H. uninervis / Zostera* with *H. ovalis*
				BH3*	Burrum Heads	152.639	-25.210	
		Mary	Hervey Bay *estuarine*	UG1*	Urangan	152.907	-25.301	*Zostera muelleri* with *H. ovalis*
				UG2*	Urangan	152.906	-25.303	*Zostera muelleri* with *H. ovalis*

Table 6-2 Extra inshore seagrass long-term monitoring sites from the Seagrass-Watch and Queensland Parks and Wildlife drop-camera programs surveyed in 2019-20

NRM region from www.nrm.gov.au. * = intertidal, ^=subtidal.

Region	NRM region	Basin	Monitoring location	Site		Longitude	Latitude	Seagrass community type
Far Northern	Cape York	Lockhart	Lloyd Bay *coastal*	LR1^	Lloyd Bay	143.485	-12.797	*H. uninervis / H. ovalis with H. spinulosa*
				LR2^	Lloyd Bay	143.475	-12.825	
		Normanby / Jeannie	Flinders Group *reef*	FG1^	Flinders Island	144.225	-14.182	*H. uninervis / H. ovalis with H. spinulosa*
				FG2^	Flinders Island	144.225	-14.182	
			Bathurst Bay *coastal*	BY3^	Bathurst Bay	144.285	-14.276	*H. uninervis with H. ovalis*
				BY4^	Bathurst Bay	144.300	-14.275	
Northern	Wet Tropics	Tully / Murray / Herbert	Missionary Bay *coastal*	MS1^	Missionary Bay	146.213	-18.216	*H. uninervis with H. ovalis*
				MS2^	Missionary Bay	146.217	-18.205	
Central	Burdekin	Ross / Burdekin	Townsville *coastal*	SB2*	Shelley Beach	146.763	-19.182	*H. uninervis with H. ovalis*
		Don	Bowen *coastal*	BW1*	Port Dennison	148.250	-20.017	*Zostera / H. uninervis with H. ovalis*
				BW2*	Port Dennison	148.252	-20.017	
	Mackay-Whitsunday	Don	Shoal Bay *reef*	HB1*	Hydeaway Bay	148.482	-20.075	*H. uninervis / C. rotundata / T. hemprichii with H. ovalis*
				HB2*	Hydeaway Bay	148.481	-20.072	
		Proserpine	Pioneer Bay *coastal*	PI2*	Pioneer Bay	148.693	-20.269	*Zostera / H. uninervis with H. ovalis*
				PI3*	Pioneer Bay	148.698	-20.271	
		Proserpine / O'Connell	Whitsunday Island *reef*	TO1^	Tongue Bay	149.016	-20.240	*H. uninervis / T. hemprichii with H. ovalis*
				TO2^	Tongue Bay	149.012	-20.242	
			St Helens Bay *coastal*	SH1*	St Helens Bch	148.835	-20.822	*H. uninervis / Zostera with H. ovalis*
			Newry Islands *coastal*	NB1^	Newry Bay	148.926	-20.868	*H. uninervis with H. ovalis / H. spinulosa*
				NB2^	Newry Bay	148.924	-20.872	
		Plane	Clairview *coastal*	CV1*	Clairview	149.533	-22.104	*H. uninervis / Zostera with H. ovalis*
				CV2*	Clairview	149.535	-22.108	

At each site, during each survey, observers record the per cent seagrass cover within a 50 cm × 50 cm quadrat every five metres along three 50 metre transects, placed 25 metres apart. A total of 33 quadrats are sampled per site. Seagrass abundance is visually estimated as the fraction of the seabed (substrate) obscured by the seagrass species leaves (living) when submerged and viewed from above. This method is used because the technique has wider application and is very quick, requiring only minutes at each quadrat; yet it is robust and highly repeatable, thereby minimising among-observer differences. Quadrat per cent cover measurements have also been found to be far more efficient in detecting differences in seagrass abundance than seagrass blade counts or measures of above- or below-ground biomass. To improve resolution and allow greater differentiation at very low percentage covers (e.g. < three per cent), shoot counts based on global species density maxima are used. For example: 1 pair of *Halophila ovalis* leaves in a quadrat = 0.1%; 1 shoot/ramet of *Zostera* in a quadrat = 0.2%. Extra information is collected at the quadrat level, including seagrass canopy height of the dominant strap leaved species; macrofaunal abundance; abundance of burrows, as a measure of bioturbation; presence of herbivory (for example dugong and sea turtle); a visual/tactile assessment of sediment composition (McKenzie 2007) and observations on the presence of superficial sediment structures such as ripples and sand waves to provide evidence of physical processes in the area (Koch 2001).

Monitoring at an extra 26 sites is conducted during the late-dry season by trained Seagrass-Watch observers (scientists assisted by community volunteers) or QPWS rangers who have demonstrated competency in the methods (see Sub-section 6.2.3; Table 6-2). Seagrass-Watch observers monitor sites using standard protocols; however, QPWS rangers use drop-cameras to collect basic site data and field imagery that is submitted to JCU scientists for post-field assessments. Digital video footage is examined post-field and seagrass abundance is visually estimated as the fraction of the seabed (substrate) obscured by the seagrass species for each clearly visible drop to the sea bed. Due to the turbid/low light conditions in the field, some post-processing may be necessary to enhance image features and improve assessments. Species visible in imagery are verified from van Veen grab samples.

Seagrass reproductive health

An assessment of seagrass reproductive health at locations identified in Table 6-1 via flower production and seed bank monitoring is conducted in late-dry season (October) of each year at each site. Extra collections are also conducted in late-monsoon (April) where possible to assist identification of peak flowering period.

In the field, 15 haphazardly placed cores (100 mm diameter × 100 mm depth) of seagrass are collected from an area adjacent, of similar cover and species composition, to each monitoring site. All samples collected are given a unique sample code/identifier providing a custodial trail from the field sample to the analytical outcome.

Seeds banks and the abundance of germinated seeds are sampled according to standard methods by sieving (1 mm mesh) 30 cores (50 mm diameter, 100 mm depth) of sediment collected across each site and counting the seeds retained in each (McKenzie *et al.* 2000). For *Zostera muelleri* subsp*. capricorni*, where the seeds are <1 mm diameter, intact cores (18) are collected and returned to the laboratory where they are washed through a 710 µm sieve and seeds identified using a hand lens/microscope.

Seagrass tissue nutrients

The collection of seagrass leaf tissue (targeted foundation genus include *Halodule*, *Zostera* and *Cymodocea*) for the analysis of tissue nutrients (C, N, P, δ^{15}N, δ^{13}C) is conducted during the late-dry season (October) sampling period at regions identified in Table 6-1. Approximately five–10 g wet weight of seagrass leaves is harvested from three to six haphazardly chosen plots (two to three metres apart) in an area adjacent, of similar cover and species composition, to each monitoring site. All samples collected are given a unique sample code/identifier providing a custodial trail from the field sample to the analytical outcome.

6.2.3   Observer training

The JCU personnel collecting data in association with this project are without exception highly experienced in the collection of seagrass monitoring data. The majority of observers have been involved in seagrass monitoring for at least a decade and were employed specifically for their skills associated with the tasks required.

All observers have successfully completed a Level 1 Seagrass-Watch training course (seagrasswatch.org/training.html) and have demonstrated competency across nine core units, achieved 80 per cent of formal assessment (classroom and laboratory) (5 units) and demonstrated competency in the field both during the workshop (1 unit) and post workshop (3 units = successful completion of 3 monitoring events/periods within 12 months). Volunteers who assist JCU scientists have also successfully completed a Level 1 training course.

Technical issues concerning QC of data are important and are resolved by using standard methods that make sure of completeness in the field (the comparison between the amounts of valid or useable data originally planned to collect versus how much were collected); using standard seagrass cover calibration sheets to guarantee precision (the degree of agreement among repeated measurements of the same characteristic at the same place and the same time) and consistency between observers and across sites at monitoring times. Ongoing standardisation of observers is achieved through routine comparisons during sampling events. Any discrepancy is used to identify and subsequently mitigate bias. For the most part, however, uncertainties in per cent cover or species identification are mitigated in the field via direct communication or by the collection of voucher specimens (to be checked under a microscope and pressed in the herbarium) and the use of a digital camera to record images (protocol requires that all quadrats are photographed) for later identification and discussion. Evidence of competency is securely filed on a secure server at JCU Cairns campus.

6.2.4   Laboratory analysis — Inshore seagrass meadow abundance, community structure and reproductive health

Seagrass reproductive health

In the laboratory, reproductive structures (spathes, fruits, female flowers or male flowers; Figure 6-2) of plants from each core are identified and counted for each sample and species. If *Halodule uninervis* seeds (brown green colour) are still attached to the rhizome, they are counted as fruits. Seed estimates are not recorded for *Halophila ovalis* due to time constraints (if time is available post this first pass of the samples, fruits are dissected and seeds counted).

For *Zostera muelleri* subsp*. capricorni*, the number of spathes is recorded, and male and female flowers and seeds are counted during dissection if there is time after the initial pass of the samples. Apical meristems are counted if possible. The number of nodes for each species is counted, and for each species present in the sample, 10 random internode lengths and 10 random leaf widths are measured. Approximately five per cent of samples are cross-calibrated between technicians. All samples, including flowers and spathes and fruits/fruiting bodies are kept and re-frozen in the site bags for approximately two years for revalidation if required. Reproductive effort is calculated as the number of reproductive structures per core.
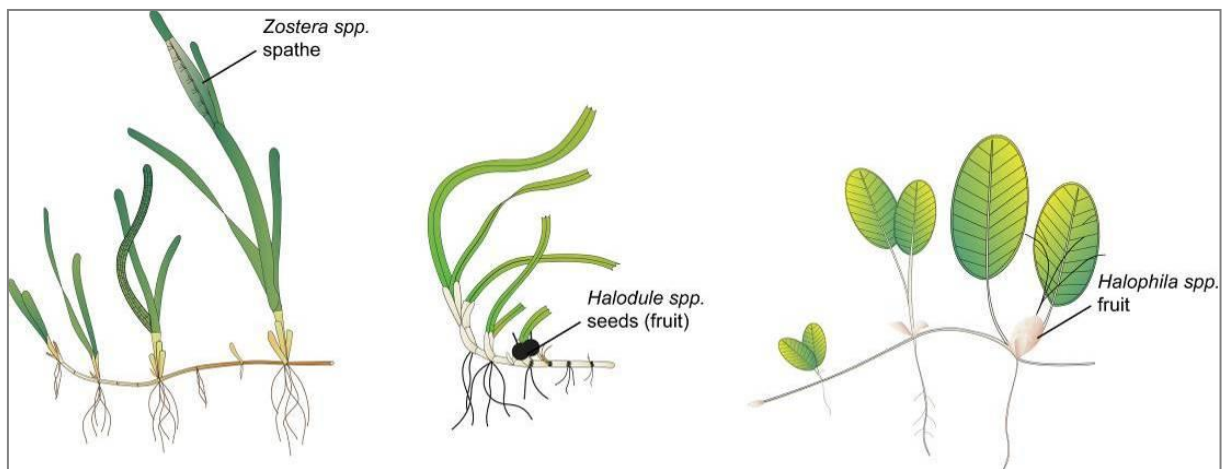
Figure 6-2 Form and size of reproductive structure of the seagrasses collected: *Halophila ovalis, Halodule uninervis* and *Zostera muelleri* subsp. *capricorni*

Seagrass tissue nutrients

Leaves are separated in the laboratory into seagrass species and epiphytic algae is removed by gently scraping the leaf surface. Samples are oven dried at 60°C to weight constancy. Dried biomass samples of leaves are then homogenised by milling to fine powders before nutrient analyses and stored in sealed vials.

The ground tissue samples are sent to ChemCentre (Western Australia) for analysis. Chemcentre holds NATA accreditation for constituents of the environment including soil, sediments, waters and wastewaters (details of Chemcentre accreditation can be found at the NATA website: http://www.nata.asn.au/). NATA accreditation held by the ChemCentre includes a wide variety of QA/QC procedures covering the registration and identification of samples with unique codes and the regular calibration of all quantitative laboratory equipment required for the analysis. ChemCentre has developed appropriate analytical techniques including QA/QC procedures and detection of nutrients. These procedures include blanks, duplicates where practical and internal use of standards. In 2010, QA/QC also included an inter-laboratory comparison (using Queensland Health and Scientific Services – also a NATA accredited laboratory) and an extra blind internal comparison.

Nitrogen and phosphorus are extracted using a standardised selenium Kjeldahl digest and the concentrations determined with an automatic analyser using standard techniques at ChemCentre in Western Australia. Percentage of C is determined using atomic absorption, also

at ChemCentre. Elemental ratios (C:N:P) are then calculated on a mole:mole basis using atomic weights (i.e., C = 12, N = 14, P = 31). Analysis of all seagrass tissue nutrient data is based on the calculation of the atomic ratios of C:N:P.

To determine per cent carbon, dried and milled seagrass leaf tissue material is combusted at 1400°C in a controlled atmosphere (for example a LECO analyser). This converts all carbon containing compounds to carbon dioxide. Water and oxygen is then removed from the system and the gaseous product is determined spectrophotometrically.

Samples are digested in a mixture of sulphuric acid, potassium sulfate and a copper sulfate catalyst (cf. Kjeldahl). This converts all forms of nitrogen to the ammonium form and all forms of phosphorus to the orthophosphate form. The digest is diluted and any potentially interfering metals present are complexed with citrate and tartrate. For the nitrogen determination, an aliquot is taken and the ammonium ions are determined colourimetrically following reduction with hydrazine to the nitrate ion, followed by diazotisation of 1-naphthylenediamine and subsequent coupling with sulphanilamide. For TP, an aliquot of the digest solution is diluted and P is determined as the phosphomolybdenum blue complex (modified from (Murphy and Riley 1962)).

Seagrass leaf isotopes

A subset of each ground tissue sample is sent to UC Davis Stable Isotope Facility (California, USA) for $\delta^{15}N$ and $\delta^{13}C$ analysis. The samples are weighed into tin capsules and combusted by an elemental analyser (ANCA-SL, SerCon Limited, Crewe, United Kingdom) to N_2 and CO_2. The N_2 and CO_2 are purified by gas chromatography and the nitrogen and carbon elemental composition and isotope ratios are determined by continuous flow isotope ratio mass spectrometry (20-22 IRMS, SerCon Limited). Reference materials of know elemental composition and isotopic ratios are interspaced with the samples for calibration.

Raw nitrogen and carbon elemental composition and isotope ratio data are corrected for instrument drift and blank contribution using Callisto software (SerCon Limited). A standard is analysed at variable weights to correct for instrument linearity, for example IAEA-N-2 and IAEA-N-1 are used to normalise the nitrogen isotope ratio, and IAEA-CH-6 and IAEA-CH-7 normalise the carbon isotope ratio, such that IAEA-N-2 ($\delta^{15}N$ = 20.32‰), IAEA-N-1 ($\delta^{15}N$ = 0.43‰), IAEA-CH-6 ($\delta^{13}C$ = -10.45‰) and IAEA-CH-7 $\delta^{13}C$ = -32.15‰).

Nitrogen isotope ratios are reported in parts per thousand (per mil) relative to N_2 in air. The nitrogen bearing internationally distributed isotope reference material N_2 in air has a given value of 0‰ (exactly). Carbon isotope ratios are reported in parts per thousand (per millilitre) relative to V-PDB. The carbon bearing internationally distributed isotope reference materials NBS19 and L-SVEC has a given value of +1.95‰ (exactly) and -46.6‰ (exactly), respectively. Compositional values are reported as percent nitrogen and percent carbon present in the sample analysed.

6.2.5   Sampling design – Inshore seagrass meadow landscape/boundary mapping

Mapping the edge of the seagrass meadow within each monitoring site (i.e. 5.5 hectares) is conducted in both the late-dry (October) and late-monsoon (April) monitoring periods at all sites

identified in Table 6-1. Training and equipment (GPS) are provided to personnel involved in the edge mapping.

Mapping methodology follows standard methodology (weblink https://www.seagrasswatch.org/seagrass-monitoring/) (McKenzie *et al.* 2001). Meadow, patch or scar edges are recorded as tracks (one second polling) or a series of waypoints in the field using a portable GPS receiver (i.e. Garmin GPSmap® 60CSx or 62s). Accuracy in the field is dependent on the portable GPS receiver (Garmin GPSmap® 60CSx is <15 metres RMS95 per cent (DGPS (USCG) accuracy: three–five metres, 95 per cent typical) and how well the edge of the meadow is defined. Generally, accuracy is within that of the GPS (i.e. three–five metres) and datum used is WGS84. Tracks and waypoints are downloaded from the GPS to a portable computer using MapSource or BaseCamp software as soon as practicable (preferably on returning from the day's activity) and exported as *.dxf files to ESRI® ArcGIS™.

Field mapping procedures at subtidal sites are altered to suit the low visibility conditions and the requirement to map by drop-camera. From the central picket (deployment location of light and turbidity loggers) straight lines of approximately 50 m lengths are traversed at an angle of 45 degrees from each other. The drop-camera and frame are a sequentially lowered and raised to and from the seabed as the boat traverses at drifting speed. As a consequence, the frame "hops" along the seabed, and the camera records images in time-lapse mode every 2 seconds. A GPS on the vessel records the track, which polls every 1 second. Prior to deployment fot he camera and frame, the camera records an image of the GPS display, to record the timestamp: this enables synchronisation of the images with the GPS to geotag each image. Eight lines at approximately 45 degrees are performed, with the first following the orientation of the monitoring transects; the others are undertaken at 45 degree angles from the first.

Mapping is conducted by trained and experienced scientists using ESRI® ArcMap™ 10.4.1 (Environmental Systems Research Institute, ArcGIS™ Desktop 10.4.1). Boundaries of meadows/patches are determined based on the positions of the geotagged photos and the presence of seagrass. Edges are mapped using the polyline feature to create a polyline (i.e. 'join the dots'), which is then smoothed using the B-spline algorithm. The smoothed polyline is then converted to a polygon and saved as a shapefile. The coordinate system (map datum) used for projecting the shapefile is AGD94.

In certain cases, seagrass meadows form very distinct edges that remain consistent over many growing seasons. However, in other cases the seagrass landscape tends to grade from dense continuous cover to no cover over a continuum that includes small patches and shoots of decreasing density. Boundary edges in patchy meadows are vulnerable to interpreter variation, however, the general rule is that a boundary edge is determined where there is a gap with the distance of more than three metres (i.e. accuracy of the GPS). Final shapefiles are overlayed with aerial photographs and base maps (AusLig™) to assist with illustration/presentation.

The expected accuracy of the map product gives some level of confidence in using the data. Using GIS, meadow boundaries are assigned a quality value based on the type and range of mapping information available for each site and determined by the distance between waypoints and GPS position fixing error. These meadow boundary errors are used to estimate the likely range of area for each meadow mapped (Lee Long *et al.* 1997).

6.2.6 Sampling design — Within seagrass canopy temperature loggers

Autonomous iBTag™ submersible temperature loggers are deployed at all sites identified in Table 6-1. The loggers record temperature (degrees Celsius) within the seagrass canopy every 30 to 90 minutes (depending on the duration of deployment and logger storage capacity) and store data in an inbuilt memory, which is downloaded every three to six months, depending on the site.

The iBCod 22L model of iBTag™ loggers is used as it can withstand prolonged immersion in salt water to a depth of 600 metres. It is reinforced with solid titanium plates and over molded in a tough polyurethane casing that can take a lot of rough handling.

The main features of the iBCod 22L include:

- operating temperature range: -40 to +85°C
- resolution of readings: 0.5°C or 0.0625°C
- accuracy: ±0.5°C from -10°C to +65°C
- sampling Rate: 1 second to 273 hours
- number of readings: 4096 or 8192 depending on configuration
- password protection, with separate passwords for read only and full access.

The large capacity of this logger allows the collection of 171 days of readings at 30 minute intervals.

The iBCod 22 litre submersible temperature loggers are placed at a permanent marker at each site for three to six months (depending on monitoring frequency). Loggers are attached to the permanent station marker using cable ties, above the sediment-water interface. This location makes sure that the sensors are not exposed to air unless the seagrass meadow is completely drained and places them out of sight of curious people.

Each logger has a unique serial number that is recorded within a central secure database. The logger number is recorded on the monitoring site datasheet with the time of deployment and collection. At each monitoring event (every three to six months), the iBTag™ temperature loggers are removed and replaced with a fresh logger (these are dispatched close to the monitoring visit). After collection, details of the logger number, field datasheet (with date and time) and logger are returned for downloading.

Logger deployment and data retrieval are carried out by JCU professional and technical personnel who have been trained in the applied methods. Methods and procedures documents are available to relevant staff and are collectively kept up-to-date. Changes to procedures are developed and discussed and recorded in metadata records.

6.2.7 Sampling design and logistics — Seagrass meadow canopy light loggers

Autonomous light loggers are deployed at selected nearshore and offshore seagrass sites in all regions monitored (Table 6-3).

Submersible Odyssey™ photosynthetic irradiance loggers are placed at the permanent marker at each of the sites for three to six month periods (depending on monitoring frequency).

Odyssey™ data loggers (Odyssey, Christchurch, New Zealand) record photosynthetically active radiation (400–1100 nm) and store data in an inbuilt memory, which is retrieved every three to six months, depending on the site. Each logger has the following technical specifications:

- cosine corrected photosynthetic irradiance sensor 400–700 nm
- cosine corrected solar irradiance sensor 400–1100 nm
- integrated count output recorded by Odyssey data recorder
- user defined integration period
- submersible to 20-metre water depth
- 64 k memory.

Table 6-3 Monitoring sites selected for light logger data collection

Region	Basin	Water body	Site	Latitude		Longitude	
Cape York	Jacky Jacky / Olive-Pascoe	Enclosed Coastal intertidal	Shelburne Bay	11°	53.251	142°	54.938
		Midshelf intertidal	Piper Reef	12°	15.352	143°	14.020
	Normanby / Jeannie	Open coastal intertidal	Stanley Island	14°	8.576	144°	14.680
			Bathurst Bay	14°	16.062	144°	13.896
North	Daintree	Midshelf intertidal & subtidal	Low Isles	16°	23.11	145°	33.88
	Mossman / Barron / Mulgrave-Russell / Johnstone	Midshelf intertidal & subtidal	Green Island	16°	45.789	145°	58.31
		Enclosed Coastal intertidal	Yule Point	16°	34.159	145°	30.744
	Tully / Murray / Herbert	Open coastal intertidal & subtidal	Dunk Island	17°	56.75	146°	08.45
Central	Ross / Burdekin	Open coastal intertidal & subtidal	Picnic Bay	19°	10.734	146°	50.468
			Cockle Bay	19°	10.612	146°	49.737
		Enclosed coastal intertidal	Bushland Beach	19°	11.028	146°	40.951
			Barratta Creek	19°	25.380	147°	14.480
	Proserpine / O'Connell	Open coastal intertidal & subtidal	Hamilton Island	20°	20.802	148°	58.246
			Lindeman Island	20°	26.293	149°	1.691
		Enclosed coastal intertidal	Midge Point	20°	38.099	148°	42.108
	Plane	Enclosed coastal intertidal	Sarina Inlet	21°	23.76	149°	18.2
Southern	Shoalwater / Fitzroy	Open coastal intertidal	Great Keppel Island	23°	11.7834	150°	56.3682

Region	Basin	Water body	Site	Latitude		Longitude	
		Macro tidal Enclosed coastal intertidal	Shoalwater Bay	22°	23.926	150°	16.366
	Calliope / Boyne	Enclosed coastal intertidal	Gladstone Hbr	23°	46.005	151°	18.052
	Baffle	Enclosed coastal intertidal	Rodds Bay	24°	4.866	151°	39.7584
	Burrum	Enclosed coastal intertidal	Burrum Heads	25°	11.290	152°	37.532
	Mary	Enclosed coastal intertidal	Urangan	25°	18.197	152°	54.364

The logger is self-contained in a pressure-housing with batteries providing sufficient power for deployments of longer than six months. For field deployment, loggers are attached to a permanent station marker using cable ties; this is above the sediment-water interface at the bottom of the seagrass canopy. Similar to the submersible temperature loggers, this location makes sure that the sensors are not exposed to air unless the seagrass meadow is almost completely drained and places them out of sight of curious people. At subtidal sites, the loggers are deployed on the sediment surface (attached to a permanent marker) with the sensor at seagrass canopy height. Two loggers are deployed at subtidal sites as there is an increased chance of logger fouling, and the dual logger set-up offers a redundant dataset in the instance that one logger fouls completely. Where possible, extra light loggers are deployed at subtidal sites 80 centimetres from the sediment surface. Data from this logger, together with data from the logger at canopy height, is used for calculation of the light attenuation co-efficient. Furthermore, another logger is deployed above the water surface at each of the subtidal monitoring stations. These extra loggers (surface and subtidal higher in the water column) allow comparison of water quality indices for some of the time.

Measurements are recorded by the logger every 30 minutes (this is a cumulative 30 minute reading). Experiments utilising loggers with and without wipers have been conducted to determine the benefits of wiper use and it was confirmed that the wipers improved the quality of the data by keeping the sensor free from fouling. Automatic wiper brushes are attached to each logger to clean the optical surface of the sensor every 15 minutes to prevent marine organisms fouling the sensor or sediment settling on the sensor, both of which diminish the light reading.

Each light logger has a unique serial number that is recorded within a central secure database. The logger number is recorded on the monitoring site datasheet with the time of deployment and collection. At each monitoring event (every three to six months) the light loggers are removed and replaced with a 'fresh' logger. At subtidal monitoring sites, the loggers are checked by scuba by JCU personnel (and replaced if fouled) every three months due to the increased fouling rates at permanently submerged sites. After collection, details of the logger number, field datasheet (with date and time) and logger are returned to JCU for downloading.

Photographs of the light sensor and/or notes on the condition of the sensor are recorded at logger collection. If fouling is major (e.g. wiper failure), data are truncated to included only that data before fouling began — usually one to two weeks. If fouling was minor (up to ~25 per cent of the sensor covered), back corrections to the data are made to allow for a linear rate of fouling (linear because with minor fouling it is assumed that the wiper was retarding algal growth rates, but not fully inhibiting them).

6.2.8   Calibration procedures — Seagrass meadow canopy light loggers

Loggers are calibrated against a certified reference photosynthetically active radiation sensor (Li-Cor™ Li-192SB Underwater Quantum Sensor) against a Li-Cor light source under controlled laboratory conditions.

The Li-192SB sensor is cosine corrected and specifications are:

- absolute calibration: ± 5 per cent in air
- relative error: <± 5 per cent under most conditions
- sensitivity: typically 3 µA per 1000 µE s^{-1} m^{-2} in water.

The reference light sensor is calibrated before deployment by JCU. The calibration of each logger is recorded within metadata and corresponds to the serial numbers attached to each logger. The calibration is performed in air and a 1.33 conversion factor is applied to the data to allow for the difference in light transmission to the sensor between air and water (Kirk 1994). This factor is not applied when the sensor is immersed at low tide and emersion is estimated from actual sea level data provided by Maritime Safety Queensland.

Logger deployment and data retrieval is carried out by scientific personnel who have been trained in the applied methods. Methods and procedures documents are available to relevant staff and are collectively kept up to date. Changes to procedures are developed and discussed and recorded in metadata records.

6.3   Data management

6.3.1   Inshore seagrass meadow abundance, community structure and reproductive health

TropWATER (JCU) has systems in place to manage the way MMP data is collected, organised, documented, evaluated and secured. All data is collected and collated in a standard format. Seagrass-Watch HQ has implemented a QA management system to make sure that the data collected are organised and stored and able to be easily used.

All data (datasheets and photographs) received are entered into a relational database on a secure server at JCU, Cairns campus. Receipt of all original data hardcopies is documented and filed within the Seagrass-Watch HQ File Management System, a formally organised and secure system. The database is routinely backed up (in multiple places). Seagrass-Watch HQ operates as custodian of data collected and provides an evaluation and analysis of the data for reporting purposes. Access to the IT system and databases is restricted to only authorised personnel.

Seagrass-Watch HQ performs a quality check on the data. Seagrass-Watch HQ provides validation of data and attempts to correct incidental/understandable errors where possible (for example blanks are entered as -1 or if monospecific meadow percentage composition = 100 per cent) (https://www.seagrasswatch.org/seagrass-monitoring/). Validation is provided by checking observations against photographic records to make sure of the consistency of observers and by identification of voucher specimens submitted.

Keeping to QA/QC protocols, Seagrass-Watch HQ informs observers via an official data error notification of any errors encountered/identified and provides an opportunity for correction/clarification (this may include extra training). Any data which does not pass QA/QC is quarantined or removed from the database.

6.3.2   Inshore seagrass meadow boundary mapping

After field collection, data points are downloaded from the GPS into computer memory and the data exported to ESRI® ArcGIS™. An administration file (*.gdb) is generated by the MapSource or BaseCamp software that contains metadata information about the tracks, waypoints, dates and times of the measurements, as well as general comments. Data and metadata are stored on the TropWATER (JCU, Cairns campus) secure server.

6.3.3   Within seagrass canopy temperature loggers

After retrieval, data are downloaded into computer memory and the data are displayed as graphs to allow visual identification of outliers. These outliers are then tagged and removed from the datasets (for example a temperature spike below -10°C or above 65°C). Other data adjustments include the removal of data points from the beginning and end of the data series, for example when the logger was not attached to the permanent peg. An administration file is generated by the logger software that contains metadata information about the deployment site, dates and times of the start and stop of measurements, as well as general comments. Data and metadata are stored in a temporary Microsoft® Access database.

Loggers are then launched for the next deployment. All data are transferred into the existing TropWATER (JCU) database.

6.3.4   Seagrass meadow canopy light loggers

After retrieval, data are downloaded into computer memory and the data are displayed as graphs to allow visual identification of outliers. These outliers are then tagged and removed from the datasets; however, such outliers have mostly not been present. During the placement and retrieval of the logger, the site or logger may suffer a short disturbance from the technician; adjustments are made to the data to remove a small number of data points from the beginning and end of the data series to account for this.

An administration file is generated by the logger software that contains metadata information about the deployment site, dates and times of the start and stop of measurements, as well as general comments. Data and metadata are stored in a temporary Microsoft® Access database.

Loggers are then launched for the next deployment. All data are transferred into the existing TropWater (JCU) database.

JCU is also working on assigning values to the level of confidence in the data. For example, sometimes corrections are made to light data to account for minor fouling. We would like to add a code to the data that indicates that we have reduced confidence in it because we have made adjustments.

6.4   Summary of Quality Control measures

6.4.1   Inshore seagrass meadow abundance, community structure and reproductive health

- Training of field staff
- Sampling guidelines
- Document control
- Analytical QC measures
- QC of data entry

6.4.2   Inshore seagrass meadow boundary mapping

- Training of deployment and retrieval staff
- Data download control
- Training of staff using ESRI® ArcGIS™ Desktop 10.7 software

6.4.3   Within seagrass canopy temperature loggers

- Training of deployment and retrieval staff
- Use of serial numbers to provide unique identification to individual loggers
- Data download control
- QC of data entry

6.4.4   Seagrass meadow canopy light loggers

- Use of serial numbers to provide unique identification to individual loggers
- Training of deployment and retrieval staff
- Calibration of loggers with certified reference light sensor
- QC of data entry

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Appendix A Detailed AIMS Manuals and Standard Operating Procedures

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Section A1: Operating instructions for the AIMS Sea-Bird CTD

1.1   Objective

To conduct vertical measurements of water temperature, salinity and other parameters throughout the water column to obtain depth profiles of water quality characteristics with a Conductivity Temperature Depth profiler (CTD) (Sea-Bird Electronics SBE19plus).

1.2   Materials and equipment

As currently configured, the AIMS SBE19plus' are set up to measure: Pressure (depth), temperature, conductivity (salinity), PAR (either downwelling [2π] or scalar [4π]), chlorophyll fluorescence (*in situ* fluorometer for chlorophyll *a*, WET Labs), turbidity (beam transmissometer, Sea Tech, 25cm, 660nm) and either oxygen or optical backscatter.

The Sea-Bird SBE19 CTD profiler is operated through two pieces of software, which may be downloaded from www.seabird.com:

- **SeaTerm V2** – a terminal program which communicates with the CTD and controls many CTD functions, and
- **SeaSave V7** – a plotting program which displays the data.

1.3   Operating the CTD

Before you start a cast:

1. Connect the CTD to the computer: The CTD communications cable has a DB9 serial connector at the computer end.
2. At the CTD end both the CTD communications cable (4 conductor) and the bulkhead connector plug on the CTD have watertight rubber dummy plugs. Remove them and plug the communications cable into the CTD plug. Check to see that the 4 connector pins (CTD side) and the corresponding socket holes (cable side) are clean. When making a connection, **make sure the thickest pin of the CTD plug aligns with the larger hole on the CTD comms cable socket**. The bump on the outside of the comms cable socket identifies the location of the larger socket hole. When you plug the cables together, **don't force or twist the connection**. They should push together with a little 'pop'.
3. Check to see that the magnetic slider switch on the side of the CTD is in the **OFF** position (**down**). This puts the CTD in the sleep mode.

Communicating with the CTD:

1. Start up the computer, launch SeaTerm V2 and connect the CTD cable.
2. Click the **Instruments** menu on the SeatermV2 window and select **C. SBE 19plus V2** from the drop-down list. SeaTerm automatically connects to the instrument if the **Options** tab has **Connect at startup** enabled. The program may cycle through several baud rates if not

already configured to 9600 under **Configure** in the **Communications** menu. Once connected, the CTD prompt will appear in the main window as **S>** or **<Executed/>**. The top status bar displays Serial Port - COM1, Baud Rate 9600.Click the **CONNECT** button on the SeaTerm window. You should get a little window with a windows moving bar that says you are trying to connect at 9600 baud.

3. In the **Send Commands** window, click **Status** and select the **Display status and configuration parameters** command, then press the **Execute** button. Alternately type **ds** (for display status) in the main display window. The information displayed includes battery voltage, check to see that **vbatt > 12** volts. With new batteries, it should read > 13.5 volts.

Performing a CTD cast:

1. Connect to the CTD as above, and check the battery voltage is >12 volts via SeaTerm V2.

2. To clear the CTD memory prior to the cast, select **General Setup** from the **Send Commands** menu of SeaTerm V2 and select **Initialize Logging**. The **Execute** button must be clicked twice to take effect.

3. Check the depth under the keel, and manoeuvre the ship so it is facing upwind and the CTD side faces the sun.

4. Connect the CTD to the hydrographic wire.

5. Unplug the communications cable and install the dummy plug and lock collar on the CTD connector.

6. Slide the magnetic switch on the side of the CTD to the **ON** position (**up**).

7. Steady the CTD by hand to stop it swinging against the boat. Lower the CTD into the water with the CTD winch so the ring at the top of the cage is just at the sea surface and the PAR sensor is just under the surface. *Some bubbles will be expelled from the tubing at the top of the CTD.*

8. Whilst keeping the CTD at the top of the profile, wait **3 minutes** for the CTD to start pumping and purge the air and water out of the salinity cell, and to equilibrate the temperature of the sensors. (*The CTD has an internal pump which pumps water through the conductivity cell at a constant rate for high accuracy salinity measurements. The pump starts when the conductivity circuitry determines it is in salt water. It needs at least 30 seconds thereafter to purge the lines.*).

9. After 3 minutes, lower the CTD to within 2-3 m of the bottom at no more than 1 m per sec. Slightly slower is preferred. *[The SBE19 samples @ 4 Hz, so a slower speed allows you to average more readings per 1 metre depth bin in the later processing step]*

10. Reel in the CTD at the same speed and retrieve to the deck.

11. Switch the CTD slider switch **OFF** (**down**) and secure the instrument.

12. Pull the dummy plug off the CTD bulkhead connector, and re-connect the communications cable.

1.4   Data retrieval and management

Retrieving the data:

1. Electronically capture the position of the CTD cast using the Access Field Data Entry System database, or record it on the field data sheets.

2. Re-establish communications with the CTD using SeaTerm V2, once the cable is connected. You should get the **S>** prompt, but if SeaTerm display window indicates 'time out' you may need to select the **Instruments** menu and click on **Disconnect and reconnect**. *If the display starts scrolling out lines of HEX characters, you've forgotten to switch the CTD off.*

3. Click the **Upload…** menu on SeaTerm V2. *The screen will display a header for the CTD cast, giving a range of metadata for the cast which is saved at the top of the uploaded file. Note the text specifying the number of samples to confirm data has been captured.* A **Save As** dialog box will open, then navigate to *C:\Field\CTD files* folder to store the file. Whilst the default file name includes the serial number and date, we type in the unique station identifier as the file name.

4. Navigate the window to the and type your desired output filename into the box. *The program will automatically append a .HEX delimiter to the filename. A small window ask which cast number you want to download.* Type in "1" to download the first cast in memory. *You should then get a progress window with a moving bar that counts the bytes transferred.*

5. When it's complete, the CTD file is now saved on the computer. Use Windows Explorer if you want to check that the datafile is stored in the correct location.

Plotting the Data

1. Launch SeaSave V7 on the computer. Normally for CTD plots, the vertical scale is depth (m).
2. Click the ArchiveData tab and then Start opens an input window that lets you select a file to plot. Navigate to your datafile using the Select Data File button in the usual Windows way and click on it to select it.
3. Click on the button at the bottom of the input box (Start Display) to plot your data using the default plot parameters stored in the computer.
4. To change the plot parameters, use the ScreenDisplay tab to select "Edit Selected Display Window". This will allow you to load another plot parameter file (.dso) that might be more appropriate to your data set, to directly modify the plot you already have or save the plot parameters for future use. There are a number of .dso files on the pathway in the box. The plotname usually gives the depth range.
5. If you select to Modify Display Parameters, you will get a menu to change the output style. The Y axis is normally depth on the CTD computer. The Select Variable button puts you in a small window that lets you select the variable (from a particular instrument type) that you want to plot.
6. Save the plot as a .dso file if you want to shut down the laptop and use it again later. After you click "OK" to close the above window, you will be in the previous dialog which lets you save the parameters in a .dso file. Exit that box and you will be looking at a changed, but blank plot.
7. To replot the data, select the ArchiveData tab and Start, check the input filename in the next dialog box and click the Start Display button again to see the plot. If you are not happy, go back to step 4 and try again.
8. Save all data on the hard drive and the back up external hard drive. Immediately upon return to AIMS transfer all data to the MMP shared file area on the AIMS Server.

1.5   Maintenance

After the CTD profiles are completed:

1. Disconnect the CTD from the communications cable and reattach the dummy plugs on both ends.
2. Wash the CTD thoroughly with fresh water.

1.6   Quality control

- When on board RV Cape Ferguson, CTD cast data are immediately plotted and checked so the cast can be repeated if there were erroneous readings or other technical problems.

- The CTD sensors are regularly sent to either the manufacturer or CSIRO Oceans and Atmosphere Oceanographic Calibration Facility for calibration (generally annually).

- Salinity and chlorophyll readings from CTD sensors are validated against results from direct water sampling using Niskin bottles conducted immediately after the CTD cast.

- Temperature readings from CTD sensors are validated against temperature readings from high quality reversing thermometers attached to the surface and bottom Niskin bottles.

Section A2: Measuring salinity using the Portasal Salinometer 8410A

AIMS-SOP-(EFWQ)-v1.0

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TASKS INCLUDED IN THIS SOP

This method outlines the operational and calibration procedures for measuring salinity using a Portasal Salinometer model 8410A.

PRINCIPLE AND RESPONSIBILITIES

Laboratory measurements of salinity with a Portasal Salinometer are based on a high-precision comparison of the conductivity of an unknown water sample with the conductivity of a well-characterised sample of sample of IAPSO (International Association of Physical Science Organisations) Standard Seawater (IAPSOSW). "The conductivity of seawater is proportional to the salinity. With the appropriate corrections for temperature and pressure, the measurement of conductivity has become the most generally used method of determining salinity. Electrical conductivity is a measure of total electrolyte concentration in seawater and it is a technique which can be performed rapidly and with great accuracy, both in laboratories and in situ. (Devlin and Lourey, 2000).

The reliability and accuracy of salinity analyses by conductivity are best if the unknown samples have a salinity that is reasonably close to that of the standard. While estuarine samples can readily be run, conductivity comparisons with very low salinity samples are progressively less reliable. In most cases, samples with salinities > 25-30 ‰ should be suitable. Low salinity "standards" can be made by precise dilution of standard seawater. In low salinity estuarine samples, the use of conductivity is confounded by the potentially differing ratios of ions in the "fresh" water which may have a different conductivity-mass relationship from seawater

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Manual handling	Biomechanical	Reduced combined weight of samples. Use proper lifting techniques. Use trolley to move
Electrical	Electrical shock	Protect electronic components from water leaks/spills and clean up any spills immediately. Disconnect instrument before conducting maintenance or servicing. Call an electrician if you are not sure you can solve the problem safely.

PERSONNEL PREREQUISITES & COMPETENCIES

Laboratory safety induction

Read and understood procedure

Training by experienced technician

TOOLS & EQUIPMENT

- 250 mL screw cap polyethylene bottles - acid washed, rinsed and air-dried
- Standard seawater IAPSO P-series from OSIL (IAPSOSW) at room temperature – 1x 250 mL bottle
- Surface sea water (SSW) from coral sea at room temperature as a secondary standard- 500 mL to 2 L
- Milli-Q Water at room temperature, approx. 500mL
- Kimwipes
- Drain bucket and salinometer drain tubing
- Guideline Portasal Salinometer

ENVIRONMENTAL CONSIDERATION

None as no reagents or preservatives are used during the measurement calibration or process.

CHEMICAL DISPOSAL

No chemicals are used the processing of samples.

REFERENCES

- Guildline Insruments (2001). Manual for Guildline Model 8410A Portable Salinometer.
- Devlin,M.J. and Lourey, M.J. (2000). Long term Monitoring of the Great Barrier Reef Standard Operational Procedure Number 6, Australian Institute of Marine Science.
- Guildline Instrument technical manual for model 8410A Portasal, TM841A-J-00, May 2006

PROCEDURE

1. SAMPLE COLLECTION AND STORAGE

- Water samples for laboratory salinity analysis are normally drawn from one to two Niskin bottles at each hydro station. The salinity sample is usually taken last from the Niskin bottle as gas exchange or contamination are not issues. Salinity samples should be taken from bottles tripped near the top and bottom of the water column to get the maximum range of salinities measured at a station.

- Salinity samples are stored in 250 mL screw-capped plastic bottles. Before a cruise, the bottles are soaked in RO water and air dried to remove old salt crystals. Before taking a sample from the Niskin Bottle, rinse the sample bottle and cap with sample water. Fill the bottle to near the top, leaving only a small air bubble (<20 mL) in the bottle to minimize evaporation. Put the cap on tightly. Store in a cool room (4-5°C), if possible, or at air-conditioned room temperature. Do not expose to full sunlight or excessive heat. Do not freeze.

- Surface sea water for secondary standards (SSW) is normally pumped into 40 L plastic drums at convenient times when an AIMS vessel is operating outside the reef. With the deck hose running, rinse out the drum thoroughly, then pump at least 30 L into the drum. Screw the lid on fully and store against the rail. At the lab, the seawater is stored in a walk-in cold room (5°C). Several days before use, some of the water should be transferred to a 5 L carboy on the lab bench next to the salinometer. The carboy is fitted with a siphon to remove water without bubbles. The

secondary water should be allowed to fully equilibrate to room temperature before use.

2. SALINOMETER SET UP AND CALIBRATION

a. Preliminary to using salinometer

- Bring the salinity samples out of the cold room at least the day before analysis and put them in the lab next to the salinometer to fully equilibrate to room temperature.

- The temperature of the bath water should be set to about 2ºC above ambient. See Appendix 1 to change bath temperature. Get a new bottle of IAPSO standard seawater (IAPSOSW) and a bottle of high-quality Milli-Q water and let both equilibrate next to the salinometer as well.

- Make sure you have enough (>2 L) secondary standard seawater (SSW) in the carboy. If you need more, put it in at least the night before to allow temperatures to equilibrate.

- Make sure the drain tubing is put in an empty bucket to collect waste water

- Before turning on power make sure the FUNCTION switch is in the STDBY or ZERO position, and the peristaltic pump speed selector is on 0.

- Before proceeding, please note that the external peristaltic pump is an after-factory modification and is not shown in the factory manual. To operate this pump requires its own power outlet and it has three speed setting. Normally the lowest speed will be sufficient. Always leave the FLOW RATE switch on the salinometer in the "on" position although its only function since the installation of the peristaltic pump is to keep the venting line open (Appendix 1).

- Samples up to 15ºC below the bath temperature or 5ºC above the bath temperature can be measured if the flow rate is reduced sufficiently to allow the sample to reach the bath temperature while in the heat exchanger, however this practice may not give the most accurate results obtainable.

b. Salinometer set up

- Power the salinometer and attached peristaltic pump by turning ON the 2 power outlet above the salinometer unit.

- Perform a flow rate check by:
  - Placing the intake pipe in the Milli-Q water bottle
  - Turning the FLOW RATE switch on salinometer
  - Ensuring the FUNCTION switch is set to STDBY

- Turning the peristaltic pump speed to 2

- Filling the conductivity cell, ensuring all four arms of the conductivity cell fill sufficiently to cover electrodes

- Covering the FLUSH vent momentarily with fingertip to allow the water to empty from the cell and then allow to refill

- Repeat this process 5 times

- Perform a conductivity cell check by:

  - Rocking the SSW bottle gently to eliminate gradients without creating air bubbles

  - Placing the intake tube in the SSW sample bottle

  - Turning the FLOW RATE switch on salinometer

  - Ensuring the FUNCTION switch is set to STDBY

  - Turning the peristaltic pump speed to 2

  - Filling and flushing the conductivity cell 2 times ensuring all four arms of the conductivity cell fill sufficiently to cover electrodes. Cover FLUSH vent momentarily with fingertip to allow the water to empty from the cell and then allow to refill

  - When cell has been filled for the third time, turn off the pump and while the cell is full, set FUNCTION switch to READ. Press COND key and observe a stable conductivity ratio measurement

  - Return the FUNCTION switch to STDBY

  - Turn the peristaltic pump speed to 0

  - Leave the conductivity cell filled with SSW for at least 30 minutes or until the temperature bath reaches the preferred temperature

  - Remove the sample bottle and wipe the pickup tube.

- This step ensures a reduction of observed salinity drift when the salinometer has not been used for a couple or days and the conductivity cell has been left dry, in Milli-Q, or has gone through a cleaning procedure.

- If air bubbles are adhering to the cell and are hard to dislodge by flushing, it may be necessary to clean the cell as per the instructions in Appendix 1.

- Each time the Portasal has been powered up or the bath temperature has been changed, allow 2 hours with the bath temperature regulating at 2 degrees above ambient before standardising or calibrating. A timer switch is available that can be utilised to turn the machine on outside of work hours so that it is ready for use at a predetermined time. If the temperature will not stabilise, do not use the machine and perform a temperature check as well as a bath temperature check (see Appendix 1)

c. Standardization

The Portasal is usually quite stable but to ensure quality control, a standardization routine should be performed in the following sequence every time the machine is powered up or the bath temperature is changed.

To perform the Reference Calibration and the Zero Calibration and then the Standardization and Drift Correction Routines the instrument needs to be powered up for a minimum of 3 hours:

i. Reference Calibration (with conductivity cell empty)

- Set the FUNCTION switch to STDBY and press REF key.
- After a delay of approximately 8 seconds, the display will read: -REFERENCE xxxxx and will update for 16 seconds. Next, the display will read +REFERENCE xxxxx and will update for 7 seconds, after which the display will read REFERENCE xxxxx for 8 seconds. This procedure will repeat itself until any key is pressed. The -REFERENCE and +REFERENCE numbers must stabilize to between 19750 and 19999. The -REFERENCE and +REFERENCE numbers must agree with each other to within ±2 counts after 10 cycles and must remain stable to within ±2 counts over 10 minutes.
- If they do not do so, please consult the machine's custodian as it may be malfunctioning.

ii. Zero Calibration (with conductivity cell filled with Milli-Q)

- Using Milli-Q, fill the cell and flush 2 times and refill cell as described in the Sample Measurement procedure (below) steps 1 through 6.
- Set the FUNCTION switch to ZERO and press COND key.
- When satisfied that the zero-conductivity ratio measurement is stable, press ZERO on the salinometer keypad. The display will then read ZERO x.xxxxx. The ZERO value should not exceed ±0.00075.
- When satisfied that this number is not drifting, press COND key on the keypad. The display should then read RATIO 0.00000.
- Turn the FUNCTION switch to STDBY
- Flush and refill the cell with Milli-Q
- Turn peristaltic pump to 0
- Turn the FUNCTION switch to read
- Press COND on keypad and confirm the value is 0± 0.00075
- Press SAL on keypad and confirm value is 0.000

- Turn the FUNCTION switch to STDBY
- Restart pump on speed 1 and flush the cell, wipe the pickup tube.

iii. Standardization Routine

Using a vial of Standard Sea Water (IAPSOSW) as the primary standard, fill and flush 2 times and fill the cell as described in the Sample Measurement procedure (below) steps 1 through 6.

- Turn the FUNCTION switch to READ and press STD on the salinometer keypad. (Please Note: Once the following procedure has commenced do not flush the cell and do not move the FUNCTION switch from READ)
- The following prompt will be displayed:
  - STD STANDARDIZE
  - Press ENTER key
- You will then be prompted with:
  - COND NO 0.99984 (example value only)
  - Enter the correct conductivity ratio as shown on the label of the IAPSOSW bottle
  - Press ENTER key
- You will then be prompted with:
  - BATCH NO P113 (example value only)
  - Enter the correct batch number as shown on the label of the IAPSOSW bottle
  - Press ENTER key
- You will then be prompted with:
  - ENTER WHEN READY
  - Ensure the conductivity cell is full and free of bubbles
  - press ENTER key
- "Measuring..." will briefly appear on the display followed by:
  - STANDARD 4.22000 (example value only)
  - When satisfied that the displayed number is stable, press COND key. This will terminate the standardisation operation and display the conductivity ratio using the new calibration values.
- Turn the FUNCTION switch to STDBY
- Flush and refill the cell
- Turn peristaltic pump to 0
- Turn the FUNCTION switch to read
- Press COND on keypad and record IAPSOSW conductivity value in the worksheet
- Press SAL on keypad and record IAPSOSW conductivity value in the worksheet
- Turn the FUNCTION switch to STDBY

- Remove the IAPSOSW bottle from holder
- Restart pump on speed 1 and flush the cell, wipe the pickup tube.

Note: Because a digital filter is applied to the measurement data during standardization, any significant change in STANDARD number will cause the new standardization to approach its final value over a period of several minutes. This apparent drift in standardization will be minimized by strictly adhering to the above procedure. Depending on the circumstances between standardizations, the change in STANDARD number may be up to ±0.0005 typical.

iv. Drift Correction Routine

- To minimise the use of expensive commercial primary standard (IAPSOSW), a secondary standard (SSW) is used. This is just clean seawater (preferably collected off the continental shelf to ensure maximum clarity). This is run directly after the SSW and every 20 samples thereafter. A quick reference guide to this procedure listing just the necessary keystrokes can be found in Appendix 2. A drift calculation is then applied to the sample readings assuming linear drift between the two substandard readings (see Appendix 3).

3. SAMPLE MEASUREMENTS

1) Rock sample bottle gently to eliminate gradients. Do not introduce air bubbles by shaking it.

2) Wipe salinometer pickup tube using a Kimwipe

3) Turn on the peristaltic pump on speed 1 so that a parcel of air is sucked into the pickup tube before opening the sample bottle and inserting the pickup tube. This will minimize the chance of backflow and contamination from the previous sample. Ensure that the pick-up tube reaches almost to bottom of bottle such that flow will not be restricted. Care must be exercised not to contaminate the sample. Do not handle the pickup tube except with clean lint-free tissue for wiping or surgical gloves for handling. Do not pump the sample through too fast or its temperature will not equilibrate with that of the water bath before reaching the cell and readings will be erratic.

4) Fill and then empty the cell by placing fingertip over FLUSH air vent. Be sure to completely fill the conductivity cell and that are no air bubbles are present.

5) Fill and flush again

6) Fill the cell again

7) Turn the FUNCTION switch to READ

8) The conductivity ratio measurement will be displayed. Wait for this number to stabilize and then record the ratio value on the sample worksheet. If the number displayed is slowly drifting, flush the cell and take a new reading.

9) To display the sample salinity in Practical Salinity Units, press **SAL** key. Allow the measurement to stabilize and record the salinity value on the sample worksheet.

10) Set the FUNCTION switch to STDBY, empty cell using FLUSH button and prepare to load the next sample (return to step 1).

⚠ When measuring IASOPSW, the pump selector can be set to 0 after the 3rd cell fill to save some of the standard water while measurement is being taken and data entered in the worksheet.

4. STANDBY OR SHUTTING DOWN:

• If another sample is not to be measured immediately, leave the pickup tube sample bottle in the sample bottle holder with the conductivity cell filled, set FUNCTION switch to STDBY, and turn off the flow rate.

• If another sample will not be measured for at least 12 hours, set FUNCTION switch to STDBY, remove the sample bottle, install a bottle of Milli-Q water, fill and flush the conductivity cell at least 3 times then, with the conductivity cell full, turn off the pump.

• If the machine is not to be used in the medium to long term, flush the cell with Milli-Q water 5 times and then empty it and store empty to avoid algal growth. Turn off the salinometer and peristaltic pump by turning the power outlet to off.

5. DATA MANAGEMENT:

The sample reading is logged with standards in the exact order indicated on the laboratory worksheet (see Appendix 3). All information from this lab sheet is then entered into a Microsoft Excel spreadsheet template called "Salinity-Portasal blank worksheet" which is a visual copy of the laboratory form but has all the calculation equations embedded (Appendix 4). It will automatically perform all salinity drift measurement calculation once the values are typed in.

6. QUALITY CONTROL

QA/QC tests undertaken as part of the data reporting include:

Assessment of accuracy of the analysis

This is generally achieved by using reference materials to assess recovery of known amount of analyte.

- The certified reference material available is IAPSO P-series Standard Seawater (www.osil.co.uk), which is included at regular intervals during the analysis.
- If during the analysis the conductivity from the IAPSOSW returns a value drifting by ±0.0005, the samples measurements are stopped and repeated after instrument re-calibration.

    a. <u>Assessment of precision of the analysis</u>

    NB: This is generally achieved by the repeated analyses of the same concentration of analyte to check for reproducibility.

- Repeated analysis of IAPSO (IAPSOSW) and SSW

APPENDICES

All appendixes must be referenced within the body of the SOP

#.1	Portasal operation and maintenance
#.2	Quick guide to standardization and sample measurement
#.3	Sample analysis worksheet
#.4	Salinity Portasal calculation sheet
#.	

RECORDS

All AIMS analyses undertaken on behalf of AIMS or a client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with [External Document and Data Control Policy](), [Data Access Policy]() and AIMS Disaster Recovery Plan, and must have an associated metadata record (see the Data Access Policy for full details).
- Raw experimental measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements).

- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A3: Automated analysis of dissolved nutrients in seawater

3.1 Objective

Analyses of concentrations of ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), silicate ($Si(OH)_4$), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) in water samples, with a focus on detecting very low concentrations of nutrients (< 0.1 µmol L^{-1}) with a high levels of precision.

3.2 Principle of analysis

Inorganic dissolved nutrient concentrations are determined by standard wet chemical methods (e.g. Parsons et al. 1984) with spectrophotometric detection based on Ryle et al. (1981) and Bran and Luebbe (1997), implemented on a Seal AA3 Analyser segmented flow analyser.

3.3 Sample analysis
3.3.1 Materials and Equipment

A nutrient analytical section is established at AIMS in a dedicated laboratory to undertake low-level nutrient analysis where all laboratory ware and equipment are used for that sole purpose to assist in avoiding contamination.

1. Continuous segmented flow autoanalyzer (SEAL AA3 Analyser and its accompanying software, (SEAL AACE Software Version 7.06).
2. Laboratory facilities and labware:
    - Lab air intakes in location likely to be minimally affected by atmospheric contaminants.
    - Sample storage in dedicated, clean, locked and alarmed freezers. The freezers are on power circuits with UPS/generator backup and are checked regularly by security personnel. Initially, racks of samples to be analysed are stored in a holding freezer. When the samples are logged into the laboratory data system, they are transferred to a pre-analysis freezer, where they are tracked by rack number.
    - Weighing balances (serviced/calibrated annually).
    - Volumetric flasks and positive displacement pipettes for preparation of standard solutions.
    - Cleaned, plastic ware is used where possible to avoid contamination from dissolution of silicate.
    - All glass and plastic ware is washed with 10%HCl and rinsed with 18MΩ water; other equipment is rinsed with 18MΩ water.
3. Reagents and Water
    - Nutrient standards are prepared using AR grade chemicals, wherever possible [(**NH$_4$**)$_2$SO4, K**NO$_3$**, Na**NO$_2$**, KH$_2$**PO$_4$**, Na**Si**O$_3$ $_9$H$_2$O].
    - Analytical reagents are prepared with AR grade chemicals wherever possible.
    - Water is 18MΩ quality.

3.3.2 Method
3.3.2.1 Samples

- Samples are received and stored frozen in dedicated freezers. Before analysis, samples are thawed overnight in 4°C refrigerator, then uncapped, placed in sample racks and covered with alfoil for analysis.
- Samples for analyses of total dissolved nitrogen and phosphorus are oxidised with persulfate reagent under alkaline conditions before analyses are carried out (Attachment 3.1).

3.3.2.2 Standards

- Primary stock standards are prepared in 18MΩ water and stored for up to 6 months at $4^{o}C$.
- Six working standards are prepared fresh from stock standards prior to each analysis run.
- Working standards are prepared over the following (low/high) ranges are:
- Working standards are prepared over the following ranges are: NH_3: 0-10 µmol-N L^{-1}; NO_3: 0-30 µmol-N L^{-1}; NO_2: 0-3 µmol-N L^{-1}; PO_4:0-3 µmol-P L^{-1}; Si: 0-30 µmol-Si L^{-1}.
- Working standards are prepared in a matrix which matches the refractive index of samples (e.g. fresh, estuarine or marine waters) by addition of appropriate amounts of AR grade NaCl to DIW.
- Certified reference standards for each nutrient analysis are purchased from Oceans Scientific International and Research Council of Canada (NRC) and prepared as a mixed standard to fit within the working concentration range of the samples being analysed
- In-house sea water storage reference samples:
  Coastal seawater obtained from the AIMS pontoon is filtered through 0.45 µM cellulose acetate filters. The final filtered volume (approx. 4 L) is spiked with stock standards to read within working concentration range (e.g. NH_3: 2.4 µmol L^{-1}, PO_4: 1.7 µmol L^{-1}, NO_2: 0.7 µmol L^{-1}, NO_3+NO_2: 2.3 µmol L^{-1}, Si: 9 µmol L^{-1}), dispensed into 10ml plastic sample tubes and frozen. Aliquots of a batch of these reference samples usually last for several years and are prepared as required.

3.3.2.3 Calibration

- Pipettes are gravimetrically calibrated before use.
- The auto-analyser is calibrated for each analysis (nutrient species) with mixed working standards. The working standards are utilised for each run to establish a standard curve from which the sample concentrations are calculated.
- The linear response of the working ranges utilises a 1st order linear regression fit, calculated as

  part of the auto-analyser software package.
- Each tray protocol as part of every analysis session consists of:
  - Six working standards for a linear calibration curve.
  - QA/QC standards: replicate in-house seawater storage reference samples and certified reference standards.
  - Blank samples for drift and baseline corrections (part of the autoanalyzer software) are included at regular intervals within the sample set (maximum up to 60 samples per interval).

3.3.3 Calculations

- Instrument charts are checked and edited upon completion of a run to ensure signals are correctly detected. Editing is performed within the standard auto-analyser software by moving the peak markers to the top of the sample peak. The auto-analyser software is used for the initial processing which include:
- Standard-curve fit, drift correction, base calibration, calculation of sample concentrations.
- Further processing is carried out using Excel functions in a spreadsheet: e.g. Data is standardised against reference standards and corrected for any dilution factors.
- If there are problems with the chart output, (excessive noise, power outages, hardware problems, software problems) sample peaks are hand measured using digital Vernier callipers and a 1st order regression calibration is performed in an Excel spreadsheet.

3.4 Instrument performance

The method for determination of method detection limits (mdl) is that adopted by Bran and Luebbe from the US Environmental Protection Agency (Bran & Luebbe 1997). Given the variability inherent in environmental samples and that conditions for each automated run are never absolutely identical, detection limits are calculated for each run. Detection limits are statistically derived from multiples of the standard deviation of the baseline readings. Increased gain with acceptable baseline noise (at lower concentration ranges) yielded detection limits of: 0.02 µmol L^{-1} NH_3 (0-4 µmol L^{-1}), 0.01 µmol L^{-1} PO_4 (0-2 µmol L^{-1}), 0.03 µmol L^{-1} NO_2+NO_3 (0-4 µmol L^{-1}), 0.01 µmol L^{-1} NO_2 (0-2 µmol L^{-1}), 0.10 µmol L^{-1} Si (0-20 µmol L^{-1}).

3.5 Data management

Unique sample identifiers for all samples are supplied by staff requesting analyses. These are provided electronically as an Excel spreadsheet linked to the request form. Sample ID's are transferred to Analytical Technology datasheets (Excel). A unique Job Number is assigned to each analytical request, under which all information associated with the analyses is filed. Spreadsheets with the nutrient data results are send to staff requesting analyses (example of client report in Attachment 3.2). All nutrient data results incl. QA/QC data are archived by the analytical section as Excel spreadsheets. For more details about AIMS Data Management procedures for Reef Rescue MMP refer to Section A12.

3.6 Quality control

QAQC tests undertaken and reporting as part of the data reporting include:

Assessment of the limit of detection (LOD)

NB: the Limit of Detection (LOD) or detection limit, is the lowest concentration level that can be

determined to be statistically different from a blank (99% confidence).

- Detection limits are reported for each analytical batch and are statistically derived from multiples of the standard deviation of the baseline readings, which are conducted during analysis.

Assessment of accuracy of the analysis

NB: This is generally achieved by using reference materials to assess recovery of known amount of analyte.

- External certified reference standards are measured at regular intervals during analysis.
- Regular validation of the analyses by continued participation in inter-laboratory trials, e.g. biannually with Quasimeme (http://www.quasimeme.marlab.ac.uk/about.htm) and annually with the national low-level nutrient collaborative trials (NLLNCT, Australia).
- As a further measure to ensure analytical accuracy, spikes of known concentration were added to natural seawater samples on board of the research vessel, during the normal sample preparation and analysed as part of the sample batch, unbeknown to the analyst

Assessment of precision of the analysis

NB: This is generally achieved by the repeated analyses of the same concentration of analyte to check for reproducibility.

- Recording of in-house seawater storage reference samples for each nutrient species analysed.

Procedural blanks:

- Blank samples for drift and baseline corrections (part of the autoanalyzer software) are included at regular intervals within the sample set (maximum up to 60 samples per interval)

Reproducibility of duplicate analytical units

NB: The variation between results for sample duplicates indicates the reproducibility of the analysis and also the effects of various sources of contamination and analytical error during collection, sample preparation and analyses. Before data analysis, results are generally averaged over duplicates.

- Coefficients of variance are calculated from analytical results of sample duplicates. Duplicates with CV>30% are discarded from the analysis and the analysis repeated using extra duplicates that are generally collected as a backup.

Other

- Spike recovery: As a further measure to ensure analytical accuracy, spikes of known concentration were added to natural seawater samples on board of the research vessel,

during the normal sample preparation and analysed as part of the sample batch, unbeknown to the analyst.

3.7   References

Bran and Luebbe (1997) Directory of Autoanalyser Methods, Bran and Luebbe GmbH, Norderstedt, Germany.

Parsons,T.R., Yoshiaki, M. and Lalli, C.M. (1984) A manual of chemical and biological methods for seawater analysis, Pergamon, London. pp22-25.

Ryle VD, Mueller HR, Gentien P (1981) Automated analysis of nutrients in tropical sea waters. AIMS Technical Bulletin, Oceanography Series No. 3, Australian Institute If Marine Science, Townsville. P. 24.

Attachment 3.1   Persulfate digestion for analysis of total dissolved nutrients

Principle

Significant pools of N and P are complexed within dissolved organic matter or organisms. To measure of the total amount of N and P within a sample, it is necessary to decompose these N and P pools to a form that can be quantified. This decomposition can be achieved by exposure of samples to an oxidising reagent with the speed to completion of the oxidation reaction being promoted by exposure to heat and pressure via an autoclave. Persulfate oxidation under alkaline conditions results in conversion to nitrate which can then be converted to nitrite for colorimetric quantitation in the Bran+Luebbe AutoAnalyzer III.

Hardware

1) Harvey SterileMax Autoclave (Barnstead, USA) with attached printer located in West First Photo-oxidiser Room
2) Sarstedt vials (Cat # 60.9922.210)
3) Sarstedt lids (Cat # 65.9923.335)

Reagents

1) Potassium peroxidisulfate ($K_2S_2O_8$) - use the highest quality reagent with low N and P
2) Sodium hydroxide (NaOH) - use the highest quality reagent with low N and P
3) Boric Acid ($H3BO3$) - use the highest quality reagent with low N and P
4) Persulfate oxidising solution: mix 7.5 g of NaOH and 25 g of $K_2S_2O_8$ and 15 g H_3BO_3 in 400 mL of reagent grade water. Stir until all reagents are dissolved. Make volume to 500mL. Make fresh daily.

Procedure

1) If samples are frozen, thaw samples by running under a continuous flow of cold water
2) Make up test samples, calibration standards, In-House Reference Materials and Certified Reference Materials
3) Thoroughly mix samples and then decant 10 mL into a Sarstedt vial. If samples contain high amounts of particulates dilution series should be carried out at this point
4) Add 5 mL of Persulfate oxidising solution to each Sarstedt vial for digestion, put the lid on tightly and mix well using vortex equipment. Loosen vial lid by half a turn back and place vial into digestion rack for the autoclave
5) Insert samples into autoclave and program autoclave cycle as detailed in Attachment 3.1.
6) Let samples cool before analysing or freeze for later analysis.

Autoclave use

1) For freshwater samples digest the samples, calibration standards, In-House Reference Materials and Certified Reference Materials in the autoclave at 121°C for a continuous period of 70 minutes

2) For saltwater samples, digest the calibration standards, In-House Reference Materials and Certified Reference Materials in the autoclave at 121°C for a period of 70 minutes,

3) For samples of unknown salinity and having a salinity between that of fresh and full seawater, treat the samples as if they were seawater i. e. autoclave at 121°C for a period of 70 minutes.

4) Ensure the external water supply to the right of the autoclave is filled with reverse osmosis water

5) To change the autoclave cycle setting to the desired conditions, do the following:

    a) Press "Optional Cycle" button
    b) Press the down arrow button (▼) to reject use of default settings
    c) Press the down arrow button (▼) to set the temperature to 121°C
    d) Press "Start" button to proceed
    e) Press Up arrow button (▲) to desired time (i.e., 35 or 70 minutes)
    f) Press down arrow button (▼) for Optional Liquid Cycle
    g) Press "Start" button for the cycle to commence

6) A printer has been attached to the autoclave which will record autoclave conditions during the cycle

7) Note the operator's name, date of use, cycle conditions and result of autoclave performance check in the log book associated with the Harvey SterileMax Autoclave.

Section A4: Ship-board ammonium analysis using the OPA method

AIMS-SOP-EFWQ-v2.0

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TASKS INCLUDED IN THIS SOP

Analysis of ammonium (NH_4^+) in seawater with a focus on detecting low level concentrations in fresh samples.

PRINCIPLE AND RESPONSIBILITIES

The objective of this method is to measure the concentration of ammonium (NH_4^+) present in fresh seawater samples with a focus on detecting low concentrations (<0.1 µM) using shipboard analysis to minimize contamination through elimination of storage and reducing handling effects.

This method is based on the fluorescent detection of ammonium which combines with the fluorochrome orthopthaldialdehyde (OPA). OPA has been widely used as a reagent for measuring primary amines (e.g., amino acids) in marine and freshwater. By altering the reagents and reaction conditions, this method inhibits the reaction of OPA with bound primary amines and encourages the binding with free ammonium.

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Spills or splashing of hazardous substances	Chemical burns	Read the relevant MSDS. Use appropriate PPE including safety glasses, lab coat, and gloves. Work within a fume hood. Store chemicals securely while at sea, whether working or stowed.
Breakage of containers or glassware	Injury	Use appropriate PPE including safety glasses, lab coat, and gloves. Prepare reagents in safe and calm conditions. Store glassware securely while at sea, whether working or stowed.
Accidental release of chemicals at sea	Environmental impact	Reduce the volume of chemicals taken to sea. Use fume hood for chemical analysis and store chemicals securely while at sea. Transport any chemical waste back to the laboratory and dispose of waste appropriately in laboratory setting.

PERSONNEL PREREQUISITES & COMPETENCIES

Laboratory safety induction
Read and understood procedure
Trained by an experienced technician

TOOLS & EQUIPMENT

Equipment:

- 2 x 100 mL acid cleaned Schott bottles for NH_4^+ stock solution
- 2 L volumetric flask for sodium tetraborate
- 100 mL volumetric flask for OPA
- 150 mL glass bottle for sodium sulfite
- 10 mL pipette
- 2 L brown polyethylene bottles for working reagent
- Magnetic stirrer to mix sodium tetraborate buffer
- Turner Designs Trilogy Laboratory Fluorometer with Ammonium module
- 10 x 10 x 45 mm cuvette for reading
- 6 x 250 mL glass or plastic volumetric flasks for standards, acid-soaked (10% HCl) before use
- 100 to 250 µL adjustable pipettes for dispensing stock solution to make standards
- 5 mL pipette to transfer sample to cuvette and dispense buffer for matrix analysis
- 1 L brown polyethylene bottle with 5 mL dispenser for working reagent
- 75 reaction tubes. Use 30 mL graduated test tubes with Teflon stoppers or 30 mL screw-top Pyrex test tubes marked at 20 mL with lid liners removed
- Dark box to hold reaction tubes
- Electronic balance to weigh chemicals

Chemicals:
1) Borate Buffer: Dissolve 80 g sodium tetraborate in 2 litres of Milli-Q. Shake thoroughly or use magnetic stirrer overnight to dissolve. Stable.
2) Sodium Sulfite: Dissolve 1 g of sodium sulfite (Sigma S4672) in 125 mL of Milli-Q. Store at room temp, stable for ~1 month.
3) OPA solution: Dissolve 4 g of OPA (Sigma P1378) in 100 mL of high grade (HPLC) Ethanol. Store in an opaque brown or grey polyethylene bottle.

Working reagent (WR): Mix 2 litres of (1) + 10 mL of (2) + 100 mL of (3). Allow this solution to "age" for 1 day prior to use. Store in a 2 L brown polyethylene bottle. Stable for at least 3 months if stored in dark at room temperature.

4) Primary NH_4^+ Stock Standard Solution (1 mM NH_4-N): Dissolve 0.0134 g of ammonium chloride (NH_4Cl) in 250 mL of Milli-Q. 1 mL of this stock contains 1 µmoL of NH_4-N.

ENVIRONMENTAL CONSIDERATION

N/A

CHEMICAL DISPOSAL

Due to their dilute final concentrations, all unused chemical waste can be disposed safely in laboratory sinks.

REFERENCES

Holmes, R.M., A. Aminot, R. Kerouel, B.A. Hooker and B.J. Peterson 1999. A simple, precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56: 1801-1808.
Solorzano, L. 1969. Determination of ammonium in natural waters by the phenolypochlorite method. Limnol. Oceanogr. 14: 799-801.

PROCEDURE

1.   SAMPLE HANDLING AND PROCESSING CONTROLS

- There is high demand for NH_4^+ by marine plants and bacteria in marine waters, and thus natural concentrations are usually very low (<0.1 µM). However, ammonium is ubiquitous in waters, on surfaces, exuded from skin, and in the atmosphere (including tobacco smoke). As a result, water samples, containers, and labware are readily contaminated with NH_4^+. The amount of NH_4^+ in samples that is derived from this contamination can be of similar order or greater than natural concentrations of NH_4^+. Accordingly, every precaution must be taken to minimize contamination of samples during the sampling process, handling, preservation, and analysis.

- People handling samples must have clean hands and should wear powder-free nitrile gloves. Smokers must wear gloves and no smoking may occur in the vicinity of samples being collected or analysed.

- Labware should be acid soaked prior to use, kept apart in a clean state to reduce contamination, and well-rinsed with Milli-Q and sample water prior to handling seawater samples. In doing analyses, it is preferable to be able to transfer the sample water directly to a graduated reaction tube without additional handling, filtration or measurement steps. Avoid touching the rims of tubes or flasks used

to contain sample water. Filtration of the sample will almost always introduce significant contamination.

- When using Pyrex analysis tubes: a) for the first time after acid cleaning, b) when there has been a significant time lag between sample runs (>2 days), or c) when the tubes have previously had high concentrations of ammonia, it is advisable to "burn" them out. This is done by filling the tubes with 20 mL of fresh Milli-Q water, adding 5 mL of working reagent, mixing the contents by inversion and soaking them for several hours. This will strip the glass walls and stopper of any residual ammonia adhering to them.

2.   LABORATORY SAMPLE PROCESSING

This procedure is adapted from Protocol A (Holmes et al, 1999) and a quick guide of this protocol can be found in Appendix 3.

⚠   Prior to commencing a new series of sampling, all reaction tubes must be cleaned. First, tubes should be acid soaked and rinsed in Milli-Q, then "burned out" by filling with 20 mL of Milli-Q and 5 mL of WR. This strips off ammonia adsorbed to the reaction tubes, minimizing contamination of the sample.

- Rinse triplicate reaction tubes with sample water and fill to 20 mL mark.
- Prepare the series of standards using Milli-Q (as outlined below).
- Rinse and fill triplicate reaction tubes to the 20 mL mark with each standard.
- Prepare the same series of standards using a representative seawater sample as the matrix instead of Milli-Q (as outlined below). Rinse and fill triplicate reaction tubes to the 20 mL mark with each seawater matrix standard.
- Add 5 mL of WR to all the reaction tubes with a dispenser, replace stoppers, shake (or mix with vortex mixer) and store in the dark at ambient temperature. Record the time of incubation on the laboratory log sheet (see Appendix 2).
- From the same representative seawater sample above, rinse and fill triplicate reaction tubes with 20 mL of seawater and add 5 mL of borate buffer. This is for a background fluorescence determination (as outlined below). Do not add working reagent.
- After incubating in the dark for 2-3 hours (2.5 hrs preferred), read fluorescence of all standards and samples on the Trilogy fluorometer, noting the time of reading on the laboratory log sheet. If samples are being run progressively throughout the day, read each batch after the same incubation interval as the initial batch of standards and samples. A quick guide for setting up the Trilogy fluorometer can be found in Appendix 1.

3.   CALIBRATIONS

⚠   Standards should be prepared and analysed at least once daily or with each discrete batch of samples if there are large time gaps between sampling events. If sample collection takes place more or less continuously over longer time periods, make sure you read each new batch of samples after the same incubation interval as the original batch of standards and samples. This ensures colour development will be at a similar stage on the reaction curve.

a.   Standard Curve – Fresh Deionised Water

- Using 1 mM Primary Standard Stock Solution make up the following Secondary Standard Solutions as follows:
- 0 µM Standard (Blank) – Milli-Q only (use freshly dispensed Milli-Q)
- 0.5 µM Standard - Add 125 µL of primary standard to 250 mL volumetric flask and make up to mark with Milli-Q
- 1.0 µM Standard - Add 250 µL of primary standard to 250 mL volumetric flask and make up to mark with Milli-Q
- Rinse tubes three times with the standard, and then pour 20 mL of each of the above standards into a corresponding series of graduated test tubes (tubes 1 to 9) as per schedule in Appendix 2.
- Add 5 mL of Working Reagent with dispenser to each tube, stopper, and vortex mix.
- Let stand in dark for 2-3 hours (2.5 hours preferred) then read on fluorometer as for samples.

b.   Control Curve (Matrix Effect) – Fresh Seawater

- Make up another set of standards in the same concentrations as for the Standard Curve but use a representative seawater sample for the diluent instead of Milli-Q. Use the fresh seawater sample as the 0 µM Secondary Standard.
- Rinse tubes three times with the standard, and then pour 20 mL of each of the above standards into tubes (10 to 18) as per Appendix 2. This is for a "control" curve to determine matrix effects.
- Add 5 mL of Working Reagent with dispenser to each tube, stopper, and vortex mix.
- Let stand in dark for 2-3 hours (2.5 hours preferred) then read on the fluorometer as for samples.

c.   Background fluorescence

- Rinse and add 20 mL aliquots of fresh representative seawater to tubes (19 to 21) for determining background fluorescence.
- Add 5 mL of borate buffer to these 3 tubes only. DO NOT add Working Reagent!
- Let stand in dark for 2-3 hours (2.5 hours preferred) then read on fluorometer as for samples.

4.   CALCULATIONS

a.   Background Fluorescence

Background fluorescence (F_{BF}) is determined by mixing the sample with borate buffer instead of working reagent. No incubation period is necessary before reading.

Sample fluorescence is calculated as:

(1) $F_{sample\ NH4} = F_{sample\ obs} - F_{sample\ BF}$

where:

$F_{sample\ NH4}$ = fluorescence of sample due to ammonium

$F_{sample\ obs}$ = measured fluorescence when incubated with working reagent

$F_{sample\ BF}$ = measured fluorescence when mixed with borate buffer

b.   Matrix Effect

Matrix Effect (ME) is determined by preparing a "standard curve" as above using natural sample water instead of Milli-Q. The difference between the <u>slopes</u> of the standard curve for standards made up with Milli-Q and representative sample seawater is the ME. Materials in the sample water may either quench or enhance the OPA fluorescence due to ammonium. The matrix effect correction is the ratio of the standard curve slope measured in Milli-Q divided by the standard curve slope measured in seawater diluted standards.

When working in an environment with different water types (e.g. salinities) or analysing batches of samples with different water characteristics (e.g. different levels of coloured dissolved organic matter), matrix effect standard curves should be prepared for each water type.

(2)   ME = [slope of standard curve in Milli-Q]/[slope of standard curve in dilutant]

c.   Corrected Fluorescence

To calculate the combined effects of background fluorescence and matrix effect, the following equation must be used:

(3) $F_{sample\ corr} = F_{sample\ NH4}$ x ME

where:

$F_{sample\ corr}$ = corrected sample fluorescence

$F_{sample\ NH4}$ = fluorescence of sample due to ammonium (from Equation 1)

The concentration of ammonium in the sample is: NH_4^+ (μmol L^{-1}) = [$F_{sample\ corr} - F_{Milli-Q\ blank}$] x slope factor from Milli-Q calibration curve

5.   DATA MANAGEMENT

The raw fluorescence reading, and analysis time are recorded against the sample identifiers on a laboratory worksheet. An example of this worksheet can be found in Appendix 2. All information from this lab sheet is then entered on to a Microsoft Excel Spreadsheet template (Appendix 2). This data entry results in other data cells being populated, plots the Standard and Control Calibration Curves, and calculates the slope and intercept of these curves. The derived values are used to calculate the original sample concentration of ammonia using calculations described above.

All the calculation equations are embedded in the spreadsheet template, except the slope and intercept of the Standard Curve must be entered into specific cells once data entry is completed.

6.   METHOD CAPABILITIES AND LIMITS OF DETECTION

The method is linear over the range 0 to 5 µmol L^{-1} NH_4-N in solution. The effective detection limit is 0.05 µmol L^{-1} NH_4^+. Samples with ammonium concentrations greater than this range will require additional calibration standards but should still exhibit a linear response.

7.   QUALITY CONTROL

- All samples are analysed in triplicate.
- Control standards are used to measure possible matrix effects.
- Background fluorescence is measured with each sample batch.
- Primary standards are used to generate a calibration curve for every batch of samples read.
- Reagent blanks are run with each sample batch.
- The Dixon Test is manually employed to check outlying results from triplicate sample sets and rejected individual sample results may be flagged with a tabulated risk of 10% false rejection.

APPENDICES

All appendixes must be referenced within the body of the SOP

1	Using the Trilogy fluorometer
2	Example of data entry and preliminary calculations
3	Quick guide to NH4 by OPA
#	
#.	

RECORDS

All AIMS analyses undertaken on behalf of AIMS or client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with External Document and Data Control Policy, Data Access Policy and AIMS Disaster Recovery Plan, and must have an associated metadata record (see for the Data Access Policy for full details).
- Raw experimental Measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements).
- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A5: Dissolved Organic Carbon and Total Nitrogen Assay in Water using the Shimadzu TOC-L

AIMS ATSOP-006

INTRODUCTION

Marine, estuarine and fresh water samples are analysed by the AIMS analytical technology laboratory for Dissolved Organic Carbon (DOC) and Total Nitrogen (TN) using the Shimadzu TOC-L with TNM-L. The instrument uses high temperature catalytic oxidation to convert organic carbon to carbon dioxide which is detected by NDIR detector, and nitrogen to nitrogen monoxide, which is reacted with ozone emitting light, which is detected by a chemiluminescence detector.

SCOPE

This procedure relates to the Shimadzu TOC-L systems in the analytical technology laboratory at AIMS. It includes the analysis of water samples for DOC and TN. Both instruments function in the same way, the procedure therefore relates to both.

RESPONSIBILITIES

It is the responsibility of the instrument user to ensure they are suitably trained and follow this procedure.

It is the responsibility of the instrument user to maintain a safe and tidy work area.

It is the responsibility of the instrument user to monitor the performance of the instrument and inform the AT lab coordinator of problems or anomalies.

It is the responsibility of the instrument user to perform routine maintenance as required.

The AT lab coordinator is responsible for scheduling service if required.

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Heat	Burn	Ensure instrument is completely cool before performing any maintenance.
Electrical	Electric shock	Clean any liquid spills immediately. Do not remove side/ back panels of instrument while connected to power supply.
Hydrochloric acid	Chemical burns Environmental damage	Always dilute acid in a fume hood. Do not allow acid release to environment. Wear gloves when handling acid

Ceramic fibre and quartz wool	Fibrous dust	Do not breathe dust, use in a fume hood.
Soda lime CO$_2$ absorber	Corrosive	Wear gloves when handling. Only open container in a fume hood.

ENVIRONMENTAL CONSIDERATION

Hydrochloric acid may cause long term adverse effects in the aquatic environment. Do not allow release of HCl to the environment. Small amounts may be washed down the lab sink with large amounts of water.

TOOLS & EQUIPMENT

- Shimadzu TOC-L with TNM-L and autosampler
- Suitable sample tubes
- Suitable pipettes
- Suitable laboratory glassware

MATERIALS AND REAGENTS

- Concentrated hydrochloric acid
- Platinum catalyst
- Instrument air and Zero air
- DOC purchased standard or Potassium Hydrogen Phthalate
- Potassium Nitrate
- MilliQ water

RECORDS

All usage, maintenance and troubleshooting of the instrument should be recorded in the instrument log. This should also include routine actions such as gas change.
Processing templates are available in the AT lab Sharepoint
Results of in-house and consensus reference materials should be recorded on the trend logs stored in the AT lab SharePoint.

PROCEDURE

Solution Preparation

1M hydrochloric acid for column regeneration
- To a 1000mL volumetric flask, add approximately 600mL of MilliQ water
- In a fume hood, using a measuring cylinder, dispense 98mL of concentrated (32%) HCl and add to the volumetric flask. Mix well.
- Allow to cool and make to volume with MilliQ water.

0.05M hydrochloric acid for B type halogen scrubber

- The procedure for making 1M HCl should be followed, substituting 5mL of concentrated HCl
- Or a 20x dilution of 1M HCl in MilliQ water can be made

25% phosphoric acid IC reagent
- To a 250mL volumetric flask, add approximately 150mL of MilliQ water
- In a fume hood, using a measuring cylinder, dispense 50mL of concentrated (85%) phosphoric acid and add to the volumetric flask. Mix well.
- Allow to cool and make to volume with MilliQ water.

Standard Preparation

Dry standard materials at 105°C for two hours, or overnight at 60°C, and allow to cool in a desiccator before use. Record the preparation of stock standards and working standards according to ATWI019.

1000ppm Stock DOC solution (certified prepared standards may also be purchased which may be of different concentration)
- To a 500mL volumetric flask, accurately weigh 1.0625g of potassium hydrogen phthalate standard material.
- Dissolve in and dilute to volume with MilliQ water.

1000ppm stock TN solution
- To a 500mL volumetric flask, accurately weigh 3.6095g of potassium nitrate standard material.
- Dissolve in and dilute to volume with MilliQ water.

Working standards
- To a 1000mL volumetric flask, add approximately 600mL of MilliQ water
- Add 10mL of concentrated HCl, mix well and allow to cool
- Add by pipette the required volume of stock DOC and TN standards (see table)
- Make to volume with MilliQ water and mix well

Standard	DOC 1000ppm stock volume (mL)	TN 1000ppm stock volume (mL)
0ppm	0	0
1ppm	1	0.1
2ppm	2	0.2

Other standard concentrations may be prepared depending on samples for analysis, it is recommended higher strength standards are used to extend the calibration curve rather than diluting samples.

Stock standards are stable in airtight glass containers for 6 months.
Working standards are stable for 2 months in airtight glass containers.

Instrument Parameters

- Refer to AT work instruction XXX for instruction to setup the instrument
- Furnace temperature 720°C
- Carrier gas flow 150 ml/min
- Sparge gas flow ~100 ml/min. Sparge flow should be manually adjusted using the sparge gas valve on the instrument to produce a steady gentle stream of bubbles
- Injection volume: 200 μL

Detection/ Integration Parameters (Sample/ Method Properties)

NPOC
- No. of Inj.: 3/4
- No. of wash: 2

- SD Max: 0.1
- CV Max: 2.0
- Sparge time: 7 minutes
- Peak Time Parameters: Use default settings
- Enable multiple injections

TN

- No. of Inj.: 3/3
- SD Max: 0.1
- CV Max: 2.0
- Peak Time Parameters: Use default settings

-

Method

Method principle: Sample is pre-acidified to pH ~1-2 by addition of concentrated HCl to make 1% v/v (0.1mL HCl to 10mL sample). Inorganic carbon is converted to CO_2 and removed from the sample by sparging with CO_2 free gas. The remaining non-purgeable organic carbon (NPOC, also referred to as DOC) and total nitrogen (TN) is measured by high temperature catalytic oxidation as described in the introduction.

Standard/ blank and reference material preparation

- Prepare 10mL plastic sample tubes for the standards and blanks by rinsing with ~2% HCl. Tubes may be left to stand with the acid. Rinse the tubes with MilliQ water and dry inverted at 60°C.
- Add approx. 2-5mL of the standard to a cleaned tube, cap and shake well. Discard the contents and repeat the procedure twice.
- Fill the tube to 10mL mark and add to the instrument carousel in the required position.

Unknown sample preparation

- Samples should already be acidified by the customer. Samples should be stored in the refrigerator and allowed to equilibrate to ambient temperature prior to analysis.
- Shake samples well, remove the cap and check the pH of residual water in the cap using indicator paper.
- If samples have not been acidified, add 100μL of concentrated HCl to a 10mL sample and shake well. Make a note on the LIMS that the samples were not acidified on receipt when entering data.
- Add the tube to the required position in the carousel.

Reference materials

- Consensus reference seawater is available from the Hansell lab at the University of Miami. Sea surface reference (SSR) seawater should be analysed during each analysis and the results trended. Other suitable reference materials may be used.
- Inhouse reference seawater should be analysed during each analysis and the results trended.
- Obtain bulk seawater (GPA from seasim is suitable) and acidify with HCl to 1% v/v.
- Store the bulk inhouse seawater in sealed glass containers.

Sample Analysis

- The analysis run should include at least five 0ppm samples at the start of the run which may be considered null or junk samples.
- Peak shapes and response should be assessed for these samples to ensure the instrument is functioning in an acceptable manner.
- Refer to appendix 1 for a typical analysis sequence.

- If samples contain more than 2ppm DOC, higher concentration standards should be added to each standard bracket.

Result processing and reporting

- Templates to process the data are available in AT lab SharePoint > Documents > Processing Templates > Template for DOC V3 (Feb2021)
- Corrections for baseline drift and response drift are included in the processing.
- Calibration curves are produced for every run using all injections of the standards. Individual results may be omitted from the curve if they are clear outliers.
- Samples need to have the result corrected for the addition of the acid - for a sample with 1% HCl added, the result is multiplied by 1.01
- Summarise the results for reference materials and add the results to trend charts stored in SharePoint – AT Lab > Documents > TOC > DOC QC data

Quality Control/ System Suitability

- The R^2 value for both C and N calibration curves should be ≥ 0.99
- Record results for all injections of the reference materials in the trend logs.
- The mean recovery of reference materials for a run should be within 2 standard deviations of the average for the material recorded in the trend log. No single injection should be >3 standard deviations from average.
- If any of these conditions are not met, a questionable result investigation should be initiated.

REFERENCES

Shimadzu Total Organic Carbon Analyser TOC-L CPH/CPN user manuals

Appendix 1

Typical sample table

Start MQ

0ppm

0ppm

0ppm

0ppm

0ppm

Inhouse seawater reference

0ppm

0ppm

1ppm

1ppm

2ppm

2ppm

Inhouse seawater reference

Hansell SSR CRM

Sample 1

Sample 2

Etc up to 12 samples

Inhouse seawater reference

Hansell SSR CRM

0ppm

0ppm

1ppm

1ppm

2ppm

2ppm

Inhouse seawater reference

Up to 12 samples

Inhouse seawater reference

Hansell SSR CRM

0ppm

0ppm

1ppm

1ppm

2ppm

2ppm

Inhouse seawater reference

The number of standard and inhouse seawater injections may be reduced to accommodate larger numbers of samples. A single injection of 1ppm, 2ppm and inhouse seawater can be made in each standard bracket if required.

Repeated injection of seawater leads to eventual blockage of the combustion column, it is therefore recommended that the maximum number of seawater sample injections for a column is 60.

Section A6: Measuring CDOM in water samples using the Shimadzu 1900UV-Vis spectrophotometer

AIMS-SOP-EFWQ-v2.0

Document Details

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TASKS INCLUDED IN THIS SOP

Measuring the chromophoric (or coloured) dissolved organic matter (CDOM) in seawater samples with a UV-Vis spectrophotometer.

PRINCIPLE AND RESPONSIBILITIES

CDOM is a term used to describe a group of mixed (and mostly undefined) organic compounds dissolved in natural waters that absorb ultraviolet or short-wavelength visible light. This method outlines the operation and calibration procedures for measuring CDOM using UV-visible absorption spectroscopy. The applied method is based on Nelson and Coble (2009) and has been partially updated based on Mannino *et. al.* (2019).

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Electrical	Electrical shock	Protect electronic components from water leaks/spills and clean up any spills immediately. Disconnect instrument before conducting maintenance or servicing. Call an electrician if you are not sure you can solve the problem safely.
Cleaning cuvettes and/or instrument with small volume of volatile flammable liquid (ethanol or isopropanol)	Electrical fire	Ensure instrument is turned off and has cooled down before cleaning it. Ensure enough time has passed for any residual flammable liquid to evaporate before turning the instrument ON.

PERSONNEL PREREQUISITES & COMPETENCIES
Laboratory safety induction
Read and understood the protocol
Trained by experienced technician

TOOLS & EQUIPMENT

Sample collection:
- 50 mL amber glass bottles, acid-washed and oven-dried (60°C)
- 60 mL sterile syringe
- 0.2 µm syringe filter (Pall Acrodisc, Supor membrane)
- Nitrile or powder-free gloves

Sample measurement:
- Two quartz cuvettes (10 cm path length)
- Kimwipes
- Milli-Q water, freshly collected from the dispenser
- Shimadzu UV-1900 Spectrophotometer
- Air-tight container containing 70% ethanol for storing cuvettes when not in use
- Isopropanol 100% (if required)
- Nitrile or powder free gloves

ENVIRONMENTAL CONSIDERATION

N/A

CHEMICAL DISPOSAL

The chemical used for storing or cleaning the instrument is used in small volumes. It is a volatile solvent, which readily evaporates during the cleaning process. Any waste from this solvent would be evaporated in a fume hood when disposal is required.

REFERENCES

Mannino, A., M. G. Novak, et al., 2019. Measurement protocol of absorption by chromophoric dissolved organic matter (CDOM) and other dissolved materials, In Inherent Optical Property Measurements and Protocols: Absorption Coefficient, Mannino, A. and Novak, M. G. (eds.), IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume ###, IOCCG, Dartmouth, NS, Canada.

Nelson, N.B., and P.G. Coble, 2009. Optical analysis of chromophoric dissolved organic matter. Chapter 5 in: Practical Guidelines for the Analysis of Seawater, O. Wurl (editor), CRC Press, 401 pp.

PROCEDURE

1.   SAMPLE COLLECTION

- Collect a sub-surface water sample (using a clean Niskin bottle or other method). Samples should not break the water's surface because of potential contamination from surface films.
- ⚠ Samplers should wear powder-free non-latex gloves (such as nitrile) while handling samples, and gloves should not come into contact with the sampled seawater.
- Rinse a sterile syringe with sampled seawater three times and empty it. Fill the syringe ~60 mL with sample and attach the 0.2 µm syringe filter. Push ~10 mL of sample through the syringe filter to prime the filter. Rinse the amber bottle with 2 rinses of ~10 mL filtered water.
- Push water through the filter and fill the glass amber bottle to the base of the bottle neck (minimum of 50 mL of sample is required). If filtering becomes very difficult (e.g., turbid water), stop filtering and replace the syringe filter with a new one. Remember to prime the filter with ~10 mL water before recommencing filling the bottle. Filtering should not require a large amount of force; pushing water through the filter with excessive force will cause the filter membrane to rupture and contaminate the sample.
- Label the bottle with unique identifier corresponding to the sample taken.
- Store sample at 4°C until analysis. Samples should be analysed as soon as possible, preferably within 4 to 24 hours, but no later than within 6 months of collection (Mannino et. al. 2019).

2.   MEASUREMENTS

The following described protocol is specific to the Shimadzu UV-1900 spectrophotometer. However, any dual cell UV-Vis spectrophotometer with a wavelength resolution of 0.5 nm can be used.

Before starting measurements, bring the samples out of the cold room or refrigerator at least 2 hours before analysis and put them in the lab next to the spectrophotometer to equilibrate to room temperature. Space the samples from one another to ensure they warm to room temperature evenly. Do not begin analysing samples until they fully reach room temperature, as cold samples will result in inaccurate analyses.

While samples are coming to temperature, prepare the pre-set template (.csv) containing your sample details (see Table 1) and runsheet (see template at C:/UVVis-Data/Text/WQQ) with batch information and notes for each sample processing batch.

a.   Initialising Spectrophotometer

- Turn on spectrophotometer (switch located lower right-hand side) and allow it 1 to 2 hours to warm up.
- Inspect the optical windows in the sample compartment. If necessary:
  - o   Remove and store safely the 1 cm cuvette inserts
  - o   Clean the light source and detector optical windows inside the sample compartment with lint-free optical lens cleaning tissue slightly moistened with isopropanol, followed by a gentle wipe with dry lens tissue to remove any visible lint on the optical windows.

- Thoroughly rinse both cuvettes and fill with Milli-Q and wipe any excess liquid from the cuvette surfaces with a Kimwipe. Ensure that the contents are bubble-free and that the cuvette windows are clean. Place the cuvettes aside to equilibrate to room temperature.
    - Reference/blank cuvette is located at the back of cuvettes compartment
    - Sample cuvette is located at the front of the cuvettes compartment
- Press the grid button on the bottom left-hand corner of spectrophotometer display and select 'PC Control'.

b. Initialising instrument PC

- Turn on the PC.
- Open 'LabSolutions UV/Vis' software.
- Select 'Spectrum' which opens another window.
- In the new window, select 'Connect' to connect to the PC.
- Set the parameters by loading (file -> open -> parameters),
    - for CDOM spectrum analysis, parameters should be set as scanning in 0.5 nm intervals between 200-750 nm wavelengths
- Check that the Go To wavelength is set to 750 nm.
    - Click W.L. to confirm the Go To wavelength is set at 750 nm. Click OK.
- Conduct a baseline correction with Milli-Q water-filled cells in the sample (UWs) and reference (UWr) beams (Milli-Q to Milli-Q baseline correction) to zero the instrument for pure water. Once filled with Milli-Q and set into the cell holder, the reference cell should not need to be adjusted or changed. For this reason, it is preferable to place the cell cap on the reference cell to ensure no debris or dust settles into the reference cell throughout the sample analysis run.
    - Click BL to run a baseline correction absorption spectrum between 200 and 750 nm. This takes a couple of minutes to complete.
    - Record time of baseline correction on the runsheet.
- Fill in the file information or use the pre-set function by importing sample information from a pre-prepared .csv file and as per Table 1.

Table 1. Example sample pre-set and nomenclature

Scan order	Sample type	Sample name	Filename_vspd	Filename_csv_autoexport
1	blank	milliq_01_start	milliq_01_start.vspd	milliq_01_start_RawData.csv
2	sample	Sample_ID1	Sample_ID1.vspd	Sample_ID1_RawData.csv
3	sample	Sample_ID2	Sample_ID2.vspd	Sample_ID2_RawData.csv
4	sample	Sample_ID3	Sample_ID3.vspd	Sample_ID3_RawData.csv
5	blank	milliq_02_mid	milliq_02_mid.vspd	milliq_02_mid_RawData.csv

c. Running Blanks

- Milli-Q water blanks are run initially, after every ten samples and at the end of every run. The blanks should be labelled according to the format "milliq_##_<position>" with the numbers being allocated sequentially from start to end and the position being either start, mid (can have multiples) or end. See the pre-set template (and Table) for further information.

- With the Milli-Q blanks in both cuvette holders (reference and sample cuvette), close cuvette compartment door.
- On PC control window press START to initialise scan. The scan will run from 750 nm to 200 nm in steps of 0.5 nm. The absorbance values must be recorded and reported to five decimal places.
- Check this initial spectrum scan to confirm that Milli-Q water absorption throughout 200 to 750 nm is close to zero.

d. Running Samples

- Open compartment door and leave open while changing samples to keep the compartment from heating up. Take the sample (front) cuvette out and rinse once with Milli-Q and twice with some sample water (these 2 rinses should be done so that there is enough sample water left to fill the cuvette entirely for sample readings).
- Fill the sample cuvette with sample water to just below the top of the cuvette. Leave the reference (back) cuvette in the spec.
- Clean the cuvette so that all external surfaces are dry and that the cell windows (ends) are free from water, fingerprints, and dust. Inspect the cell windows. Inspect the cell contents for small bubbles or particles. Place in the sample cuvette holder. Close compartment door.
- Make sure that the spectrophotometer reads 0.000 ± 0.002 at 750 nm (Check the "live" absorbance reading on PC window). If not, remove the sample cell and check that there are no bubbles and that the cuvette windows are clean. Wipe the cell windows again with a fresh Kimwipe. Inspect the cell contents for contamination and if necessary, re-filter the sample using a fresh syringe and 0.2 µm filter as per the field method above. If you are still having trouble, a new Milli-Q blank can be scanned before analysing more samples. You can also check the sample details as sometimes flooding event samples or river mouth samples with high absorbance may have live absorbance values at 750 nm of 0.002-0.003; otherwise, you may have to run a new Milli-Q baseline.
- On the PC control window press START to initiate scan.
- Check the scan is satisfactory and does not have any sharp peaks, cut-offs, or a shape that diverges from an exponential decay. If the scan is unsatisfactory then you may need to re-run the scan. Check the sample cell and reference cell as per the previous step and then re-run the scan. You will need to modify the pre-sets sheet to insert a line for the additional re-run scan. If unsatisfactory results persist there may be a problem with the sample or spec and you should speak with the lab manager. Make notes on the re-run in the sample batch runsheet.
- Once a satisfactory scan has been achieved, discard the sample, and rinse the cuvette three times with Milli-Q water and gently tap dry on a Kimwipe.
- Tapping should be done on a thick and soft pile of Kimwipes to avoid cracking or damaging the quartz cuvette. Microscopic cracks can occur in cuvettes, which are not visible to the naked eye, but nonetheless affect sample readings. Any tapping must be done with extreme care.
- Rinse the cuvette twice with some of the next sample water (ensure enough water is left for 3rd fill) then fill to just below the label on the cuvette with sample water and read as before.
- Repeat reading steps for remaining samples, ensuring a Milli-Q sample blank is read after every ten samples and at the end of the run.
- When finished, flush the cuvettes with Milli-Q water 3 times and store them in 70% ethanol to avoid algal growth.

⚠ If air bubbles are adhering to the cell and are hard to dislodge by flushing, it may be necessary to clean the cell by soaking in 10% hydrochloric acid and rinsing with Milli-Q water.

- Close the 'LabSolutions UV/Vis' program and turn off the spectrophotometer.

3.   CALCULATIONS

First, the absorption spectrum of the Milli-Q blank is subtracted from the sample absorption measured between 200 and 750 nm. The absorption coefficient at any wavelength, $a_{CDOM}(\lambda)$ (in m^{-1}), is hereafter calculated as:

$$a_{CDOM}(\lambda) = 23.03 \times [Abs(\lambda) - Abs(600-750)]$$

where:

- $Abs(\lambda)$ is the absorbance at wavelength λ,

- $Abs(600-750)$ is the average absorbance between 600 and 750 nm, which corrects for the residual scattering by fine size particle fractions, micro-air bubbles, colloidal material present in the sample, or refractive index differences between the sample and the reference, and

- the factor 23.03 converts from decadic to natural logarithms and furthermore considers the cell path-length of 10 cm.

The CDOM spectral properties can thereafter be modelled as:

$$a_{CDOM}(\lambda) = a_{CDOM}(\lambda_0) \times EXP[-S \times (\lambda - \lambda_0)]$$

where:

- $a_{CDOM}(\lambda)$ is the absorption coefficient at wavelength λ,

- $a_{CDOM}(\lambda_0)$ is the absorption at reference wavelength λ_0, and

- S is the spectral slope coefficient.

4.   DATA MANAGEMENT

The data acquired during analysis will be automatically saved locally on the PC controlling the spectrophotometer as:

- a .vspd file stored in "C:/UVVis-Data/Data/".

- a .csv file (this is stored in "C:/ UVVis-Data /Text/").

       o   If required, csv file contents/formatting can be changed by clicking on Tools -> User Settings -> Text Output. However, this should not need to be changed frequently.

Both type of files can then be exported for further analysis if required.

5.   QUALITY ASSURANCE AND CONTROL

CDOM samples are only analysed if the spectrophotometer has successfully passed its start-up sequence checks.

Special attention is taken toward the light source lamps (halogen and deuterium bulbs) which are changed according to manufacturer recommendation every 2000 hours.

During sample measurements, the sample absorption at 750 nm should be 0.000 ± 0.002 nm. If different from this value, the samples and reference cuvettes are inspected for the presence of bubbles and cuvettes are wiped using Kimwipes. If required, a new Milli-Q blank can be scanned before analysing more samples. In severe cases, a new Milli-Q baseline correction can be conducted. Under some circumstances, samples with very high absorbance (i.e. samples from flood plumes) may give higher live absorbance readings at 750 nm, this should be taken into consideration if live absorbance values are high and persist despite checks.

APPENDICES

All appendixes must be referenced within the body of the SOP

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RECORDS

All AIMS analyses undertaken on behalf of AIMS or client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with External Document and Data Control Policy, Data Access Policy and AIMS Disaster Recovery Plan, and must have an associated metadata record (see for the Data Access Policy for full details).
- Raw experimental Measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements).
- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A7: Analysis of particulate phosphorus in marine waters

AIMS-SOP-EFWQ-v2.0

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TASKS INCLUDED IN THIS SOP

Analysis of total particulate phosphorus in seawater with a focus on detecting low level concentrations of bioavailable phosphorus.

PRINCIPLE AND RESPONSIBILITIES

The method is based on the oxidation of organic phosphorus and the extraction of acid-labile inorganic phosphorus from particles by hot acid persulfate (Menzel and Corwin, 1965) and the subsequent colorimetric determination of the phosphorus released as orthophosphate (Parsons et al, 1984).

Phosphorus occurs in many organic and inorganic forms in the environment. Forms available for quantitation by instrumental means depend strongly on the extraction method employed (e.g. Ruttenberg, 1992).

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Working with electrical appliance in close proximity to water	Electrocution	Use appropriate PPE including safety glasses, lab coat, and gloves. Use a RCD safety power outlet. Clean water spills immediately.
Exposure to hazardous chemical during preparation and dispensing	Chemical burns, chemical inhalation	Read the relevant MSDS. Use appropriate PPE including safety glasses, lab coat, and gloves. Label chemical solutions correctly. Clean any spills immediately and work with chemicals in a fume hood.
Boiling or heating water and hazardous chemical	Burns	Use appropriate PPE including safety glasses, lab coat, and gloves. Use marble stoppers to avoid vial reflux of solution without bumping. Allow samples to cool before removing from heating block.

Homogenizer shattering sample vial	Injury	Use appropriate PPE including safety glasses, lab coat, and gloves. Inspect vial integrity before use, turn homogenizer on before shaft inserted in vial and off before being removed. Perform homogenization while vial is in the foam holder.

PERSONNEL PREREQUISITES & COMPETENCIES
Laboratory safety induction
Read and understood procedure
Trained by an experienced technician

TOOLS & EQUIPMENT

Sample collection
- 25 mm Whatman GF/F glass fibre filters, pre-combusted (4 hours at 450°C)
- Squares of aluminium foil (~8 x 8 cm), pre-combusted (4 hours at 450°C)
- Millipore forceps (2 pairs)
- Fine-tip permanent labelling marker
- 250 mL plastic graduated cylinder
- Filter funnels (25 mm) and filter supports
- Vacuum manifold, water trap and pump
- 60 mL sterile syringe and 0.2 µm syringe filter (Pall Acrodisc or similar)

Sample analysis
- ⚠️ *All plasticware and glassware should be well-rinsed, soaked in dilute Hydrochloric acid (HCl), rinsed again in Deionised Water and heat dried (where possible). Detergents, which may contain phosphates, must be avoided.*
- Automatic pipettes (50 µL, 100 µL, 250 µL, 500 µL, 1 mL, 5 mL and 10 mL)
- 2.5 mL dispenser on a reagent bottle for the persulphate digestion reagent
- 5 mL dispenser on a reagent bottle for deionized water
- 50 mL stoppered graduated measuring cylinder for making up mixed reagent
- 200 x 7 mL glass scintillation vials in 2 foam trays. One tray of vials (100) is to be used for the sample digestions and the other for the colorimetric analyses. Clean the glass vials in 10% HCl and oven dry. It is convenient to bake the vials at 450°C in a muffle furnace to remove the inked labels on used vials rather than wiping them off with solvent.
- 100 position heating block in a sand-filled electric frypan
- 100 small glass marbles (acid washed in 10% HCl, rinsed with deionised)
- Mini homogeniser with beaker of Milli-Q for rinsing
- Reagent bottles
- Millipore forceps
- 3 x 100 mL and 1000 mL volumetric flasks
- Safety glasses

- Spectrophotometer with 1 cm cell (capable of reading to 900 nm). Although not essential, an automated, low-volume, sipper cell attachment for the spectrometer will speed up analysis time considerably.
- Centrifuge (Eppendorf 5810 or equivalent)

Chemicals and reagents

- Milli-Q water and reverse osmosis (RO) water

- Potassium persulphate ($K_2S_2O_8$, CAS No.77727-21-1): 15 g in 300 mL of Milli-Q water. Make fresh daily.

- Colour reagent:

a. Ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}$, CAS No. 12027-67-7]: 15 g in 500 mL of Milli-Q. This solution is reasonably stable (~3 months), but if kept for too long, a precipitate will form. If this happens, discard and make up a fresh solution, though small amounts of precipitate will not affect the analysis.

b. Sulphuric acid (H_2SO_4, CAS No.7664-93-9): Add 140 mL of concentrated (98%) sulphuric acid to 900 mL Milli-Q. Stable.

c. Ascorbic acid ($C_6H_8O_6$, CAS No.50-81-7): 2.4 g in 50 mL of Milli-Q. This solution is unstable. Freeze at -20°C in 10 mL portions and defrost as needed.

d. Potassium antimonyl tartrate [K (SbO) $C_4H_4O_6$, CAS No. 28300-74-5]: 0.34 g in 250 mL of Milli-Q. Stable.

  Mixed Reagent:

   - mix 10 mL of (a) with 25 mL of (b),

   - add 10 mL of (c), and

   - add 5 mL of (d).

  This solution will remain stable for no longer than 4 hours.

- Standard solutions:
   - 1 mmol L^{-1} PO$_4$ primary standard: Dissolve 0.136 g of potassium dihydrogen phosphate, anhydrous (KH_2PO_4, CAS No. 7778-77-0) in 1000 mL of deionised. Freeze at -20°C in 10 mL portions and defrost as needed.
   - Blank- Milli-Q
   - 5 µmol L^{-1} PO$_4$: Pipette 0.5 mL of 1 mM PO$_4$ into a 100 mL volumetric flask and make to the mark with deionised water.
   - 10 µmol L^{-1} PO$_4$: Pipette 1.0 mL of 1 mM PO$_4$ into a 100 mL volumetric flask and make to the mark with deionised water.
   - 20 µmol L^{-1} - PO$_4$: Pipette 2.0 mL of 1 mM PO$_4$ into a 100 mL volumetric flask and make to the mark with deionised water.

ENVIRONMENTAL CONSIDERATION

Avoid release of concentrated forms of used chemicals (reagents) into the environment.

CHEMICAL DISPOSAL

Only small quantities of very dilute hazardous chemicals are used and as such do not require registered disposal methods. All liquid waste can be disposed of by dilution and flushing down laboratory sinks that flow to chemical waste sumps. These sumps are emptied periodically by a licenced contractor. Solid waste from GF/F filters is collected in traps and disposed in general laboratory waste.

REFERENCES

Menzel, D.W. and Corwin, N. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulphate oxidation. Limnology and Oceanography 10: 280

Parsons, T.R., Yoshiaki, M. and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis, Pergamon, London. pp22-25

Ruttenberg, K.C. 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. Limnology and Oceanography 37(7): 1460-1482.

Thompson M. and Walsh J. N. 1993 Handbook of Inductively Coupled Plasma spectrometry. 2nd ed. Chapman and Hall.

PROCEDURE

SAMPLE COLLECTION

- Filter 250 mL of seawater sample through a 25 mm Whatman GF/F glass fibre filter (through vacuum manifold or syringe filtering). Duplicate samples are recommended.
- The filter is then wrapped in aluminium foil, labelled with collection information and the volume filtered. When sampled in duplicate, there should be 2 filters per foil packet.
- Freeze the filters immediately (-20°C).
- Duplicate wet filter blanks should be collected once per day of sampling by filtering ~20 mL of filtered seawater (0.2 μm syringe filter) through a GF/F following the same procedures as for regular samples. Typically, sample

water is used for this from the final sampling site of the day. Wet filter blanks are stored in the same manner as regular samples.

ANALYSIS

- Place each sample filter in a uniquely identified 7 mL scintillation vial.

  - ⚠ A pair of deionised water blank filters and two pairs of digestion/recovery standards (in duplicate) should be run in each digestion. The suggested range of controls is 0, 50, and 100 µL of the 1 mM primary standard solution per vial, corresponding to 0, 10, and 20 µM phosphate, respectively.

  - ⚠ Sample vials should be numbered from 1 to 100 to be individually identifiable.

- Add 2.5 mL of potassium persulfate solution to each vial.

- Place vials in hot plate holder and put a clean glass marble on each vial to allow the sample to reflux while gradually evaporating to dryness.

- Turn on the fry pan/hot plate and adjust the temperature so the vials reflux gently (approx. 80 to 90ºC, thermostat 7 to 8)

- When all vials are refluxed dry (~7 hours to overnight), allow to cool and then add 5 mL Milli-Q water to each vial. Cover with plastic wrap (e.g. Gladwrap, zip lock bag) and let stand overnight.

  - ⚠ After refluxing, vials can be kept for 24 hrs in fridge before overnight Milli-Q incubation or alternatively kept in the freezer a few days before overnight Milli-Q incubation.

- The filters in each vial are homogenized using a small, motorized mixer made from a hobby engraver. Rinse the head of the mixer between samples in a beaker of clean Milli-Q water.

- After mixing, the solid particles in the vials are spun down in a centrifuge (2 x 3 minutes at 3500 rpm in Eppendorf 5810).

  - ⚠ It is advisable to stop the centrifuge midway through and gently shake the vials to "tap down" any filter particles that adhere to the sides. This will reduce the likelihood of particles contaminating the supernatant.

- Pipette exactly 2.5 mL of the supernatant into a clean 7 mL scintillation vial with the same numbering as the original scintillation vial.

  - ⚠ 4 x pairs of calibration standards need to be prepared at this stage. See Calibration section below.

- Add 0.25 mL of colour reagent to each vial and mix gently.

- Allow the colour to develop for at least one hour. It may be necessary to remove them from the cool of the lab to a warm space outside as cooler temperatures will retard colour development leading to longer development times. The intensity of colour will plateau for a short while before slowly fading. Finding this optimal temperature window for colour development can sometimes prove problematic and so the "control standards" are essential to estimate

efficiency.

- After 60 minutes (but in less than 2 hours) measure the absorbance of the solutions in the vials at 885 nm on a spectrophotometer using a 1 cm cell.

  ⚠ The colorimetric method is linear over the range 0 to 20 µmol L^{-1} phosphate in solution corresponding to spectrophotometer readings of 0 to 0.400 AU. Hence, readings above 0.400 AU will not yield accurate phosphate estimation and in this case the samples need to be diluted.

  For these samples as well as recovery standard (vial 1-3 pairs), within 1 hour of initial preparation:

  - Re-spin the original sample vial in which the filter was ground for 3 minutes @ 3500 rpm

  - Carefully pipette out 0.8333 mL of sample into a clean 7 mL vial and add 1.66 mL of Milli-Q water (corresponding to a 3-fold dilution factor)

  - Prepare a fresh batch of calibration standard

  - Add 2.5 mL of colour reagent (can be used for 4 hours from preparation time)

  - Incubate for for 1 hour and repeat spectrophotometer reading

- All measurements should be recorded on the sample analysis logsheet (Appendix 1).

CALIBRATION

- A series of phosphate standard concentrations is run each time a batch of sample is analysed to create a standard curve which should be linear up to at least 20 µmol L^{-1}. This curve measures the level of colour development but does not account for matrix effects caused by persulphate digestion and sample handling etc. Digestion/recovery standards are to be run with each batch of samples for this purpose. With each batch, a series of phosphate standard concentrations and a set of blank filters are digested and treated in the same way as the samples to determine efficiency of the analysis process. An efficiency factor is then imposed on the whole batch.

Standard Curve

A standard curve of 0, 5, 10 and 20 µmol L^{-1} phosphate (prepared from the 1 mM primary standard) is run with each sample plate.

The slope is calculated and used to convert the sample absorbance (with correction for blank) to phosphate concentration in the sample.

The efficiency factor and a dilution factor are then applied to calculate the phosphorus content of the sample.

The final value should be the concentration of particulate phosphorus, in µmol L^{-1}.

Blanks

Wet filter blanks are collected during sample collection and are run with each sample batch. The average absorbance is subtracted from both the samples and the recovery standards to calculate the effects of handling and suspended particle interference.

CALCULATION

Phosphorus concentration may be calculated using the following equations. The equations assume that the filtered sample volume is 250 mL and that there is 5 mL of Milli-Q resuspension. The calculation spreadsheet (Appendix 2) allows for variation of these numbers for individual samples.

$$F = \frac{\text{concentration of standard } (\mu mol\ L^{-1})}{Abs_{Std} - Abs_{Blank}}$$

For a given standard concentration and a 1 cm spectrophotometer cell, the value of F should be very close to 50. When using spec cells of other pathlengths, divide F by the pathlength in cm.

$$\text{efficiency} = \frac{\text{measured concentration of control standard}}{\text{real concentration of control standard}}$$

$$\text{dilution factor} = \frac{\text{initial volume of sample (i.e. 250 mL)}}{\text{concentrated volume of sample (i.e. 5 mL)}}$$

⚠️ For samples that have undergone further dilution due to initial spectrophotometer readings >0.400 AU, calculations are similar to previously described but based on diluted recovery and calibration standard. Final phosphorus concentrations are multiplied by 3 to account for extra dilution.

METHOD CAPABILITIES

The colorimetric method is linear over the range 0 to 20 µmol L^{-1} phosphate in solution (or 0 to 0.100 µmol of total phosphorus per filter).

Samples with phosphate concentrations above this range (as is often the case with river or estuarine samples) will need to be diluted before the colour reagent is added.

Where high phosphorus concentrations are likely, it may be beneficial to filter a few extra samples that can be used to establish approximate concentration ranges.

DATA MANAGEMENT

- The sample identifier and volume filtered is logged on a laboratory worksheet against the vial into which it is being placed (Appendix 1). The

spectrophotometer absorption reading for each sample is then written on this sheet.

- All information from this lab sheet is then entered into a Microsoft Excel spreadsheet template called "Particulate phosphorus calculation sheet" (Appendix 2) which is a visual copy of the laboratory form but has all the calculation equations embedded.

QUALITY CONTROL

QA/QC tests undertaken as part of the data reporting include:

Assessment of the limit of detection

- The Limit of Detection (or detection limit), is the lowest concentration level that can be determined to be statistically different from a blank (3 standard deviations = 99% confidence).

- Minimum detection limits were calculated for this method using repetition of blanks.

- The effective detection limit is 0.010 µmol P (0.310 µg P) on a filter.

Assessment of accuracy of the analysis

- This is generally achieved by using reference materials to assess recovery of known amount of analyte.

- There is no certified reference material available in the particulate phosphorus concentration range of Great Barrier Reef marine samples. Hence, no direct assessment of accuracy is possible for this method.

- As an indirect assessment, a set of separate phosphate recovery and calibration standards is analysed to determine a method efficiency factor for each analytical batch. Analytical data are adjusted using the batch-specific efficiency factor.

Assessment of precision of the analysis

- This is generally achieved by the repeated analyses of the same concentration of analyte to check for reproducibility.

Procedural blanks

- Filter blanks are run with every batch (generally daily) from dry, fresh GF/F filters. These are used as blank readings in the calculation of recovery.

- Wet filter blanks are collected during sample collection to measure contamination of GF/F filters or through handling.

<u>Reproducibility of duplicate analytical units</u>

- The variation between results for sample duplicates indicates the reproducibility of the analysis and the effects of various sources of contamination and analytical error during collection, sample preparation and analysis. Before data analysis, duplicate samples are used to calculate the coefficient of variance (CV) for each sample. Duplicates with CV outside a pre-determined confidence range (20%) are marked for re-run analysis performed using duplicate spare samples.

<u>Others</u>

- Intercalibration of this method with the ICP method used by AIMS laboratories (Thompson and Walsh, 1993) showed agreement of +/- 15%.

- The linear range of this method was established utilising increasing concentrations of primary standard. Samples are diluted if above this range.

- A reference curve is generated with every sample batch.

APPENDICES

All appendixes must be referenced within the body of the SOP

#.1	Phosphorus analysis logsheet
#.2	Particulate phosphorus calculation sheet
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RECORDS

All AIMS analyses undertaken on behalf of AIMS or client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with <u>External Document and Data Control Policy</u>, <u>Data Access Policy</u> and AIMS Disaster Recovery Plan, and must have an associated metadata record (see for the Data Access Policy for full details).
- Raw experimental Measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements)
- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A8: Carbon and Nitrogen Assay in Solids using the Shimadzu TOC-L with SSM

AIMS ATSOP-003

INTRODUCTION

Sediment and filters are analysed by the AIMS analytical technology laboratory for Carbon and Nitrogen using the Shimadzu TOC-L with TNM-L and solid sample module (SSM). Samples may be analysed for total carbon (TC), organic carbon (OC) or inorganic carbon (IC). The SSM can be used for acidification of samples and direct measurement of IC, however this method is not currently being utilised in the AT lab and is therefore not included in this procedure. TC and OC can be measured, and the IC content calculated as the difference.

SCOPE

This procedure relates to the Shimadzu TOC-L with SSM in the analytical technology laboratory at AIMS. It includes the analysis of sediments and filters for TC, OC, N, and the indirect analysis of IC in sediments as the difference between TC and OC.

RESPONSIBILITIES

It is the responsibility of the instrument user to follow this procedure
It is the responsibility of the instrument user to obtain the appropriate training in the use of the equipment
It is the responsibility of the instrument user to maintain a safe and tidy work area
It is the responsibility of the instrument user to monitor the performance of the instrument and inform the AT lab coordinator of problems or anomalies.
The AT lab coordinator is responsible for scheduling maintenance and arranging service if required

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Ceramic boats are heated to 950°C during and prior to analysis	Burn	Always use tools to move boats. Wear heat resistant gloves when removing boats from furnace. Allow furnace to cool to <200°C before removing boats.
SSM chamber heated to 950°C	Burn	Ensure SSM has completely cooled and power supply is disconnected before performing any maintenance
Hydrochloric acid	Chemical burns Environmental damage	Always use acid in a fume hood. Exercise extreme caution and wear suitable PPE when adding acid to boats, particularly to sediments containing carbonates. Do not allow acid release to environment.

ENVIRONMENTAL CONSIDERATION

Hydrochloric acid may cause long term adverse effects in the aquatic environment. Do not allow release of HCl to the environment. Small amounts may be washed down the lab sink with copious amounts of water.

TOOLS & EQUIPMENT

- Shimadzu TOC-L with TNM-L and SSM
- Labec muffle furnace
- Suitable tweezers, tongs, heat resistant gloves
- Hot plate
- Fume hood
- Ceramic boats
- Pipette

MATERIALS AND REAGENTS

- Concentrated hydrochloric acid
- Sediment reference materials
- Platinum and Cobalt oxide catalysts
- Instrument air
- High purity oxygen

RECORDS

All usage, maintenance and troubleshooting of the instrument should be recorded in the instrument log. This should also include routine actions such as gas change. Processing templates are available in the AT lab Sharepoint. Results of in-house reference sediments should be recorded on the trend log stored in the AT lab SharePoint

PROCEDURE

Preparation of ceramic boats

Boats should be freshly prepared for analysis, they may be used up to 1 week after burning if covered by foil. The boats used for a days analysis must all come from the same tray.

- Used ceramic boats should have any residue brushed away or scraped using tweezers.
- Wash boats with tap water, scrub any residue with a brush
- When clean, rinse boats twice with RO water
- Place in drying oven until dry
- Arrange boats facing upwards in a ceramic tray suitable for the furnace. Boats may be in two layers if there are gaps in the top layer
- Place in furnace and heat to 950°C for 4 hours
- Boats may be removed when furnace has cooled to <200°C
- Using long handled tongs and heat resistant glove, remove tray of boats and place on ceramic mat and cover with a double layer of aluminium foil.
- Allow to cool to ambient temperature before use
- Storage boxes with 10 spaces are available to aid sample identification and preparation
- Use tweezers to place boats into storage boxes

Instrument Start-up

In this configuration, the SSM is connected directly to the detectors of the TOC-L, the sampler and column of the TOC are therefore not used

- Check the instrument air and oxygen cylinders are sufficiently full for analysis, turn on the air cylinder, the oxygen should already be on
- Instrument air regulator set point – 200kPa
- Oxygen regulator set point – 200kPa
- Check the halogen scrubbers are suitable. The copper wool should be a bright copper colour with only slight blackening.
- Check the SSM boat holder is functioning correctly and is not too loose or tight when sliding in and out of the furnace.
- Increase flow of carrier gas to 0.6 on the controller on front of SSM
- Ensure there is power to TOC and SSM, turn on TOC. SSM temperatures should begin to increase
- Turn on PC (password Abcde123) and open TOC-L software Sample Table Editor. Click 'OK' without entry in username pop-up
- Click 'New' in left column or 'File' → 'New' → 'Sample Table'
- Select TOC TN SSM then click Ok
- Click 'Insert' → 'Multiple Samples'
- Select TC_TN SSM.met as the method from the drop-down list, click 'Open'
- Click 'Next' and enter the number of samples (E.g. 60), click 'Finish'
- Click 'Connect' to connect to the instrument, the SSM will begin to heat if it has not already started to do so.
- SSM temperature: TC/TOC (left) – 950°C, TIC (right) – 200°C
- Click 'Monitor'. The baseline of the N and C channels will be displayed and the status of these. Zoom to an appropriate level. Typically the N baseline will be flat while the C baseline tends to slope downwards and becomes more stable with time. If there is a lot of noise in the baselines, troubleshooting may be required.

Standard/ Reference Preparation

- A reference material with certified amounts of C and N is used to construct a calibration curve by weighing varying amounts of the material.
- A six point calibration is used in addition to an empty boat as zero point.
- The mass of the highest calibration point should be selected to be close to the maximum peak height detectable by the instrument without truncation.
- The most accurate calibration will be achieved by uniform spacing of the standard weights.
- Record weights to an accuracy of 0.01mg
- Weigh 2 or 3 replicates of in-house reference materials and 2 or 3 additional replicates of the calibration standard
- These reference materials should be run periodically through the day to monitor drift
- All reference materials are stored in desiccators away from direct sunlight.

Procedure

- Using tweezers, remove a ceramic boat from the covered storage box, place on the balance and tare

- Add the required amount of material to the boat and record mass.
- Return boat to covered storage box to prevent airborne contamination

Note – do not attempt to remove material from the boat to reduce mass, reject and re-weigh if mass is too high.

Sample Preparation

1.1   Sediment Samples Total Carbon and Nitrogen

- Using tweezers, remove a ceramic boat from the covered storage box, place on the balance and tare
- Add the required amount of material to the boat and record mass.
- Return boat to covered storage box to prevent airborne contamination

1.2   Sediment Samples Total Organic Carbon and Nitrogen

- Using tweezers, remove a ceramic boat from the covered storage box, place on the balance and tare
- Add the required amount of material to the boat and record mass.
- Return boat to covered storage box to prevent airborne contamination
- To each sample, in a fume hood, add 100μL of concentrated HCl. The sample will fizz as inorganic carbon is dissolved and evolved as gas. More acid may be required if the IC content is high.
- Place the acidified sample on a hot plate at 80°C in the fume hood and allow to dry
- Return boat to covered storage box to prevent airborne contamination once dry

1.3   Filter Samples (Particulate Carbon and Nitrogen)

- Filters should be received frozen and remain so until analysis
- Using tweezers, remove a filter from the foil wrap and place in a ceramic boat
- In a fume hood, add 100μL of concentrated HCl to the filter (if the filter is 45mm diameter, more acid may be required)
- Place the acidified sample on a hot plate at 80°C in the fume hood and allow to dry
- Return boat to covered storage box to prevent airborne contamination once dry

Sample Analysis

- When ready to start analysis, check the baselines are stable and close the monitor window
- Click 'View' → 'Sample Window'
- Click 'Start', enter the date followed by incremental number as file name e.g. YYYY_MM_DD_001. This will be selected by the software by default. If a second run is started on the same day it will be named YYYY_MM_DD_002
- Click 'Save'
- Sample names are not entered into the Shimadzu software, the injection number of each analysis should be noted on the excel sheet used for processing.

Procedure

1.   Open the sample chamber and, using tweezers, place ceramic boat in the holder. Close the chamber and set alarm for 2 minutes. Click 'Set', the start button should be grey at this time and the software will indicate instrument not ready

2.   After 2 minutes the instrument should be ready and the start button active – if not, troubleshooting will be required

3.   Click 'Start' and push the sample into the furnace.

4.   Peaks will appear after about 30 seconds

5.   When the C and N traces are both more than halfway back to the baseline, pull the sample slide back to the cooling position. Set a timer for 2 minutes.

6.   After 2 minutes the sample slide may be pulled completely out

7.   A results box will popup when the sample analysis is finished

8.   Record the C and N peak areas on the excel spreadsheet

9.   Remove the analysed boat with tweezers, put in heat proof tray

10.   Continue from step 1.

Analysis sequence

- Begin the analysis with an empty boat. This may be considered a 'junk' sample
- Re-analyse the same boat that has just been run without removing from the system. This will indicate the system blank, response should be very small, if peaks are present it may indicate the instrument has not settled yet. Repeat the analysis of the same boat.
- Analyse another empty boat from the same batch used to prepare the samples for the day's analysis. This is the calibration blank used as the 0mg point in the calibration curve.
- Analyse the calibration standards in ascending weight order, the curve fit for C should be linear, and for N quadratic
- Analyse 1-2 inhouse reference materials to check response then proceed with sample analysis
- Reference materials should be analysed approximately every 10 samples to check instrument performance and drift.

Quality Control/ System Suitability

The R^2 value for both calibration curves should be ≥0.99. Points may be removed if they are obviously outliers, but the curve should contain at least 5 points in addition to the 0 point.
The recovery of inhouse reference materials should be 90 - 110% of expected based on the running average recorded in the trend logs.
Recovery of drift samples of the calibration material run throughout the day should be 95 - 105% of expected value

If any of these criteria are not met, investigation should be carried out and a nonconforming results investigation initiated.

Shutdown

- When the last sample is finished, click 'Stop' in the measurement window
- Click 'Shutdown' then 'Ok'. The instrument will start to cool
- The software may be closed, and the computer turned off at this point
- Turn off the Instrument Air cylinder
- Reduce flow of oxygen to approximately 0.1 using the regulator on the SSM
- Allow the SSM to completely cool before power is turned off

Maintenance

- Replace the halogen scrubbers every 12 months or when excessive blackening is apparent
- Replace the CO_2 scrubber every 12 months
- Replace the airlock o-rings as required (if leaking)
- Ensure the burred nut where the slider enters the airlock is tight

- A build up in back pressure may indicate the combustion column is partially blocked, this is most likely to occur in the narrow tube after the furnace.   A slight hiss when the airlock is opened is normal, if this becomes louder or the sample slide is pushed back by the gas, perform maintenance as follows:
- Ensure the SSM is completely cool and disconnected from power source
- Lift the top cover and unplug the fan to allow complete removal of the cover
- Loosen the 2 screws on the front of the sample slide
- Loosen the 4 large screws on the sides of the airlock
- Open and slide back the airlock cover
- Loosen the nut on the rear of the combustion tube and carefully disconnect the elbow fitting. This fitting commonly becomes slightly blocked it should be cleaned by sonication in 1M HCl
- Undo the large nut on the rear of the airlock
- The airlock assembly may be removed leaving access to the combustion column which may be removed from the furnace
- The catalyst should not need replacing, however if a decline in performance is suspected of being caused by poor catalyst function it may be replaced. The catalyst is a mixture of; 50g of Cobalt tetraoxide and 20g of Platinum on aluminium support. Approximately 45g is needed for the combustion tube.
- To pack the column, insert a mesh screen followed by 2.5mm of quartz wool. Add approximately 75mm of catalyst followed by 2.5mm of quartz wool and another mesh screen
- The combustion tube may be cleaned by soaking in 1M HCl
- Following repacking or removal of the combustion column, the system should be heated to working temperature for a few hours before samples are analysed, trial samples should be run during this time to check performance.

Troubleshooting

- Build-up in back pressure and/or poor peak shape may occur with time due to partial blockage of the combustion column. Follow maintenance in section 9
- Leaks may occur particularly at any o ring or junction around the sample block. Leaks may cause poor peak shape or changes to response. Follow procedures in SSM-5000A manual for maintenance and troubleshooting if suspected
- Poor peak shape, such as double peaks may be caused by:
    - Insufficient acid or drying time
    - 45mm filters generally produce poor peak shape, typically with an early C spike
    - Contamination of boats
    - Gas leaks
    - Some sediments will have a double C peak in TC analysis, where the organic C is combusted first followed by the inorganic C. This is normal, in-house sediments which show this pattern are available and should be analysed for reference.
    - If a problem with the detector is suspected due to a high background and all other troubleshooting has not identified an issue, contact Shimadzu support. A re-zero of the detector may be possible.

REFERENCES

Shimadzu Total Organic Carbon Analyser TOC-L CPH/CPN and SSM-5000A user's manuals

Section A9: Analysis of Chlorophyll-a in marine waters

AIMS-SOP-EFWQ-v1.0

Document Details

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TASKS INCLUDED IN THIS SOP

To measure the concentration of chlorophyll-*a* and phaeophytin-*a* present in seawater to obtain an estimation of phytoplankton biomass.

PRINCIPLE AND RESPONSIBILITIES

This method utilises the natural fluorescence of chlorophyll-*a* (Chl-*a*) to derive the concentration of the pigment present in an extract of material filtered from seawater. The fluorescence of decomposition products (phaeophytin-*a*) can cause significant interference. This method compensates by taking an additional fluorescence reading after acidification of the original sample (by which all pigments present are decomposed) and using the decreased fluorescence reading to derive the original concentration of live pigment.

This method follows the basic fluorometric method set out in Strickland and Parsons (1972). The concentration of the primary standard used for calibration is determined spectrophotometrically using the equations laid out in Jeffrey and Humphrey (1975).

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Chemical preparation and handling (90% Acetone and 10% HCl)	Chemical splash, spillage, or fume inhalation	Knowledge of the MSDS. Use appropriate PPE including safety glasses, lab coat, and gloves. Work with chemical materials within a fume hood. Ensure samples are capped properly when handling outside of the fume hood.
Manual handling	Biomechanical	Reach ergonomic position when working within the fume hood and using the tissue grinder. Organize the workstation to avoid repetitive strain injury. Take regular breaks.
Shattering glass tubes during sample processing	Injury	Use appropriate PPE including safety glasses, lab coat, and gloves. Inspect glass tubes and grinder before use.

Unbalanced centrifuge	Injury	Receive training in centrifuge use by experienced technician. Ensure centrifuge is balanced before closing the lid.
Electrical	Electrical shock	Protect electronic components from water leaks/spills and clean up any spills immediately. Disconnect instrument before conducting maintenance or servicing. Call an electrician if you are not sure you can solve the problem safely.
Exposure to UV light	Injury	Do not attempt to open the fluorometer housing while the instrument is running.

PERSONNEL PREREQUISITES & COMPETENCIES
Laboratory safety induction
Read and understood protocol
Training by experienced technician

TOOLS & EQUIPMENT

EQUIPMENT
- 25 mm Whatman GF/F glass fibre filters, pre-combusted (4 hours at 450°C)
- Millipore forceps (2 pairs)
- Squares of aluminium foil (~8 x 8 cm), pre-combusted (4 hours at 450°C)
- Labels for tubes (pre-printed)
- 100 mL graduated measuring cylinder
- 25 mm filter funnels and supports
- Vacuum manifold, water reservoir, vacuum tubing, and vacuum pump
- Turner Designs Model 10-AU fluorometer
- 10 mL round quartz cuvette
- High speed tissue grinder comprising motor, pestle, and tube (Potter Elversham No. 23)
- 12 mL centrifuge tubes with caps pre rinsed with 90% acetone
- Centrifuge with 12 mL holders
- Plastic Pasteur pipette
- Kimwipes

CHEMICALS
- 90% acetone – 900 mL of analytical grade acetone in 100 mL of Milli-Q water
- 10% hydrochloric acid – 10 mL of analytical grade hydrochloric acid in 90 mL of Milli-Q water

ENVIRONMENTAL CONSIDERATION

Avoid the release of concentrated hazardous chemicals (100% acetone, 37% HCl) in sewage waste stream by either evaporation or dilution.
Avoid release of ground GF/F filters in sink by filtrating and discarding appropriately the 90% acetone/ground GF/F waste.

CHEMICAL DISPOSAL

90% acetone is evaporated in fume hood for 24 hours before water residue is poured down the laboratory sink.

REFERENCES

Jeffrey, S.W. and G.F. Humphrey, (1975). New spectrophotometric equations for determining chlorophylls a,b,c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen, 167: 191-194

Strickland, J.D.H. and T.R. Parsons, (1972). A Practical Handbook of Seawater Analysis, Bull. Fish. Res. Board Can.167.

USEPA METHOD 445.0: In vitro Determination of chlorophyll a and phaeophytin a in marine and freshwater phytoplankton by fluorescence, United States Environmental Protection Agency Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples, EPA/600/R-92/121.

Tuner Design Model 10AU fluorometer user's manual, April 1999

PROCEDURE

SAMPLE COLLECTION

- Filter 100 mL of seawater sample through a 25 mm Whatman GF/F glass fibre filter (through vacuum manifold or syringe filtering). Duplicate samples are recommended.
- The filter is then wrapped in aluminium foil and labelled with collection information. The volume of seawater filtered is recorded. When sampled in duplicate, there should be 2 filters per foil packet.
- Freeze the filters immediately (-20°C).

TURNER DESIGNS 10AU FLUOROMETER CALIBRATION

The Turner Designs 10AU Fluorometer needs to be turned "ON" for at least 1 hour before any calibration or measurement can be undertaken. This ensures that the UV light source has reached the appropriate spectrum and temperature for accurate measurements.

A simple way to optimise the fluorometer for this analysis method is to use a factory-supplied Solid State Standard (SSS) and set it to read 1.000 Mv in its "L" position (please note that not all SSS are the same and cross calibration may be required if a new SSS is purchased). The routine for adjusting the fluorometer using this SSS is shown in Appendix 1. You will need to have the machine manual on hand to do the initial set up of the machine.

Although very stable, the calibration of the fluorometer should be affirmed every 12 months using the chlorophyll-*a* calibration and door factor assessment methods described in Appendix 1. A worksheet showing the calibration method is shown in Appendix 2.

Fluorometer recalibration and door factor assessment is also required if the factory solid state standard (SSS) no longer reads 1.000 +/- 0.050, the lamp has been changed, service has been done, or the instrument has been moved.

a. Calibration Control (daily)

Each fluorometer has a manufactured fluorescence SSS that can be used for a daily working calibration or to calculate drift. Insert the standard at the beginning and end of each set of samples read and at regular intervals throughout the set. Make sure the "L" marking on the barrel is placed toward the left-hand side of the holder. A record is kept of the Solid Standard measured at the time of machine calibration and drift should be corrected referring to this original measurement (Appendix 3).

Drift corrected C_a = (Calculated C_a x Original Solid Standard) / (Measured Solid Standard)

where:
C_a = concentration of chlorophyll-*a* in µg L^{-1}

If at the beginning of the day the reading shows greater than 5% variation from the original, the emission and excitation filters of the fluorometer may need to be cleaned with an ethanol dampened tissue.

SAMPLE ANALYSIS

⚠ Chlorophyll-*a*, especially when extracted, is extremely light sensitive. All extractions must be carried out in the dimmest light possible. When not using extracted solutions, keep them wrapped to exclude light and store in a refrigerator. Keep extracted samples in a closed box or cover with a black cloth.

⚠ Chlorophyll-*a* is rapidly and irreversibly degraded by acid, which converts active chlorophyll-*a* to phaeophytin-*a*. Do all sample grinding, extractions, and analyses in the designated chlorophyll-*a* fume hood. Do not use acids (especially HCl) in this fume hood.

⚠ Keep all equipment used for chlorophyll-*a* analysis free of acid.

1) Grind the filters in 90% acetone in a tissue grinding tube, including a dry filter blank sample for calibration and calculations.
2) Pour suspension into a labelled graduated 12 mL centrifuge tube and make up to 10 mL with fresh 90% acetone while cleaning grinding tube.
3) Cap graduated tube and shake.
4) Clean and rinse tissue grinding tube with 90% acetone and remove any excess filter from tissue grinding pestle after each sample.
5) Leave the tubes to stand in the dark for 2 hours. Either cover the box of extracted samples or cover the tubes with a thick black cloth to block out ambient light.
6) Centrifuge for 10 minutes at 3500 rpm.
7) Rinse round quartz cuvette 3 times with 8 mL of 90% acetone and discard acetone in an evaporation beaker.
8) Dry the rim of the cuvette with Kimwipes.
9) Transfer approximately 8 mL of the sample extract supernatant to the quartz round cuvette (up to the arrow marking on cuvette).
10) Wipe the side of the round quartz cuvette with Kim wipes.
11) Insert in the cuvette holder and replace cap on the holder.
12) Read the fluorescence of the sample. Make note of reading on "F-orig" column on worksheet (Appendix 3)
    a. If the reading on the fluorometer exceeds 1.5 Mv, sample dilution will need to be carried out to obtain accurate results.
    b. According to the initial fluorometer readings, dilute 1 to 5 mL of sample supernatant in a known volume of 90% acetone using a clean 12 mL tube and an accurate pipette.
    c. Repeat sample analysis from step 6.
    d. Write down the dilution factor for this sample on the worksheet (Appendix 3).
13) Add 2 drops of 10% HCl in the sample cuvette using a plastic Pasteur pipette. Allow the acid to react and take another reading. Make note of reading in the "F-acid" column on the worksheet (Appendix 3)
14) An unused blank GF/F filter should also be processed in the same manner to give a blank value.

A simplified flow chart of sample analysis is shown in Appendix 5.

CALCULATIONS

Chlorophyll-*a* and phaeophytin-*a* may be calculated using the following equations. The equations assume that the filtered sample volume is 100 mL and that there is 10 mL of acetone extract. The calculation spreadsheet (Appendix 4) allows for variation of these numbers for individual samples.

$$C_a = F_D"(R_B - R_A)$$
$$Phaeo = F_D"(2.2R_A - R_B)$$

where:

C_a = concentration of chlorophyll-*a* in µg L^{-1}

Phaeo = concentration of phaeophytin-*a* in µg L^{-1}

$F_D"$ = door factor (see Appendix 1)

R_B = reading before acid addition-blank

R_A = reading after acid addition-blank

If volume filtered or volume of acetone were to differ, the values for R_B and R_A are calculated as followed:

$$R_B = (Fo_s - Fo_{db}) \times (V_x \times 10)/V_F$$
$$R_A = (Fa_s - Fa_{db}) \times (V_x \times 10)/V_F$$

where:

Fo_s = F - orig sample reading

Fo_{db} = F - orig dry blank reading

Fa_s = F - acid sample reading

Fa_{db} = F - acid dry blank reading

V_x = volume of acetone extract

V_F = filtered sample volume

METHOD CAPABILITIES

Chlorophyll-*a* concentrations ranging from 0.1 to 50 µg L^{-1} in the acetone extract (i.e., a 100 mL sample ranging in concentration between 0.01 to 5 µg L^{-1}) may be measured by this method without recalibration of the instrument. Detection capability for water samples with very low concentrations can be improved by increasing the volume of water filtered. Likewise, in high concentration samples, either smaller volumes may be filtered, or the extracted sample may be diluted.

QUALITY CONTROL

QA/QC tests undertaken as part of the data reporting include:

<u>Assessment of the limit of detection</u>

- The Limit of Detection (or detection limit) is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence).

- Minimum detection limits were calculated for this method using repetition of blanks.
- The effective detection limit for a 100 mL sample is 0.1 µg L^{-1}.

Assessment of accuracy of the analysis

- This is generally achieved by using reference materials to assess recovery of known amount of analyte.
- The Solid Secondary Standard (SSS) is run daily at the beginning and end of the sample run. For each sample set, an average of the readings as a percentage of the true value is used to determine accuracy.
- A yearly instrument calibration against a commercially available Chlorophyll-*a* standard is performed to ensure the accuracy of fluorometer readings and associated measurements (Appendix 1).

Assessment of precision of the analysis

- This is generally achieved by the repeated analysis of the same concentration of analyte to check for reproducibility.
- The coefficient of variance between repeated readings of the SSS at the beginning and end of an associated set of samples is calculated to assess precision.

Procedural blanks

- Filter blanks are run with every batch (generally daily). These are used as blank readings in the calculation of analytical results.
- Wet filter blanks are collected during sample collection to measure contamination of GF/F filters or through handling.

Reproducibility of duplicate analytical units

- The variation between results for sample duplicates indicates the reproducibility of the analysis and the effects of various sources of contamination and analytical error during collection, sample preparation and analysis. Before data analysis, duplicate samples are used to calculate the coefficient of variance (CV) for each sample. Duplicates with CV outside a pre-determined confidence range (20%) are marked for re-run analysis performed using duplicate spare samples.

Other

- Inter-calibration of this method with the HPLC method used by AIMS laboratories showed agreement of +/- %10.
- The linear range of this method was established utilizing increasing concentrations of primary standard. Samples are diluted if above this range.
- Calibrations cross-referenced by computer to analysis date.

- External calibration is run periodically or if SSS detects machine drift greater than 5%.

APPENDICES

All appendixes must be referenced within the body of the SOP

#.1	Solid state standard adjustment, chlorophyll-*a* calibration, and door factor assessment
#.2	Chl cal worksheet template V2 example
#.3	Chlorophyll-*a* worksheet
#.4	Chlorophyll-*a* calculation template
#.5	Flow chart for chlorophyll-*a* analysis

RECORDS

All AIMS analyses undertaken on behalf of AIMS or client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with External Document and Data Control Policy, Data Access Policy and AIMS Disaster Recovery Plan, and must have an associated metadata record (see for the Data Access Policy for full details).
- Raw experimental Measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements)
- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A10: Measuring total suspended solids in seawater

AIMS-SOP-EFWQ-v2.0

Document Details

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TASKS INCLUDED IN THIS SOP

This method outlines the field and laboratory procedures for determination of the concentration of total suspended solids by gravimetry in a sample of seawater.

PRINCIPLE AND RESPONSIBILITIES

The method is based on gravimetrically determining the dry weight of particulate material collected from a known volume of water onto a pre-weighed membrane filter.

Unlike samples from freshwater environments, those collected in a marine environment may have high levels of dissolved salts that need to be either minimised or compensated for by accurate measurement of the weight of salts retained. This method utilises the first strategy through minimal interstitial retention by filter choice and rinsing. Polycarbonate filters are used (rather than glass fibre filters) to optimise the effects of rinsing and minimise interstitial capacity.

SIGNIFICANT HEALTH & SAFETY RISKS

Hazards	Risk	Control
Electrical	Electrical shock	Protect electronic components from water leaks/spills and clean up any spills immediately. Disconnect instrument before conducting maintenance or servicing. Call an electrician if you are not sure you can solve the problem safely.

PERSONNEL PREREQUISITES & COMPETENCIES
Laboratory safety induction
Read and understood procedure
Training by experimented technician

TOOLS & EQUIPMENT

- 1 litre plastic bottle or jar
- Polycarbonate membrane filters - 0.4 µm pore size x 47 mm diameter (Nuclepore, Poretics, etc….)
- Clean 20 mL scintillation vials with caps
- Millipore forceps (2 pairs)
- Filter funnels (47 mm), filter supports, and clamps
- Vacuum manifold, water trap and pump
- Analytical balance (5 decimal places)
- 100 mL, 250 mL or 1 litre plastic graduated cylinder
- 60 mL sterile syringe and 0.2 µm syringe filter (Pall Acrodisc or similar)
- Drying oven set at 60°C
- Milli-Q water in squeeze bottle, ~30-60 mL needed per filter
- Fine-tip permanent labelling marker

ENVIRONMENTAL CONSIDERATION

None as no reagents or preservatives are used during the sampling or measurement processes.

CHEMICAL DISPOSAL

No chemicals are used the processing of samples.

REFERENCES

Neukermans, G., K. Ruddick, H. Loisel and P. Roose, 2012: Optimization and quality control of suspended particulate matter concentration measurement using turbidity measurements; Limnology and Oceanography: Methods 10, 2012, pp1011-1023.

PROCEDURE

1.   CAPABILITIES AND LIMITS OF DETECTION

As with any gravimetric measurement the limiting factor for detection is often sampling technique, the homogeneity of a sample and the level of reproducibility that can be achieved when subsampling. Following a series of wet filter blank trials, it was concluded that the smallest amount of solid that can be confidently measured is 0.10 mg. Therefore, when one litre of seawater is filtered, the lower limit of detection is 0.10 mg L^{-1}. The upper limit is dependent on particle load and size. Turbid water with a lot of fine material will block the filter prematurely, allowing only a small volume to be filtered. River water or sediment trap water samples with large concentrations of fine particles may clog the filter after as little as 10 mL is filtered. Such small volumes will lead to a greater relative error

due to small variations in the volume filtered and degree of homogeneity within the sample. Thus, this method is not suitable for turbid waters and is only appropriate in the coastal or open ocean.

Wet filter blanks are produced by following the same preparation steps as for the samples but using ~20 mL of 0.2 μm filtered seawater (typically filtered sample water is used). The average weight of these sets of blanks is subtracted from the final dry weight of sample to account for salts not washed out in the Milli-Q water rinse step. If no rinsing is done, then the salt retained in the filter can sometimes weigh as much as the sample dry weight. Polycarbonate filters are used not only because they are less hydroscopic than the traditionally used glass fibre filters (i.e., do not require a desiccator for storage) but also because minimal interstitial volume allows only minute traces of salt to be retained in a filter after rinsing. They also dry more easily, reducing drying times and the necessity for re-weighing to guarantee dryness as is necessary for glass fibre filters.

2.   PREPARATION PRIOR TO FIELD WORK

- Pre-label the lids of one or more trays (100) of clean, dry 20 mL scintillation vials with an alphanumeric sequence (e.g. JC100, JC101, JC102……) using a fine tip marker pen.

- Using a 5-place analytical balance weigh a filter and transfer the weighed filter to a numbered vial without wrinkling or creasing the filter. (photo 1). Handle the filters only with the forceps.

- Make a record of the filter weight against the vial number (Appendix 1).

- Repeat for required number of filters.

3.   SAMPLE COLLECTION AND HANDLING

⚠️   Pictures and details of the following process are described in Appendix 3

- TSS bottles can be filled on site and then transported back to land in the dark on ice. TSS can be filtered within 24 hours of collection as long as it is kept refrigerated. This will inhibit the growth of microbes and the formation of colloidal masses that block the pores of the filter.

- Set the vacuum pump and manifold up using 47 mm filter funnels that are held in place with clamps. Check that the water trap is empty before beginning filtering.

- Take a pre-weighed polycarbonate membrane filter out of its scintillation vial using forceps. Place the filter on the filter support. Wet the filter with a small amount of Milli-Q water and place the filter funnel on top, securing with a clamp.

- Before beginning filtering, make sure to record the code written on the scintillation vial in the Field Datasheet (Appendix 2).

- Collect 1 litre sample of seawater in the 1 L plastic bottle. Before drawing off the sample to the bottle make sure that the sample is homogenous by thoroughly mixing/shaking the Niskin or collection vessel if it has been sitting undisturbed for >5 minutes. If the 1 L plastic bottle has been sitting >5 minutes, make sure to shake it well before beginning filtering so that all material is resuspended.

- Turn on the vacuum pump and up-end the 1 L bottle on top of the filter funnel. The neck of the bottle will stop water flowing over the top of the filter funnel as long as you don't disturb the bottle. Open the plastic tap on the manifold to begin filtering.

- If the water is very turbid (Secchi depth < 3 m) and it is unlikely that the full amount of sample with pass through the filter without clogging, it may be necessary to reduce the volume filtered. In this case, use a graduated cylinder to measure a smaller volume for filtering. Ensure you shake the sample well before pouring into the graduated cylinder and record the volume filtered on the Field Datasheet. It should take no longer than 20 minutes to filter a sample.

- After the sample has completely filtered through and the filter is sucked dry, wash down the sides of the funnel and rinse the filter with ~20 mL of Milli-Q water. Ensure that Milli-Q covers the entire filter surface (you may need to turn off the manifold tap temporarily to reduce suction to achieve this). Allow the filter to suck dry and repeat this rinse process two more times.

- Wait until all water has filtered through and turn the vacuum suction almost all the way off by using the plastic tap at the base of the filter funnel. Leaving the vacuum on very slightly helps to keep drying the filter while you remove it from the filter support. It also prevents water sitting in the manifold base from refluxing back onto the filter. Remove the filter funnel and gently lift one side of the filter with the forceps to break the vacuum. The filter should be perfectly dry with no moisture on it.

- Using forceps, fold the filter in half with the filtered material on the inside, then in quarters. The forceps should not touch the coloured central part of the filter, but only the clean outer rim. Return the folded filter to the numbered scintillation vial from which it came.

- Record the vial number, volume sampled and replicate number against the station name and depth on the Field Datasheet.

- The filtered samples can be stored at room temperature for short periods (1-2 days) if kept dry but avoid hot or humid storage areas as mould may become a problem. If possible, dry the filters at 60ºC before extended storage. If conditions are humid and a drying oven is not available, store the filters in fridge or freezer.

4.   ANALYSIS

- Loosen the caps on the scintillation vials to allow some air transfer, but do not separate them from their vial. Dry at least overnight at 60°C in a drying oven. After the filters are dry, re-tighten the caps on the vials and allow them to cool to room temperature.

- Re-weigh the dried filter on a 5-place analytical balance. Record the loaded filter weight (final weight) on the worksheet next to the initial filter weight (Appendix 1). If working at AIMS, use the filter weight system to record this information.

5.   BLANKS

- A set of wet filter blanks should also be run with collection as part of the sample processing routine. This involves putting a filter on the filtering funnel and wetting with Milli-Q (this saturates the filter under the "lip" of the funnel, minimizing saltwater intrusion). Next, pour a small volume (~20 mL) of 0.2 µm filtered seawater into the filter funnel. Typically filtered sample water from the last sampling site of the day is used for this.

- After the sample has completely filtered through and the filter is sucked dry, wash down the sides of the funnel and rinse the filter with ~20 mL of Milli-Q water. Ensure that Milli-Q covers the entire filter surface (you may need to turn off the manifold tap temporarily to reduce suction to achieve this). Allow the filter to suck dry and repeat this rinse process two more times.

- This procedure combines both a transport and handling blank and corrects for the very small amount of salt left behind in and on the polycarbonate filter. A duplicate set of blanks should be taken with each collection trip. The average weight of the duplicates then becomes the wfb value for that collection trip.

6.   CALCULATION

Suspended Solids = $\dfrac{\text{(final weight – initial weight) – wfb}}{\text{Sample volume}}$

Where:

initial weight = weight of unused filter (g)

final weight = weight of loaded filter (g)

wfb = weight of averaged duplicate wet filter blanks (g)

Sample volume = volume of sample filtered (litres)

Suspended solids = concentration (g L^{-1}) of particulate matter in sample with a particle size greater than 0.4 µm.

REPORTING

Results are generally reported in mg L^{-1} for seawater. If this is the case, the following additional calculation will need to be made.

Suspended Solids (mg) = Suspended Solids (g) x 1000.

APPENDICES

All appendixes must be referenced within the body of the SOP

#.1	Example of log sheet for recording suspended solid filter weights
#.2	Example of field data sheet
#.3	Quick reference for filtering total suspended solids samples
#4	
#.	

RECORDS

All AIMS analyses undertaken on behalf of AIMS or client must be recorded and stored as follows:

- Data must be lodged in a manner that complies with External Document and Data Control Policy, Data Access Policy and AIMS Disaster Recovery Plan, and must have an associated metadata record (see for the Data Access Policy for full details).
- Raw experimental Measurement data must be stored as hard copy and digitally as specified by the External Document and Data Control Policy, and data from processing samples must be stored in the Data Centre with appropriate metadata (see this policy for other approved storage locations and requirements)
- The data custodian(s) is/are responsible for lodging the data and generating the metadata record.

Section A11: AIMS In-house procedures for WET Labs FLNTUSB ECO logger

11.1   Set up WET Labs FLNTUSB ECO logger:

11.1.1 Start ECOView and the logger
- Connect the battery leads on the test cable to the 12V battery (adaptation of the test cable by AIMS). Connect the DB-9 connector to a computer with the ECO View program installed.

- Start up the ECO View software:
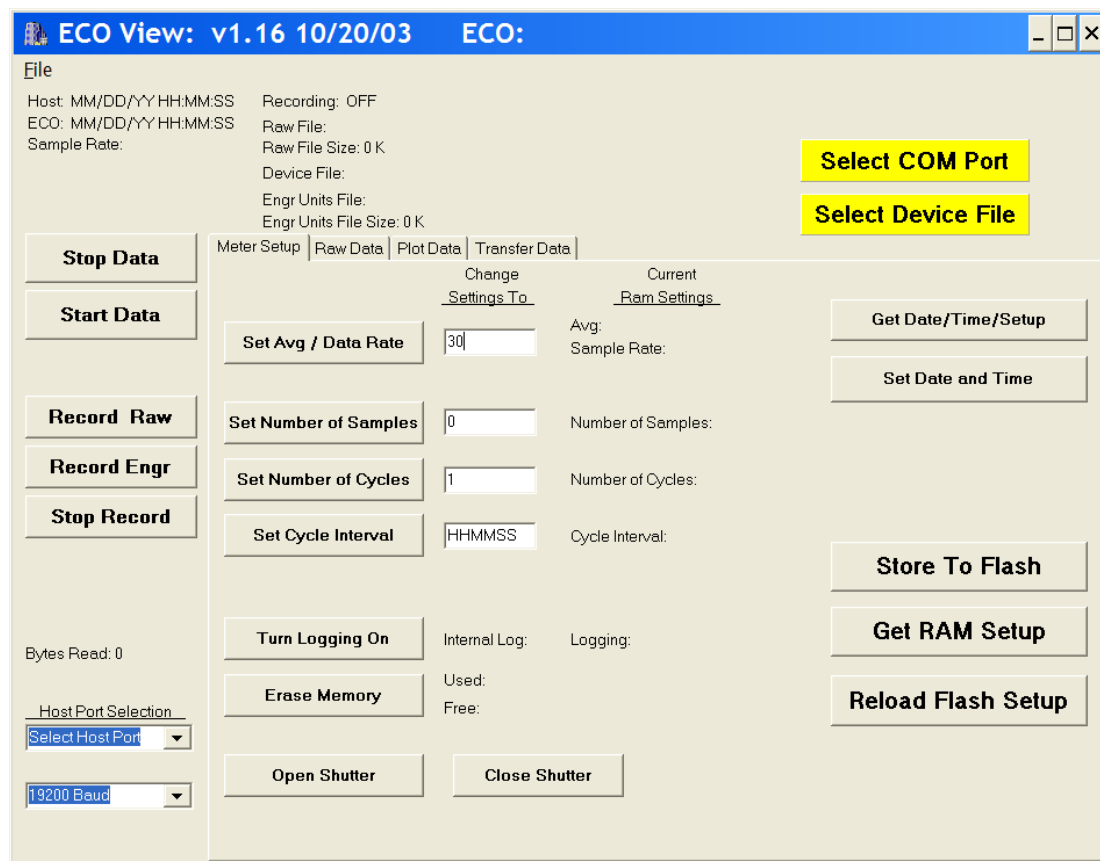
- Click [Host Port Selection / Select Host Port] and select appropriate port for the DB-9.

- Click **Select Device File** and browse for the most recent version of the device file (.dev) appropriate to the logger you are running.

The logger with serial number 818 requires device file FLNTUSB-818.dev. Device files are updated by WET Labs when the logger is returned the manufacturer for calibration and maintenance. (The device file is specific to the logger if creating output in 'Engineering units', but not for 'Raw' output. However a device file must still be selected to create raw output.)

Note that ECO View cannot read files stored in folders containing spaces in the folder names, therefore do not store sub-folders in the 'My Documents' folder or others with spaces in the name.

- Connect the 6-socket connector on the test cable to the logger to provide power to the LEDs and electronics. Light should emanate from the optical window of the logger, the frequency determined by the setup. **Quickly** stop the logger (you can only stop the logger when the light is flashing during a reading, so if the logger is set to read at 10 minute intervals you have limited opportunities.)

- To stop logger, click **Stop Data** multiple times whilst the instrument is emanating light during the reading. The logger should stop running.

11.1.2 Check ECO View settings.

In 'Meter Setup' tab view, ensure the logger is setup to sample correctly during lab calibration.

- Click **Reload Flash Setup**

- Then **Get RAM Setup**

The 'Current Ram Settings' column of the parameter list is now updated with the current settings of the logger:

If the logger was set for logging in the field, the settings will need to be altered for laboratory calibrations.

Settings for laboratory calibration checks:

- Enter '50' as Avg / Data Rate.

Set Avg / Data Rate   50   Then click 'Set Avg / Data Rate' button to set.
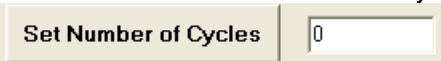
- Enter '1' as Number of Samples.

Set Number of Samples   1   Then click 'Set Number of

Samples' button to set.

- Enter '0' as the Number of Cycles.

Set Number of Cycles | 0   Then click 'Set Number of Cycles'

button to set.

- Enter '000005' as the Cycle Interval.

Set Cycle Interval | 000005   Then click 'Set Cycle Interval' button

to set. (Samples every 5 seconds.)

The instrument will average 50 samples, for one reading, once every 5 seconds; beginning on power- up.

Settings for field logging:
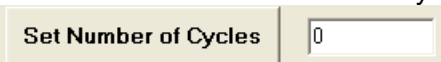
- Enter '50' as Avg / Data Rate.
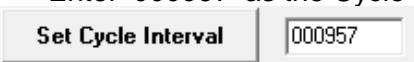
Set Avg / Data Rate | 50   Then click 'Set Number of

Samples' button to set.

- Enter '1' as Number of Samples.

Set Number of Samples | 1   Then click 'Set Number of

Samples' button to set.

- Enter '0' as the Number of Cycles.

Set Number of Cycles | 0   Then click 'Set Number of Cycles'

button to set.

- Enter '000957' as the Cycle Interval.

Set Cycle Interval | 000957   Then click 'Set Cycle Interval' button

to set. (Records every 10 minutes.)

- Alternatively you may need to enter '000958' in older instrument versions.

- **IMPORTANT: Turn logging on**, click   **Turn Logging On**   so that the data is stored in the logger memory.

The instrument will average 50 samples, for one reading, recording every 10 minutes; beginning on power-up.

Set Avg/Data Rate = 50   Average 50 Samples for one

measurement Set Number of Samples = 1   Take 1 Measurement (takes 2

seconds) Set Number of Cycles = 0   Cycle continuously

Set Time Interval = 000957   (HHMMSS) 9 minutes 57 sec per cycle.

Commit logger settings:

To commit the above settings to the logger, click **Store To Flash**

- When settings committed, the yellow warning symbol **Setup Not Stored** should disappear from the top right hand corner of the screen.

- To ensure flash storage, first click **Reload Flash Setup**

- Then click **Get RAM Setup** and check that 'Current RAM settings' have been changed to the correct values.

- To align time on logger with time on laptop click **Set Date and Time** (This will not work if the instrument is currently logging.)

11.2   AIMS Laboratory calibration checks

To determine instrument drift between field deployments, several checks can be performed.

1. Dark counts (in fresh water)
2. Determination of maximum fluorescence (in air)
3. In-house turbidity and chlorophyll calibration checks (*in situ* in seawater, co-deployment from AIMS pontoon)
4. In-house temperature calibrations (in calibration bath).

The settings used for the checks 1 and 2 are as per Section 11.1.2 'Settings for laboratory calibration'. Field sampling and AIMS pontoon co-deployment readings occur every 10 minutes which is too infrequent for laboratory tests.

11.2.1 Dark counts

Pre-logging preparation

The 'dark cap' is used for dark counts performed as a comparison to WET Labs factory calibration dark counts and therefore carried out as in WET Labs servicing laboratory. The external battery source is used and the bio- wiper is removed.

- Setup logger as per Section 11.1 and settings as per Section 11.1.2 'Settings for laboratory calibration'
- Stop the logger if it is running.
- Remove the bio-wiper.

Remove the bio-wiper. IMPORTANT the logger must not be running when you do this. **Note, manually turning the motor shaft can damage the wiper motor and void the warranty.**

- Apply black electrical insulation tape to cover the optical window.
- Fill large plastic beaker with water and immerse the 'back cap'.
- Insert the optical head of the logger into the 'black cap' immersed in water.

AIMS Water Quality group standard height of the logger collar is positioned 6cm from the optical end of the logger, which locates the logger at a suitable standard height within the calibration

cap. (The height is not relevant for dark counts, but it is for protection of the bio-wiper in transit to the field.)

Logging

Run logger in ambient light.

- Click **Start Data**

- View 'Plot data' menu tab:
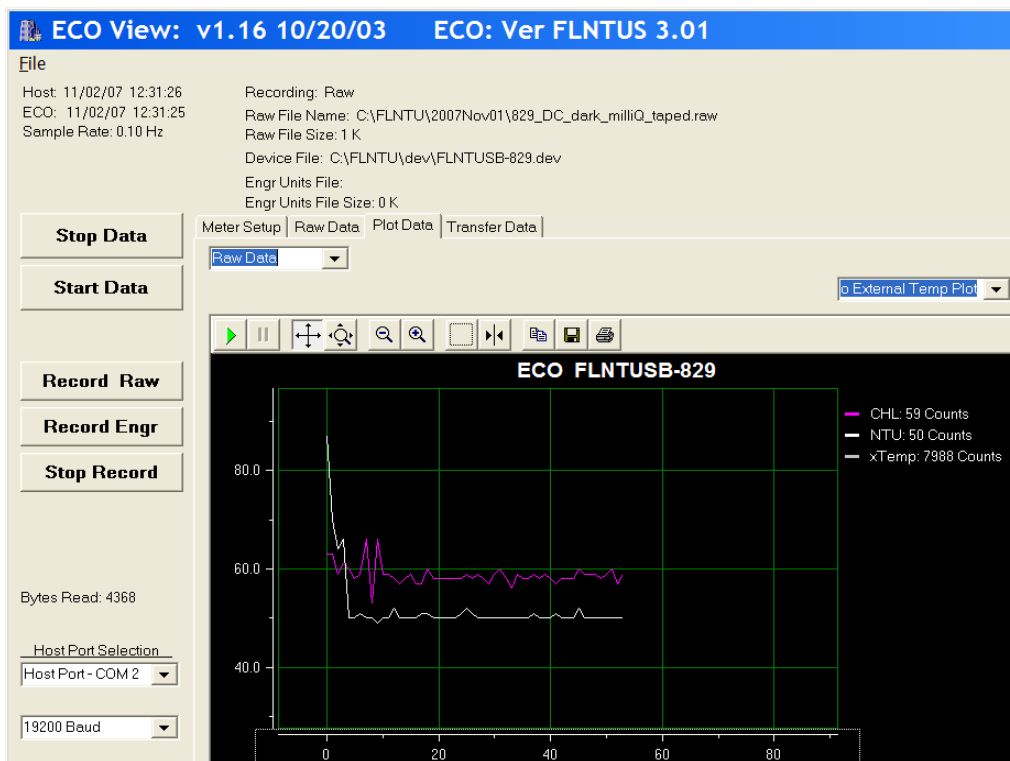
- Once plot has settled to consistent readings, click **Record Raw**

- Browse to the appropriate folder to store the .raw file, and 'save'.

- Record this start time on the data sheet.

- File name convention: 829_DC_2007Dec06.raw Ie. Logger 829, Dark Count, year, month, day.

- Continue logging for 5 minutes to obtain ample readings to calculate a minimum dark count.

- Stop logging, click **Stop Record** Then click **Stop Data** multiple times whilst the instrument is emanating light during the next reading. The logger should stop running.

- Record this end time on the data sheet.

- Remove the logger from the calibration cylinder and remove the black tape from the

optical port.

- Unplug the logger.

Your raw data file can be found in the folder you specified.

11.2.2 Determination of maximum fluorescence.

WET Labs provides a pink fluorescence stick as one of the 'spare parts'. This can be used to check whether the maximum fluorescence output is reading the same as the output specified in the FLNTU Characterization Sheet for each logger.

Pre-logging preparation

To measure maximum fluorescence the external battery source is used, and the bio-wiper is removed.

- Setup logger as per Section 11.1 and settings as per Section 11.1.2 'Settings for laboratory calibration'
- Stop the logger if it is running.
- Remove the bio-wiper.

Remove the bio-wiper. IMPORTANT the logger must not be running when you do this. **Note, manually turning the motor shaft can damage the wiper motor and void the warranty**.

Logging

Run the logger in ambient light.

- Click **Start Data**
- View 'Raw data' menu tab:

- Hold the fluorescent stick approx. 1 cm from the optical port, in the air parallel to the logger optics as per the Wet Labs information sheet.

When it is apparent that the position of the stick is suitable (by reading maximum counts on the Raw data' window in 4th column)

- Click **Record Raw**

Example above indicates maximum output for chlorophyll signal 4120 counts.

- Browse to the appropriate folder to store the .raw file, and 'save'.

- File name convention: 829_fluor_2007Dec06.raw, i.e. Logger 829, Fluorescence determination, year, month, day.

- Record three maximum output readings to compare with FLNTUSB Characterization Sheet. Enter maximum value on data sheet.

- Stop logger, click **Stop Record**, then click **Stop Data** multiple times whilst the instrument is emanating light during the next reading. The logger should stop running.

- Unplug the logger.

Your raw data file can be found in the folder you specified.

11.2.3 AIMS In-house 'Pontoon' turbidity and chlorophyll calibration checks

To test the logger data output against laboratory analyses, as well as to compare logger output relative to each other, AIMS has adopted a co-deployment of loggers in addition to laboratory checks. Results from AIMS Pontoon deployments provided to WET Labs initiated a new calibration procedure in their factory.

- All available ECO loggers are co-deployed for a 2–3 day period prior to servicing and between redeployment cruises
- they are placed in a rack and hung in coastal seawater off the AIMS Pontoon
- they are set to log in 'field mode' with readings at the same times, every ten minutes

During this period the loggers are exposed to varied turbidity and chlorophyll levels, due to the tides and relatively high turbidity of the location of the pontoon.

- Water samples are collected to coincide with the logger readings, (3–6 times per day) at the same 10 minute intervals that the loggers are set to record
- Water samples are collected adjacent to the logger rack with weighted bottle holders which have acid washed bottles inserted and suspended by stainless cable
- The water samples are filtered immediately after collection, in the AIMS laboratory
- These water samples are filtered and analysed for Chl-*a* and TSS following the same procedures as AIMS MMP field sampling.
- 2 replicate Chl-*a* samples are collected at each time
- 2 replicate TSS samples are collected at each time
- The water sample collection times, volumes filtered, filter numbers are recorded and entered into the Oracle database using the same procedures as the AIMS field water sampling
- The logger data is downloaded following deployment as per field deployment procedures
- The data records from the loggers are loaded into the database with the site name 'AIMS' and the filter samples collected identified with the 'ECO' sample i.d. code to distinguish them from field cruise samples
- The logger data is plotted together to determine differences between loggers, and against the laboratory samples how they sit within the range of the laboratory analyses

The logger rack was built by the AIMS workshop, specifically for the purpose of co-deployment. The loggers are in close proximity and effectively measure the same water body.

11.2.4 AIMS in-house temperature calibrations

AIMS has a high precision temperature calibration facility and thus performs reliable temperature calibrations on loggers starting in early 2015.

Setting up the temperature bath for calibration

- Plug the sensor measurement module into the temperature reference thermometer
- Turn on the temperature reference thermometer by turning on the green switch on the face of the reference measurement module
- Place the sensor measurement module of the temperature reference thermometer into the water bath using the clamp.
- Remove a small amount of water using a bucket and mix hot water from the tap into the bath to raise the temperature to approximately 33°C
- Ensure the water level is within 2–3cm of the lip of the bath
- Turn on the temperature bath
- Set the bath set point to 33°C as follows:

      Press the SETPNT key

      Enter 33.00

      Press <Enter>

- Wait until the set point is achieved and holding steady in the bath.

Setting up the Temperature Bath Calibration Tool for calibration

- Turn on the PC attached to the temperature reference thermometer and log in.
- Start Temperature Bath Calibration Tool on the desktop
- Choose a file location for temperature cycle calibration. Name the file with a .txt extension. The file will be created by the software. e.g. 20160319-FLNTUSB.txt
  - Sample Interval 1 Minutes
  - Setpoint Duration 1 Hour
  - Start Setpoint 33 Degrees
  - End Setpoint 16 Degrees
  - Setpoint change 1 Degree
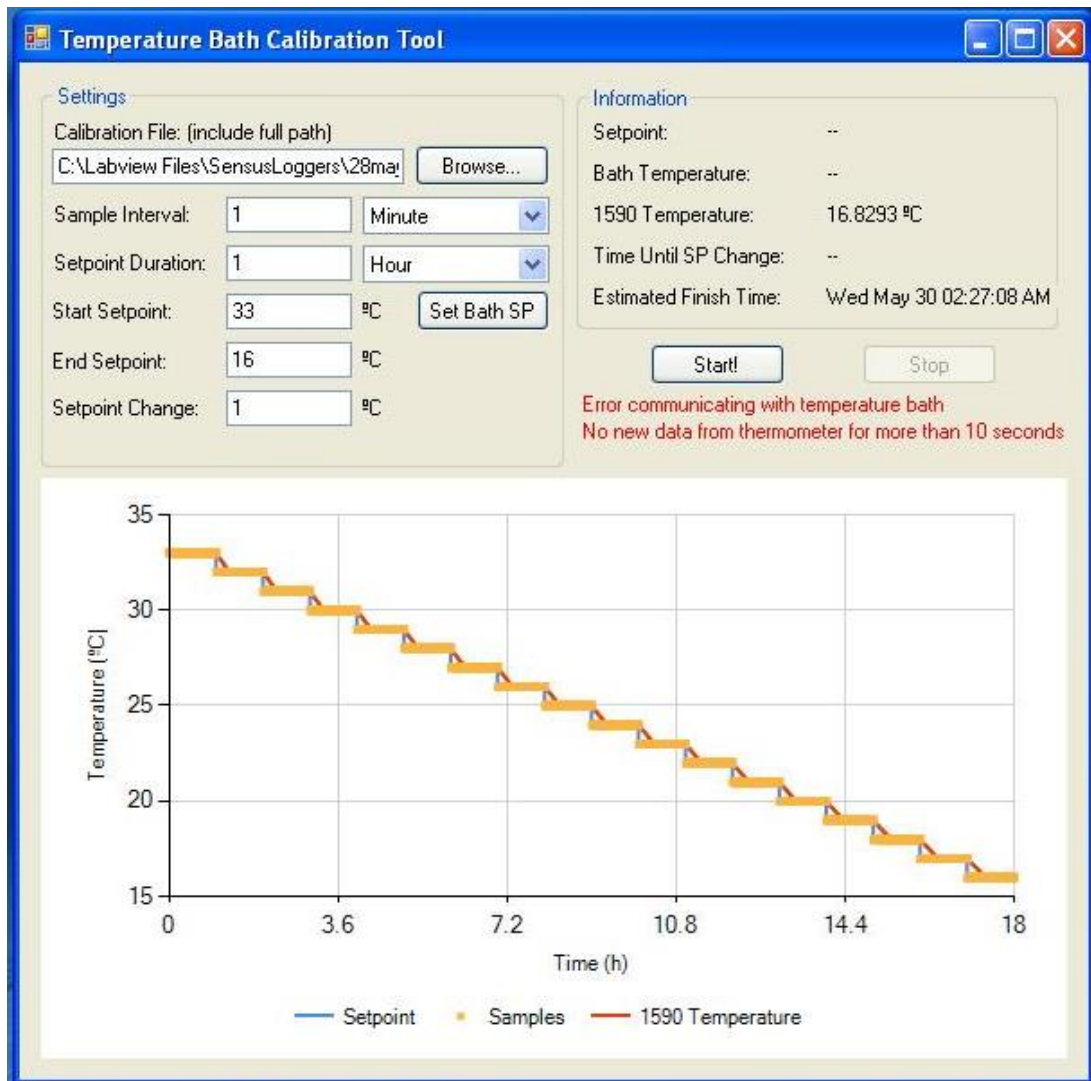
Note: the above image was taken after the bath had finished the calibration from 33 to 16 degrees

Running the calibration

- Run essential.exe in C:\temp This program mimics key strokes on your pc to stop it hibernating.
- Start the calibration on the 30 second mark of the minute. (this is to assist in the data

management of the calibration data.)

- To start the calibration, simultaneously plunge the sensors into the calibration bath and press the Start! button. The calibration will now be underway.
- The calibration should take approximately 18 hours to complete. Once it has finished:
  **Remove the loggers from the water
  **Create a folder with the datestamp in \\pearl\temp\FLNTU
  **Save the calibration file created by the Calibration Tool in the folder above.

Calculating the temperature calibration slope and intercept

- Use the STDMS Sea Temperature Data Management System (STDMS)
- Put a valid folder in "working directory" Eg C:\underway\Calibration
- Press "set up folders"
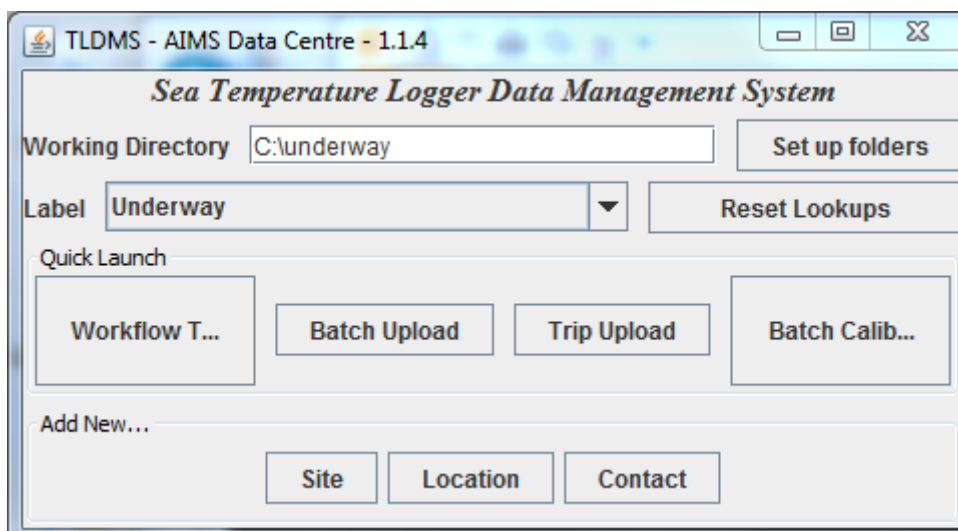
- Copy your data files to a sub-folder of <working directory>/calibration/cals
- Eg C:\underway\Calibration\Cals
- Press the button "batch calibration"
- Use the "…" buttons to choose a folder and a reference file
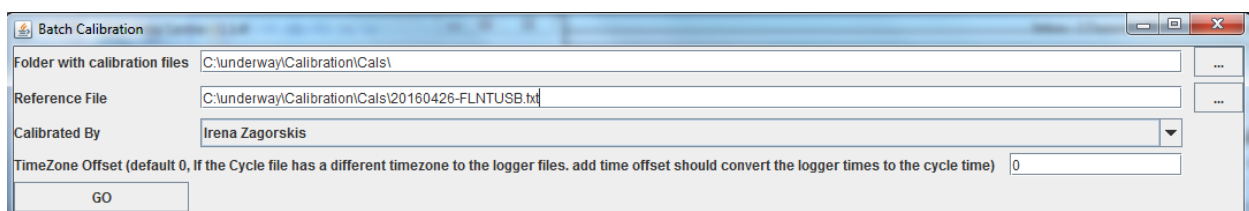
- Press the "GO" button.
- Find your calibration files in the "cals" subdirectory of the data directory.
- Eg C:\underway\Calibration\Cals\cals

Example calibration file output:

Sea Temperature Data Logger Calibration File for a Sensus Logger Calibration			
Logger serial number	818		
Logger Type	flntu		
Calibration Date	26/04/2016		
Linear Regression Calibration Coeffients			
equation:	y = Slope.(x) + Offset		
Slope	-0.005564551		
Offset	71.29684193		
RSquared	0.999846376		
Anova SSE	0.741959946		

11.4   Field deployment of ECO logger

11.4.1 Final logger preparation for deployment

The final preparation of the logger for deployment includes:

- The 6-pin dummy plug must be connected.

- Apply insulation tape to parts of the instrument to be taped with copper tape. This protects the logger casing from the significant adhesive residue of the copper foil tape.

- Subsequently copper tape (3M 1194 copper foil with non-conductive adhesive) is applied to the outer casing of the instrument adjacent to the faceplate.

- Connect the 3-pin power plug (identified by red or orange tape). This turns on the internal battery. N.B. For autonomous operation, the 'power plug' must be connected to the 3-pin bulkhead connector. This 'jumper' shorts pins 1 and 3, connecting the internal battery supply to the logger.

- Record the connection time on the deployment datasheet.

- Ensure the lock collars of the dummy and power plugs are screwed firmly.

- Check the vent seal plug for full insertion.

- Wrap the remainder of the exposed logger casing and plugs with plastic (we use cut up garbage bags) and secure firmly with insulation tape.

- Wrap tape over most of the logger deployment clamp to protect from fouling.

- Attach plastic AIMS identification label to clamp with cable tie.

- Attach the logger clamp to the logger, securing firmly with the allen key.

- Ten minutes after the logger was turned on, check the bio-wiper and logger function and ensure it is running (the logger is set to read at 10-minute intervals for field deployments).

- Protect the logger face with the field deployment black cap, to ensure the bio-wiper is not touched.

- Place the logger in the padded case.

11.4.2 Deployment equipment list

When loading the work boat the following gear is required:

- Logger (in protective padded case)
- SCUBA gear including dive watch
- Dive log sheet and pencil
- GPS (with dive site loaded)
- Marker buoy with rope and chain weight attached
- Niskin bottles x 1
- Large rubber bands to secure Niskin closed during handling back onto boat
- Spanner to attach logger clamp to star picket (plus a spare)
- Safety gear according to AIMS Field Work Procedures.

11.4.3 Logger deployment and recovery dive

The logger site is located by GPS and the marker buoy deployed to mark the site. The work boat is anchored up close to the site, typically up-current but mindful the anchor should not be dropped near the underwater benthic survey transect lines.

Once the divers are kitted up to dive, they enter the water and the boatperson passes 1 Niskin (filling with surface water to allow the Niskins to sink) and the logger to the divers on the surface. Care is taken that the bio-wiper of the logger does not contact anything, to ensure it is not disabled. The divers proceed down the buoy line and locate the logger star picket.

The divers are careful not to disturb the bottom sediment or disturb fouling on the star picket until the 'ground truth' water sample is collected. The Niskin is used to sample the water immediately adjacent to the logger. Both ends of the Niskin bottle are opened, and the diver swings the Niskin through the water to ensure the Niskin water is flushed, and the water representing that adjacent to the logger is contained in the Niskin. This Niskin is closed and placed gently on the substrate until logger changeover complete. The depth of the water sample is checked on the SCUBA depth gauge.

The new logger to be deployed is placed gently on the substrate with the bio-wiper clear from obstruction. The diver uses the spanner to detach the clamp of the logger to be recovered from the star picket. It may be necessary to remove fouling such as barnacles, hydroids and algae from the star picket. The face of the logger is inspected to assess whether it is still functioning, and the recovered logger placed gently on the substrate whilst the new logger and clamp attached to the star picket.

The loggers are mounted with the optical window facing downward, so that the infrared LED source will not 'see' any part of the deployment hardware. A final function check of the newly deployed logger is done by checking that the bio-wiper turns and the logger samples underwater after the appropriate 10-minute time interval. See photo of deployed logger below.
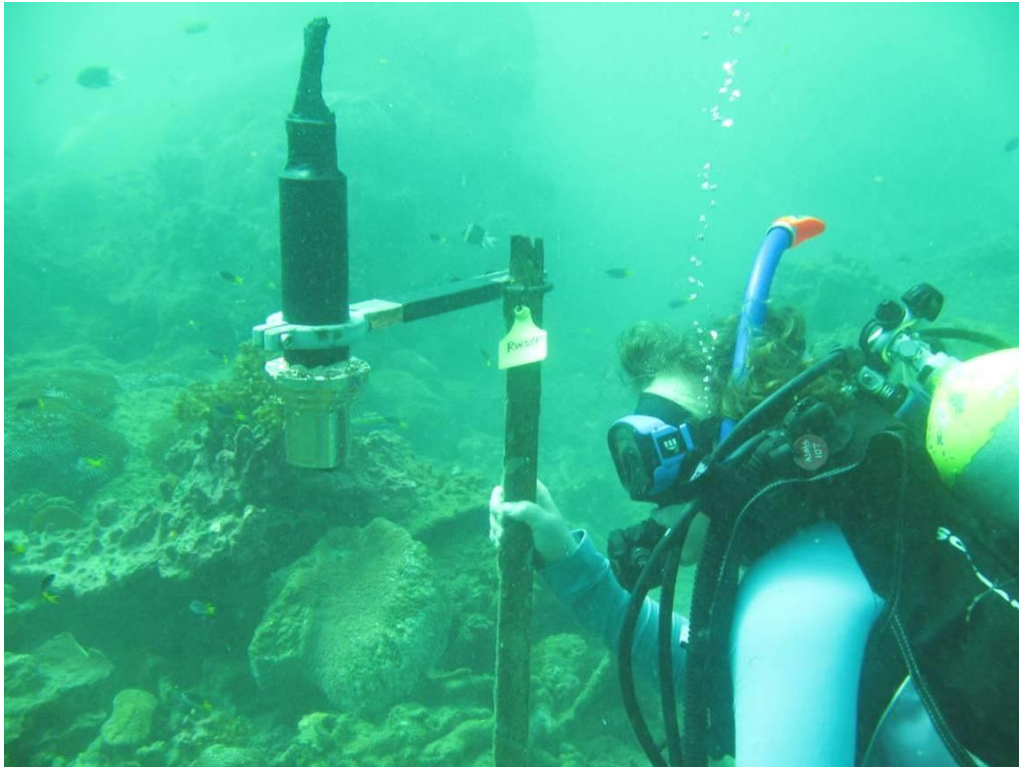
The divers return to the work boat with the recovered logger, and Niskin bottle. The boatperson carefully collects these from the divers at the surface. The field deployment black cap pre-filled with seawater and the logger placed upright with the logger face immersed in the seawater.

AIMS Water Quality group standard height of the logger collar is positioned 6 cm from the optical end of the logger, which locates the logger at a suitable standard height within the black cap used for field deployment. This protects the bio-wiper from being bumped and contains seawater to allow the logger optical face to be protected until the logger can be switched off. Do not fill cap with fresh water, as this appears to react with the corrosion on the recovered logger and may cause damage to the optical window, if the bio-wiper is operating.

The 5 L Niskin is clamped shut using No.109 rubber bands used to ensure the sample water is not lost until placed in the Niskin rack on board the boat.

The Niskin seawater samples are processed without delay as per shipboard filtering procedures for Chl-*a* and TSS, and salinity sample is collected.

11.4.4 Post recovery logger handling on board boat

Once the logger is on board the boat, the logger is switched off as soon as possible. To do this:

- The face of the logger should remain immersed in seawater in the cap, with the top (connector end) of the logger out of water.

- Using a Stanley knife, cut the protective tape and plastic from the connecter end of the logger to expose the connectors and plugs.

- Dry the connector end and remove the 3-pin power plug (identified by red or orange

tape, or the newest plugs now have blue ends).

- Replace this with the 3-pin dummy plug (identified by green tape).
- Record the time the logger is switched off on the logger recovery datasheet

Once there is time available, following processing of the water samples, other tasks with the recovered logger include:

- Photograph the logger to show the degree of fouling on the instrument. (Example photo below showing fouling on a recovered logger).

- Carefully remove the plastic and tape from the logger casing.

- Rinse the logger clean with fresh water (now that the bio-wiper is no longer functioning because the logger is switched off). Allow to dry.

- Take further macro photographs to record the condition of the faceplate, bio-wiper and optical window of the logger.

Once there is time available, following processing

- Remove the bio-wiper carefully with the allen key.

- Download the data and complete details in the logger download datasheet including a function check of the shutter

- When the data download is complete, the rinsed and dry logger should be returned to a padded Pelican case for return to the laboratory.

- Details of the dive time and depth should be transferred from the dive log sheet to the logger recovery datasheet.

11.5   ECO logger data download and storage

11.5.1 Data retrieval from ECO logger

Data is downloaded from the logger using the ECO View software on the computer.

- Connect the logger to the computer and software package.

- View 'transfer data' menu tab:
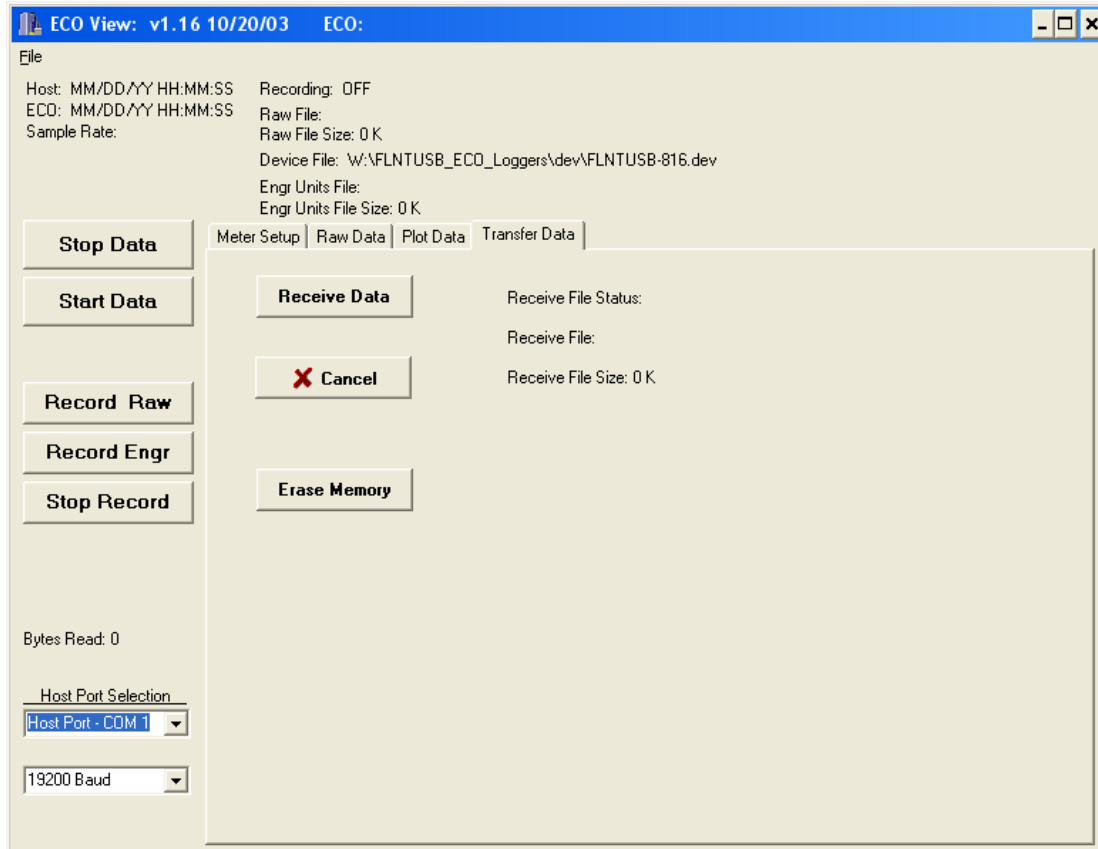
- Click ![Receive Data] and the file naming window will appear.

- Browse to the appropriate folder and name the raw data file.

- File name convention: 817_reef_2014Sep22_Dunk.raw [i.e., Logger 817, reef deployment, year, month, day (**RECOVERY** date), site name].

A typical logger field deployment download may take about 10 minutes with a file size of 700–800 kb for a 4-month deployment. The 'Receive File Status' will indicate 'Complete'.

- Fill in details on the logger download datasheet

- Disconnect the logger from the computer connection cable and reconnect the 6-pin dummy plug.

11.5.2 Upload raw and QA/QC files to AIMS Data Centre Water Quality Environmental Logger Data Management System

The raw field data files are uploaded into the AIMS Data Centre Water Quality Environmental Logger Data Management System (ELDMS) and is discussed in more detail in Section A12.

The ELDMS currently has the structure as in the following screen view:

Environmental Logger Data Management
List Loggers
List Logger Event History
List Calibrations
List Locations
List Deployments
Location Data Exports
Location Data Charts

Information about the loggers and the deployment locations (example screen shots below) is held in this database as well as the device calibration files and logger data files.

Deployment List

Create

Restrict by Location: Dunk   AND/OR Logger:

Order By: Location StationId, Deployment Date

27 Deployments found, displaying 20 Deployments, from 1 to 20. Page 1 / 2.

|< << < 1 2 > >> >|

Actions	Location	Logger Serial#	Deploy Date	Deploy Trip	Deploy WQ Station Name	Retrieval Date	Retrieval Trip	Retrieval WQ Station Name	Status	Raw File Uploaded	QA File Uploaded	Version Tag
View QA Delete	Dunk	824.01	21/02/2015 14:50	6162	WQP068	No Date Available	0		C	No	No	UR
View QA Delete	Dunk	843.0	22/09/2014 08:50	6069	WQP022	21/02/2015 14:50	6162	WQP068	C	Yes	Yes	UR

View Deployment

Deployment Id: 23284
Logger Serial#: 843.0
Location: Dunk
Sample Date: 22/09/2014 08:50

In-Situ WQ Stations:

Comments on Deployment: Maxed out records NTU ~4-13 Feb 2015

Logger Deployment Details		**Logger Retrieval Details**	
Deploy Date:	22/09/2014 08:50	Return Date:	21/02/2015 14:50
Deploy Trip:	6069	Return Trip:	6162
Deploy WqSationName:	WQP022	Return WqStationName:	WQP068

Data and QA Details
Calibration File Name: FLNTUSB-843_comb-2014May8.pdf
Calibration Date: 08/05/2014 00:00
RAW Filename: 843_reef_2015Feb21-Dunk.raw
QA Filename: QA_Edit1_Dunk_2014_09_22-08_50_843.0.xml
Status: C
Version Tag: UR

Edit Deployment QA Show All

Upload raw data file to the ELDMS

- Login to the ELDMS.

- Click 'List Deployments'.

- Select site by 'Restrict by Location' and/or Logger to be uploaded.

- Select 'QA' Action for the chosen deployment.

- Click 'Upload Raw Data File'.

- Browse for raw file, then 'Upload'

- Click 'Create Raw Data Records' which will automatically calculate 'engineering' units.

- The record processing will indicate it is 'running in the background' until the process is complete, and then a 'pop-up' window will confirm this.

Back to Deployment View | Back to Deployment List

Deployment 23284 Data QA

Location: Dunk, Deployed Date: 22/09/2014 08:50, Retrieval Date: 21/02/2015 14:50, Logger Serial: 843.0

Process Raw Data

Upload Raw Data File: [_____] Browse... | Upload

Raw Data Filename: 843_reef_2015Feb21-Dunk.raw

Create Raw Data Records | Calculate Engineering Units

Manual Data QA

Export XML Schema: [____ ▼] Export XML Data File For QA

Upload QA Data File: [_____] Browse... | Upload

QA Data Filename: QA_Edit1_Dunk_2014_09_22-08_50_843.0.xml

Import XML Schema: [_____ ▼] Update Data From QA Data File

- Click 'Export XML File for QA'

The logger data files are stored on the AIMS network Water Quality share area.

Once edits using WISKI-TV have been performed the QA/QC edited files are re-imported to the ELDMS.

Upload QA/QC XML files to the ELDMS

- Login to the ELDMS.

- Click 'List Deployments'.

- Select site by 'Restrict by Location' and/or Logger to be uploaded.

- Select 'QA' Action for the chosen deployment.

- Click 'Upload QA Data File'.

11.5.3 WISKI-TV ECO logger data quality control

Sub folders on the AIMS network Water Quality share area contain the XML files in two main stages of the QA edit process. The folders and example file names are listed in the table below.

The XML file exported from the Data Centre ELDMS is saved to the folder 1QA_export_xml

The files exported are stored into folders ordered by their file type, deployment location and

DEPLOYMENT date and time, ending with the logger serial number.

N.B. Raw logger field deployment data files are named with the **recovery** date to avoid errors, but the exported files from the ELDMS are named with the convention including the **deployment** date.

The next step is the QA WISKI-TV editing process.

- Original exported files are left untouched as reference in 1QA_export_xml (with the file name convention format as per table below).

- Using Windows Explorer, copies made to folder 2QA_edit1_xml (using the file name convention as per table below).

- Using WISKI-TV the first edit (edit1) we delete start and end records of the logger when the logger was not in the water or when the logger had a physical malfunction. The information supplied on the logger deployment and logger recovery datasheets is used in the first edit.

- Once WISKI-TV QA/QC editing is complete, the XML files are re-imported to the AIMS Data Centre ELDMS and final individual point edits may occur in Oracle via the ELDMS.

11.6   References

WET Labs Combination Fluorometer and Turbidity Sensor ECO FLNTU User's Guide. Revision Y, 12 November 2008. WET Labs Inc., Philomath, OR, USA www.wetlabs.com

WET Labs ECO View Host Software for ECO Meters User's Guide. Revision I, 18 March 2008. WET Labs Inc., Philomath, OR, USA www.wetlabs.com

Anderson, C.W., 2005, Turbidity (version 2.1): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., section 6.7, July, accessed November 2007 from http://pubs.water.usgs.gov/twri9A6/

Section A12: AIMS Data Management Procedures for the Marine Monitoring Program

12.1   Introduction

Data Management practices are a major contributor to the overall quality of the data collected; poor data management can lead to errors, lost data, and can reduce confidence in the data. This appendix details the overall approach AIMS takes to develop data management practices that actively protect and enhance the quality of the data collected as part of the MMP.

12.2   Basic principles

The fundamental goal is to ensure that at each step where data is transformed, modified or 'handled' the process is understood, documented and that there is a clear logical connection between the raw data coming into the system and the final processed data. It should always be possible to back-track from the final processed data to the raw data from the instrument or data sheet.

The MMP data management system centrally stores all the generated data in an integrated data system designed to allow cross-referencing and access to related data. The data are described in a metadata system and can be queried to access the data, both internally to AIMS as well as externally.

12.3   Data storage

All data are stored in AIMS' centralised Oracle 9i database. This is located on a secured central UNIX server which is protected by a UPS power supply and fire suppression systems. The Oracle database operates in a 'zero data loss' mode with the following backups:

- Nightly backups to tape via Export of the main database.
- Mid-day 'hot-backup' to disk using Oracles backup utility.
- Log files are stored on a separate server allowing for complete roll-back and roll-forward of data to the database.

The backup tapes are stored off site (initially at the AIMS Marine operations building and then at the Recall service in Townsville) and a Disaster Recovery disk facility is being set up at James Cook University for remote storage of on-line backups.

12.4   Metadata

Metadata are a critical part of the data record and must be stored, related to any data they represent, and discoverable as part of a robust data management practice. In the case of the MMP, metadata include: location information and date/time of sample collection; weather and sea state conditions during collection; volumes of water sampled or filtered; equipment and vessels used to collect samples; personnel involved in sample collection; sample identification codes; and laboratory methods used to process samples. Metadata related to sample collection are recorded on a Field Datasheet each time field sampling occurs. These records are entered into a centralised database (see below) and are also retained in hard copy. Metadata related to laboratory processing is recorded in analytical reports, which are entered into a centralised database and raw reports are saved on AIMS' internal servers.

12.5   Data management of biogeochemical data

Entry of data must be captured in a process that does its utmost to eliminate error as manual entry of data can be a major source of error in data collection. For this reason, various specific data management applications for the MMP have been developed at AIMS. These are:

- The Field Data Entry System (FDES)
- The Filter Weight Management Web Application, and a
- The Water Quality Data Management System which provides a web-based portal to ingest analytical results into the Oracle database.

The **Field Data Entry System (FDES)** is an implementation of a Microsoft Access Database which has two main components. A database schema that models the data collection process to provide data integrity, and a set of forms that control the data entry process by providing drop down selection lists, radio buttons of predefined selection options. This document will not provide in-depth details on the schema or form design. The FDES is integrated with a labelling system for the samples taken, allowing unique and traceable labels to be printed prior to going in the field. The label format includes the Station Name, a 6-digit alphanumeric code (e.g., WQQ001), which is unique to location and time, allowing all samples to be traced back to their original sampling event. Label information also includes the depth and duplicate number of the sample taken. Following sample collection, all information from the Field Datasheet is entered into FDES, checked, and then imported into the centralised Oracle database.

The **Filter Weight Management Web Application** is a J2EE based application that is accessed via any browser within the AIMS internal network. Using a set of forms with drop pre-defined selection lists, data entry error is kept to a minimum. The software forming the application's backend is integrated with the five decimal place precision weight scales and an Oracle database dedicated to storing filter weights. The integration with the scales is such that when the weight is finalised on the scales, that value is

automatically placed in the web applications form for the particular weight with no manual weight entry required. For every filter, an initial weight is taken before the sampling, and a final weight is taken after sampling; both weights are performed in the laboratory using the same scales and Web Application.

The **Water Quality Data Management System** is a web-based portal that can be accessed via any browser within the AIMS internal network by a select group of MMP staff. Data entry error minimised by not allowing manual data entry. The process for data entry for the analytical results into Oracle is through a file import task using pre-formatted Sample Results Feeder Sheets. The Web Application is integrated with a dedicated Oracle database (the AIMS Nutrients Database). The Nutrients database schema is complex and ties in with an AIMS workflow schema for tracking and recording when particular database manipulation actions take place. This document does not cover in-depth design of this database. The Sample Results Feeder Sheets are a pre-formatted Excel spreadsheet that are created by the Data Management System. Analytical results for all water quality parameters are then placed in these sheets and ingested into Oracle via this system. The System logs each time data are ingested, keeping a record of all changes to the database. Every time data are ingested, a copy of the Feeder Sheet is stored in the Nutrients Database.

12.1   Coral monitoring data management

All field data is recorded on pre-printed datasheets. The use of standard data sheets aids in ensuring standard recording of attributes, and ensures required data are collected.

Upon return from the field, all data is entered on the same day into database forms linked directly to an Oracle Lite database. Each field on these forms mirror those on pre-printed data sheets and include lookup fields to ensure data entered is of appropriate structure or within predetermined limits. For example, entry of genera to the demography data table must match a pre-determined list of coral genera.

Upon return to the office, the data is uploaded to an Oracle Database using the Oracle Lite synchronization process. All keyed data is printed and checked against field data sheets prior to final logical checking (ensuring all expected fields are included and tally with number of surveys). The Oracle database is backed up on a daily basis. Photo images are burnt to DVD prior to analysis this copy to DVD doubles as a backup of the photo images. Photo images are stored on a server that is included in a routine automatic back up schedule.

Image analysis is performed within the AIMS monitoring data entry package "reefmon". This software contains logical checks to all keyed data and is directly linked to a database to ensure data integrity. The directory path to transect images is recorded in the data base. This functionality allows the checking of benthic category identification. All photo transect data is checked by an experienced observer prior to analysis and reporting of results.

12.2 Instrument data management

Data from instruments is currently limited to a set of Environmental Loggers, CTD Profilers and Temperature Loggers.

12.7.1 Fluorescence and turbidity loggers

Wet Labs Eco FLNTUSB instruments are used to measure temperature, fluorescence, and turbidity (see Section 11 for details). After retrieval, the raw data are extracted in CSV format using Wet Labs software and a serial communication port on a laptop. The calibration files and raw data files are backed up on a UNIX server which has a comprehensive backup regime. The data from the loggers are managed by the 'Environmental Logger Data Management' J2EE based web application that is accessed via any browser within the AIMS internal network by a select group of MMP staff. Data entry error is kept to minimum using pre-defined selection lists, and a set of import tasks. The Web Application is integrated with a dedicated Oracle database (the AIMS EnvLogger Database). Data import is discussed in Appendix A11.

12.7.2 CTD Profilers

At the MMP sites where Niskin samples are collected, Sea-Bird CTD Profilers are deployed to measure vertical profiles of salinity, temperature, conductivity, turbidity, fluorescence, and other parameters. Also some time-series CTD instruments (Sea-Bird SBE37) are deployed at logger sites to record changes in salinity and temperature over time. Both instruments record data in the same format and are processed in a very similar fashion, the main difference being vertical casts are over depth (distance in meters) and *in situ* deployments are over time. For vertical and *in situ* deployments, Sea-Bird Sea-Save and SBE-DataProcessing software modules are used to produce data files in the Sea-Bird HEX format. These HEX files contain the raw data readings and are stored on a server which has a backup and retention policy that ensures the data files are retrievable as required over the long term. Each Sea-Bird CTD instrument (SBE37, SBE19plus, etc), calibration information is kept in a CON file.

The processing of the data is done via the CTD Data Management J2EE web services application, and the Sea-Bird DataProcessing Software. The HEX files and CON files are used as input to these. During the process, four PSA files are created for each data file, and these represent the initial QAQC process.

1. Raw data conversion PSA: Conversion of raw HEX characters to ASCII characters
2. Wild Edit PSA: 2 pass running mean filter/de-spike.
3. Loop Edit PSA: vertical shift correction (boat rocking due to swell)
4. Bin Average PSA: Data is averaged into 1 meter vertical bins for each parameter.

The creation of the four PSA files is automated by the CTD Data Management System using the HEX and CON files are input. Once the data has been Bin Averaged, the data is then ingested into an Oracle 9i database schema, within the same schema used to house the biogeochemical data.

12.7.3 Temperature loggers

Temperature loggers are deployed at, or in close proximity to, all locations at both 2m and 5m depths and routinely exchanged at the time of coral surveys.

There are two types of Temperature loggers used for the sea surface temperature logger program. The first type is an Odyssey temperature logger that comes with either 64 kb or 128 kb of memory (http://www.odysseydatarecording.com/). These are currently being phased out. The second type is a Sensus Ultra Temperature logger (http://reefnet.ca/products/sensus/). The odyssey Temperature loggers are set to take readings every 30 minutes. The Sensus Temperature loggers are set to take readings every 10 minutes.

Prior to deployment, the Temperature loggers are calibrated to within 0.02 Degrees Celsius using a temperature bath and a Hart Scientific 1522 thermometer.

The deployment process involves deploying two Temperature loggers at a location designated by the experimental scientist. The first logger is deployed in shallow water on a reef flat. The second logger is deployed in deeper water on a reef slope. The temperature loggers are deployed for a period of approximately one year.

After the temperature loggers are retrieved the data is downloaded and imported into an Oracle database as raw or level 0 data. To accomplish this task the Odyssey loggers have proprietary software to download and transfer their data into a Microsoft Access database via a serial port. The software then uploads this data from the Access database into the Oracle database. The Sensus loggers download their data to a Microsoft Excel spreadsheet via a serial port. The spreadsheet is uploaded into the Oracle database using an Java application written by the AIMS data centre.

Quality control is performed on all temperature logger data using Wiski Time Series Viewer (www.kisters.de/english/html/WISKI/WiskiTV.html). The Wiski input files are generated using an Java application written by the AIMS data centre. Data from the same location (the flat and the slope) and nearby locations are plotted together for comparison. Any bad points are removed. If a logger has suffered any drift in its recorded data points this can be corrected using Wiski Time Series Viewer. After this process the data is inserted into the database as quality controlled or level1 data.

12.8   Data validation

As the previous section describes, the resulting data from sampling and instrumentation is ingested into several Oracle databases. Once the data are in Oracle, views are created of the data to simplify processing and hide the complexities of the underlying database schemas. The views are made available 3 mechanisms:

1. On-line forms via the Intranet.
2. On-line file downloads/exports.
3. Microsoft Access Databases.

All 3 give the scientist the data validation capability, and when issues with the data are found, they are addressed by fixing/removing the inconsistency in its raw format, then re-importing the values back into Oracle. Once back in Oracle, the provided views will reflect the changes made via data validation. Mechanisms for data validation are as follows;

- Bounds checking – is the data within acceptable limits.

- Logical checking – is the data logical (is the value possible).

- Comparative checking – is the data similar to other comparable data (such as previously collected data or better previous equivalent data).

- Correlative checking – if there is a relationship between two variables, if so is the relationship valid, if sample data should exist for this data, is it present.

12.9   Data integration

Once data is available in the Oracle databases after the quality assurance and validation processes have gone through the cycles, the data is then consolidated in an Access Database via Oracle views. The Access Database product was chosen as the delivery mechanism for its simplicity, and with a view that most are familiar with the software. Providing the Oracle database lends its elf to database schema complexity and licensing issues for the software required to use the exported Oracle databases.

It is AIMS policy that all data collected have a metadata record created for it. The metadata record is created using a Metadata Entry System where the metadata is in the form of ISO19139 XML. This is the chosen format for many agencies across Australia and the International Community that deal with spatial scientific data. The AIMS Data Explorer is searchable by keyword and location and is found here: https://apps.aims.gov.au/metadata/search.

12.10 Outputs

The outputs from the data management system include Standard Operating Procedures for all data manipulation, transfer, uploading and processing, a full database description, the metadata records, the data quality reports from the automated checking programs, the progress statistics from the data tracking system and a copy of the data in the appropriate format (such as an Access Database).

Appendix B James Cook University TropWater Appendices

B1: TropWATER Water Sampling Procedures
B2: TropWATER Water Sampling Field Sheet - Example
B3: Metadata for flood plume data entered into TropWATER flood database
B4: TropWATER Water Sampling Input Sheet
B5: TropWATER Auto-analysis methods – Nitrogen – Ammonia
B6: TropWATER Auto-analysis methods – Phosphorus
B7: TropWATER Auto-analysis methods – Nitrogen – Nitrate and Nitrite
B8: TropWATER Auto-analysis methods – Nitrogen – Total Alkaline Persulfate
B9: TropWATER Auto-analysis methods – Phosphorus – Total Alkaline Persulfate
B10: TropWATER Auto-analysis methods – Chlorophyll
B11: TropWATER Auto-analysis methods – Total Suspended Solids
B12: TropWATER Auto-analysis methods – Coloured Dissolved Organic Matter
B13: TropWATER methods – Laboratory salinity

Appendix B1 TropWater Water Sampling Procedures

Nutrient Sampling

Dissolved nutrients (DIN's [NO_3, NO_2, NH_4^+,] FRP (PO_4),TDN/TDP, ($Si(OH)_4$)

Six 10 mL vials, yellow lids plus one 60 mL vial for silica

Samples need to be FILTERED.

- Sample is collected from the 10 L container using sterile 60 mL syringes.
- Rinse <u>syringe</u> at least once with the water to be sampled. Discard rinse water <u>away</u> from sampling area.
- Attach yellow minisart 0.45 μm filter to tip of syringe.
- Fill syringe with sample water and push some sample water through to prime the filter.
- Minimise the air gap between water sample and black syringe plunger to prevent contamination.
- Do not rinse vials with sample water.
- Fill the vials to the line (10 mL or 60 mL) (prefer to be just below the mark to avoid loss of sample). Do not overfill, this may cause the vials to split when frozen – destroying the sample.
- To minimise contamination please keep fingers away from all tops and lids (wear gloves if available).
- If possible, freeze the 10 mL samples before sending to the laboratory. Otherwise, store in the dark on ice for transport the laboratory as soon as possible. The 60 mL vial for silica analysis is not frozen, just kept in fridge or on ice.

Total Nitrogen/Total Phosphorus (TN/TP)

One 60 mL plastic vial

Filtering NOT Required

- Sample is collected from the 10 L container using sterile 60 mL syringes. Water in the bucket is rigorously stirred before being collected using the syringe.
- Requires 60 mL plastic vial.
- Do not rinse the vial with the water to be sampled.
- Fill the vial leaving approx. a 3cm air-gap from the top. Do not overfill, this may cause the vial to split when frozen – destroying the sample.
- To minimise contamination please keep fingers away from all tops and lids.
- If possible, freeze samples before sending to the laboratory.
- Otherwise, store in the dark on ice for transport the laboratory as soon as possible.

Chlorophyll *a*/Phaeophytin (Chl-*a*)

One 1 L black plastic bottle

Filtering not required on vessel, but must be filtered within 24 hours of collection.

- Sample is collected directly *in situ* over the side of the boat or from 10 L sample container (stirred rigorously) into collection bottle (requires a one-litre black plastic bottle to reduce the effect of sunlight on the phytoplankton species in the interim between collection and filtration).
- Rinse each container at least once with the sample water, taking care to avoid any contact with the sample (gloves must be worn at all times).
- Fill to overflowing and seal. <u>Do not</u> leave an air gap.
- Once sample is taken it should be kept in the dark on ice.
- The filtering is undertaken off the vessel either in the laboratory or beforehand depending on the time required for transport.
- Filter using manifolds, provided – ensure manifold cups are washed with deionised water between samples to avoid contamination. Record volume of sample filtered.
- Wash cups with deionised water to ensure the capture of the entire sample.
- ***Important – add magnesium carbonate to wash (~0.2 mL is required to preserve/fix Chl-*a* on the filter paper).
- Fold filter papers in half and wrap in aluminium foil to avoid loss of sample on the filter paper.
- Place wrapped filter paper in envelope with site no. reference (i.e. MMP 68) and the volume of sample filtered is written on the envelope.
- Store papers dry and frozen or as cold as possible prior to analysis in the laboratory.

Total Suspended Solids

One 1 L plastic bottle

Filtering not required on vessel, but must be filtered within 7 days of collection.

- Sample is collected directly *in situ* over the side of the boat or from 10 L sample container (stirred rigorously) into sample collection bottle.
- Rinse each container at least once with the sample water, taking care to avoid any contact with the sample (gloves must be worn at all times).
- Fill to overflowing and seal. Do not leave an air gap.
- Once sample is collected, it should be kept on ice and stored at 4° C.
- TSS sampling requires filtering within 7 days of collection. The filtering is undertaken off the vessel either in the laboratory or beforehand depending on the time required for transport.
- Filter using manifolds, provided – ensure manifold cups are washed with deionised water between samples to avoid contamination. Record volume of sample filtered.
- Wash cups with deionised water to ensure the capture of the entire sample.
- At least 500 mL of deionised water is rinsed through the sample to rinse the salts from the filter papers.

CDOM (Coloured Dissolved Organic Matter)

120 mL glass amber bottle

- Sample is collected from the 10 L container using sterile 60 mL syringes.
- Requires 120 mL amber (glass) bottle.
- Rinse <u>syringe</u> at least once with the water to be sampled.
- Attach 0.45 μm (yellow) to syringe, fill with sample and insert plunger; air contact must be minimised so filter needs to be removed at this point to expel any trapped air.
- Place filter back onto syringe and push some sample through to prime the filter.
- Place a 0.2 μm filter (blue) onto the yellow filter; ensure they are secure and fitted onto the syringe by turning them until there are 'locked' together – at this point the syringe should have two filters attached with the yellow next to the syringe. If syringes and filters aren't fitted together correctly there may be a risk of contamination.
- Push the sample water through both filters into the glass amber bottle– a minimum 100 mL filtered sample is required.
- Samples are stored at 4° C and need to be analysed within 7 days of sample collection.
- When there is too much back pressure on the syringe, the yellow filter will need replacing first — if this does not alleviate the back pressure, the blue one also needs replacing; however, always replace the yellow filter first.

Salinity

One 60 mL plastic vial

Filtering NOT Required

- Sample is collected from the 10 L container using sterile 60 mL syringes.
- Rinse <u>syringe</u> at least once with the water to be sampled.
- Fill the syringe with water from the 10 L container and fill the vial to just below the line.

Pesticide sampling

One 250 mL or 1 L amber glass bottle (obtained from Queensland Health laboratory)

Filtering NOT Required

- Sample is collected directly *in situ* over the side of the boat or extended pole sample collection bottle.
- Do not rinse bottles. Fill them to the neck of the bottle leaving an air gap.

- Immediately place samples in an esky on ice until being returned to laboratory. Samples should be refrigerated if not transported immediately. Do not freeze the bottles.

Appendix B2 TropWater Water Sampling Field Sheet - Example

Date _____

Samplers _____

Weather _____

Vessel _____

															Extra samples			
Site	Lat	Long	Sample ID	Time	Site Depth	Sample Depth	Temp	SPC	Sal	pH	Chl	DO	DO%	Duplicate	Pesticide	Phytoplankton	Trace Metals	Comment

Appendix B3 Metadata for flood plume data entered into TropWater flood database

Metadata for flood plume data entered into TropWater flood database

Water quality data for flood plumes 1991 – 2008

Mandatory fields

Field	Description
Unique ID	Id that links the sample and water quality data. Note all duplicates have been combined
Cyclone	Name of Cyclone associated with flood event (non cyclone = low pressure)
Cyclone number	Number associated with the cyclone [Joy = 1, Sadie = 2, Violet = 3, Ethel = 4, Justin = 5, Sid = 6, Rona = 7, Steve = 8, etc.]
Catchment	Catchment adjacent to sampling station
Catchment number	Number associated with the catchment
River	Most closely located flooding river (if able to identify)
Year	Year of sample
Month	The month that the sample was taken in
Dist_Mou	Distance from river mouth (as plume moves)
Reef	Identifies if sample is located near a reef (within 1km) (R)
Agency	Agency responsible for sampling of data [string]
T_B_S	Denotes location in water column. S(surface sample < 1m), top (3 meters below surface, W (water column) , B (bottom)
S_Depth	Surface depth of sample
Latitude	Latitude in decimal degrees [integer]
Longitude	Longitude in decimal degrees [integer]
DIN	Dissolved Inorganic Nitrogen (addition of $NO_2 + NO_3 + NH_4$) measured in µM [integer with 2 decimal places]
NO_x	Nitrate (addition of $NO_2 + NO_3$) , measured in µM[integer with 2 decimal places]
NO_2	Nitrite, measured in µM [integer, 2 decimal places]
NO_3	Nitrate, measured in µM [integer, 2 decimal places]
NH_4	Ammonium
DON	Dissolved organic nitrogen , measured in µM [integer with 2 decimal places]

Field	Description
TDN	Total dissolved nitrogen (DIN + DON)
PN	Particulate Nitrogen (measured in µM[integer with 2 decimal places]
TN	Total Nitrogen (PN plus TDN)
DIP	Dissolved inorganic phosphorus (measured in µM[integer with 2 decimal places]
DOP	Dissolved organic phosphorus, (measured in µM) [integer with 2 decimal places]
PP	Particulate Phosphorus
TP	Total phosphorus
DIN%TN	
DIN:DIP	**Ratio**
NOx:DIP	
SPM	**Suspended particulate matter**
Salinity	
Chlorophyll	
Phaeo	
Chl method	

(Quality control identified by laboratories and need further explanation).

1. Historical data

1(a) Flood plume data collected during 1991 – 2001

Program: Great Barrier Reef Marine Park Authority flood plume monitoring.

Duration: 1991 – 2000

Organisation: GBRMPA, AIMS

Individuals: Michelle Devlin, Jane Waterhouse, David Haynes

Area: Various catchments – see below

Reports: Devlin et al. (2001) Flood plume monitoring in the Great Barrier Reef: spatial and temporal patterns in composition and distribution

Devlin et al. 2002

Devlin et al. 2004

Events: 8

[Cyclone Joy – 1991]

[Cyclone Sadie – 1994]

[Cyclone Violet – 1995]

[Cyclone Ethel – 1996]

[Cyclone Justine – 1997]

[Cyclone Sid – 1998]

[Cyclone Rona – 1999]

[Cyclone Steve – 2000]

For further information on cyclones, refer to Devlin et al. 2001 Parameters for each cyclone.

2. 2001 – 2007 data

2.1 Flood plume data collected during 2002

Program: National Action Plan for Salinity and Water Quality (NAP)

Duration: 2002 – 2005

Organisation: TropWater (JCU), ANU.

Individuals: Jon Brodie, Malcolm McCulloch

Area: Burdekin catchment

Events: 1

[Major flow event in February 2002, no cyclone associated]

Description:

Water samples collected from multiple sites within flood plume in transect heading out from river mouth. Majority of samples collected inside the visible area of the plume though some samples taken outside of plume for comparison. Surface samples were collected at 0.5 metres below the surface, with either a reversing thermometer Niskin Bottle for a rinsed clean sampling container with temperature measured by thermometer. Samples taken at depth were collected with Niskin Bottles. Salinity and temperature profiles were measured at all sites with an YSI salinity meter. Water samples for nutrient and chlorophyll analysis were collected, filtered and stored for further analysis. Volumes filtered for all analyses were dependent on turbidity of the water. Sub samples were filtered through GF/F (glass fibre) filters for chlorophyll and phaeoyphytin, and the filter immediately wrapped in aluminium foil and frozen. The second sub-sample was filtered through 0.45µM membrane filter for SS.

Samples were collected at 13 stations in the plume covering range of salinities on February 20 and 21, 2002. The Burdekin flow in this event was moderate at Home Hill (Clare), the total February flow being 3.6 million ML, compared to a long term median flow at Clare of 1.2 million ML. Exact latitude and longitude can be found in database. While profiles for salinity and temperature with depth were taken only surface samples were collected for SS, nutrients and chlorophyll analysis.

Data conversion:

Latitude and longitude converted to degrees and minutes.

Comments:

Only chlorophyll, phaeophytin, SPM and salinity data (nutrient data lost in analysis process)

SPM – mg L^{-1}

Chl - µg L^{-1}

Salinity

2.2 TropWater flood plume data collected 2007

Program: MTSRF 3.7.2

Duration: 2007

Organisation: TropWater (GBRMPA)

Individuals: Steve Lewis, Zoe Bainbridge**,** Jon Brodie,

Area: Burdekin catchment

              Black and Bohle River (Townsville)

Events: 1

              [February 2007]

Description:

Data conversion:

Nutrient concentrations (measured in µg L^{-1}) were converted to µM.

- TN, NO$_2$, NO$_3$, NO$_X$, DON, PN converted by multiplying by 14.0067.
- TP, FRP (DIP), PP, DOP converted by multiplying by 30.97376 (for description of parameters, see Appendix 1).
- DIN calculated by addition of NO$_X$ and NH$_4$.

Comments:

Three separate events were sampled, being in the Burdekin flood plume, off the mouth of the Haughton (Barrattas) and the Bohle and Black river.

Unique id and sites for each event are listed below:

- Burdekin samples collected on 6th and 7th February 2007
- Townsville-Thuringowa (Ross and Black River) collected on 2nd February 2007
- Lower Burdekin (Haughton) collected on 3rd February 2007

Location	unique id	Location	unique id	Location	unique id
Ross River	16J350-100	Burdekin	15J390-345	Haughton	15J390-641
Ross River	16J350-113	Burdekin	15J390-346	Haughton	15J390-645
Bohle River mou	16J350-195	Burdekin	15J390-347	Haughton	15J390-648
Black River mou	16J350-196	Burdekin	15J390-348	Haughton	15J390-649
Ambient	16J350-197	Burdekin	15J390-349	Haughton	15J390-650
Bohle River mou	16J350-200	Burdekin	15J390-350	Barratta	15J390-651
Ross River	16J350-201	Burdekin	15J3990-351	Ambient	15J390-652
Ross River	16J350-202	Burdekin	15J390-352	Barratta	15J390-653
Black River mou	16J350-204	Burdekin	15J390-353	Barratta	15J390-654
Ross River	16J350-206	Burdekin	15J390-354	Barratta	15J390-656
Black River mou	16J350-207	Burdekin	15J390-355	Ambient	15J390-659
Sandfly Creek	16J350-210	Burdekin	15J390-356	Haughton	15J390-660
Bohle River mou	16J350-211	Burdekin	15J390-357	Haughton	15J390-666
Ambient	16J350-213	Burdekin	15J390-390	Barratta	15J390-673
Ross River	16J350-214	Burdekin	15J390-359		
Ross River	16J350-215	Burdekin	15J390-360		
Black River mou	16J350-219	Burdekin	15J390-361		
Black River mou	16J350-205	Burdekin	15J390-362		
Bohle River mou	16J350-220	Burdekin	15J390-363		
Sandfly Creek	16J350-225	Burdekin	15J390-364		
		Burdekin	15J390-368		
		Burdekin	15J390-374		
		Burdekin	15J390-383		
		Burdekin	15J390-384		

NO TN data available for Townsville (Ross and Black Rivers) and for Burdekin plume samples. Need to check with lab.

Parameters measured in the 2007 flood plume data (x denotes if the parameter was appended to the overall flood plume dataset.

Fields in JCU 2007 flood data

Sample ID	Ametryn (µg/L)
Catchment	Atrazine (µg/L)
Kit	Desethyl Atrazine (µg/L)
River	Desisopropyl Atrazine (µg/L)
unique id	Diuron (µg/L)
Location	Fluometuron (µg/L)
Date collected	Hexazinone (µg/L)
Year	Prometryn (µg/L)
Month	Simazine (µg/L)
Cyclone	Tebuthiuron (µg/L)
Catch_no	Oil & Grease (mg/L)
Cyclone_No	Total Silver (µg/L)
T_B_W_S	Filterable Silver (µg/L)
S_Depth	Total Aluminium (µg/L)
Agency	Filterable Aluminium (µg/L)
Dist_mou	Total Arsenic (µg/L)
Lab salinity	Filterable Arsenic (µg/L)
Refractometer	Total Barium (µg/L)
YSI	Filterable Barium (µg/L)
TSS	Total Beryllium (µg/L)
Ba-137	Filterable Beryllium (µg/L)
Y	Total Bismuth (µg/L)
Chlorophyll a (µg/L)	Filterable Bismuth (µg/L)
Phaeophytin (µg/L)	Total Cobalt (µg/L)
Total Nitrogen (µg N/L)	Filterable Cobalt (µg/L)
TN uM	Total Chromium (µg/L)
Particulate N (µg N/L)	Filterable Chromium (µg/L)
PN uM	Total Copper (µg/L)
Total Filterable N (µg N/L	Filterable Copper (µg/L)
Total Filt N uM	Total Iron (µg/L)
DON (µg N/L)	Filterable Iron (µg/L)
DON uM	Total Manganese (µg/L)
Ammonia (µg N/L)	Filterable Manganese (µg/L)
NH4 uM	Total Molybdenum (µg/L)
Nitrite (µg N/L)	Filterable Molybdenum (µg/L)
Nitrate (µg N/L)	Total Nickel (µg/L)
NOx	Filterable Nickel (µg/L)
Nox uM	Total Lead (µg/L)
NOX proportion of TN	Filterable Lead (µg/L)
DIN	Total Antimony (µg/L)
Total Phosphorus (µg P/L)	Filterable Antimony (µg/L)
TP uM	Total Selenium (µg/L)
Particulate P (µg P/L)	Filterable Selenium (µg/L)
PP uM	Total Thallium (µg/L)
Field36	Filterable Thallium (µg/L)
TFP uM	Total Vanadium (µg/L)
DOP (µg P/L)	Filterable Vanadium (µg/L)
DOP uM	Total Zinc (µg/L)
FRP	Filterable Zinc (µg/L)
FRP uM	Total Uranium (µg/L)
TN:TP Molar Ratio	FilterableUranium (µg/L)
DIN:FRP Molar Ratio	Total Cadmium (µg/L)
longitude	Filterable Cadmium (µg/L)
latitude	

2.3   AIMS data collected during 2007

Program: Catchment to Coast MTSFR 3.7.2

Duration: 2007

Organisation: JCU

Individuals: Britta Schaffelke

Area: Burdekin and Mackay Whitsundays catchment

Events: 1

[Major flow event in February 2007, no cyclone associated]

Description:

For description of samples in each trip refer to the table below.

Data conversion:

Latitude and longitude converted to degrees and minutes

Nutrient Laboratory results given in µg L^{-1} – converted to µM

Comparison between *in situ* salinity and laboratory salinity

CHECK TDP (DOP plus DIP)

TDN (DIN plus DON)

unique id	Depth Code	Sample depth	Duplicate	Sample Time	Lat	Long	Position
WQS107	D0	0	1	06-Feb-07	-19.2795	147.0783	AIMS jetty
WQS108	D0	0	1	06-Feb-07	-19.30733	147.3798	Green
WQS109	D0	0	1	09-Feb-07	-19.5675	147.6233	mouth
WQS109	D2	12	2	09-Feb-07	-19.5675	147.6233	mouth
WQS110	D0	0	1	09-Feb-07	-19.69433	147.7488	Cape Upstart
WQS111	D0	0	1	09-Feb-07	-19.97733	148.3252	Edgecumbe Bay
WQS112	D0	0	1	09-Feb-07	-19.96567	148.4483	Island
WQS113	D0	0	1	10-Feb-07	-20.37283	148.8482	Long Island
WQS114	D0	0	1	10-Feb-07	-20.46283	149.0338	(Seaforth)
WQS115	D0	0	1	10-Feb-07	-20.3345	148.9262	(Dent)
WQS116	D0	0	1	10-Feb-07	-20.28717	149.0697	Island
WQS117	D0	0	1	11-Feb-07	-20.254	149.1717	Edward Island
WQS118	D0	0	1	11-Feb-07	-20.16433	149.0755	Deloraine Island
WQS119	D0	0	1	11-Feb-07	-20.16933	148.9557	Whitsunday
WQS120	D0	0	1	12-Feb-07	-20.24267	148.8105	Molle Island
WQS121	D0	0	1	12-Feb-07	-20.29183	148.7975	Shute Island
WQS121	D0	0	2	12-Feb-07	-20.29183	148.7975	Shute Island
WQS122	D0	0	1	12-Feb-07	-20.608	148.8985	Repulse Bay
WQS123	D0	0	1	12-Feb-07	-20.8585	149.2848	open water
WQS124	D0	0	1	13-Feb-07	-21.63333	150.1045	Percy Isles
WQS125	D0	0	1	13-Feb-07	-21.47	149.9642	to Whitsundays
WQS126	D0	0	1	13-Feb-07	-21.34817	149.8487	to Whitsundays
WQS127	D0	0	1	13-Feb-07	-21.23867	149.7238	to Whitsundays
WQS128	D0	0	1	13-Feb-07	-21.13217	149.6033	to Whitsundays
WQS129	D0	0	1	13-Feb-07	-21.0295	149.4745	to Whitsundays
WQS130	D0	0	1	13-Feb-07	-20.92333	149.3488	to Whitsundays
WQS131	D0	0	1	13-Feb-07	-20.814	149.2232	to Whitsundays
WQS132	D0	0	1	13-Feb-07	-20.7155	149.1032	to Whitsundays
WQS133	D0	0	1	13-Feb-07	-20.60033	149.0063	to Whitsundays
WQS134	D0	0	1	13-Feb-07	-20.4645	148.9498	to Whitsundays
WQS135	D0	0	1	13-Feb-07	-20.32967	148.8417	Long Island
WQS136	D0	0	1	14-Feb-07	-20.32967	148.8417	Long Island
WQS137	D0	0	1	14-Feb-07	-20.21183	148.7697	Whitsundays
WQS138	D0	0	1	14-Feb-07	-20.10133	148.6947	Island
WQS139	D0	0	1	14-Feb-07	-19.9655	148.4622	Cone to
WQS140	D0	0	1	14-Feb-07	-19.88833	148.2927	Cone to
WQS141	D0	0	1	14-Feb-07	-19.82017	148.116	Cone to
WQS142	D0	0	1	14-Feb-07	-19.745	147.877	Cone to
WQS143	D0	0	1	14-Feb-07	-19.6495	147.7683	Cone to
WQS144	D0	0	1	14-Feb-07	-19.5705	147.622	mouth
WQS145	D0	0	1	14-Feb-07	-19.4205	147.5255	Burdekin to
WQS146	D0	0	1	14-Feb-07	-19.2935	147.4028	Burdekin to

2.3   AIMS data collected during 2007

Program: Catchment to Coast MTSFR 3.7.2

Duration: 2007

Organisation: JCU

Individuals: Katarina Fabricius

Area: Tully Murray catchment

Events: 1

[February 2007, Cyclone Nelson]

Description:

For description of samples in each trip refer to the table below.

Data conversion:

Latitude and longitude converted to degrees and minutes.

Nutrient Laboratory results given in µg L^{-1} – converted to µM.

Comparison between *in situ* salinity and laboratory salinity.

Unique sample id	Lat	Long	Catchment	Date
KF 0207-01	17°59.55'	146°05.34'	Tully-Murray	02-Feb-07
KF 0207-2	18°00.36'	146°05.43'	Tully-Murray	02-Feb-07
KF 0207-3	18°02.11'	146°06.78	Tully-Murray	02-Feb-07
KF 0207-4	18°00.52'	146°07.82'	Tully-Murray	02-Feb-07
KF 0207-5	18°00.46'	146°08.38'	Tully-Murray	02-Feb-07
KF 0207-6	18°00.53'	146°09.99'	Tully-Murray	02-Feb-07
KF 0207-7	17°58.28'	146°10.89'	Tully-Murray	02-Feb-07
KF 0207-8	17°57.01'	146°07.48'	Tully-Murray	02-Feb-07
KF 0207-9	17°57.40'	146°07.09'	Tully-Murray	02-Feb-07
KF 0207-10	17°57.26'	146°06.42'	Tully-Murray	02-Feb-07
KF 0207-11	17°59.56'	146°05.30'	Tully-Murray	04-Feb-07
KF 0207-12	17°57.11'	146°06.48'	Tully-Murray	04-Feb-07
KF 0207-13	17°57.41'	146°07.25'	Tully-Murray	04-Feb-07
KF 0207-14	17°57.16'	146°07.50'	Tully-Murray	04-Feb-07
KF 0207-15	18°00.33'	146°05.44'	Tully-Murray	04-Feb-07
KF 0207-16	18°00.51'	146°07.82'	Tully-Murray	04-Feb-07
KF 0207-17	18°00.80'	146°09.34'	Tully-Murray	04-Feb-07
KF 0207-18	17°59.92'	146°08.28'	Tully-Murray	18-Feb-07
KF 0207-19	18°00.22'	146°05.67'	Tully-Murray	18-Feb-07
KF 0207-20	18°00.27'	146°06.78'	Tully-Murray	18-Feb-07
KF 0207-21	18°00.51'	146°07.82'	Tully-Murray	18-Feb-07
KF 0207-22	18°00.80'	146°09.34'	Tully-Murray	18-Feb-07
KF 0207-23	17°57.36'	146°06.55'	Tully-Murray	19-Feb-07
KF 0207-24	17°57.42'	146°07.13'	Tully-Murray	19-Feb-07
KF 0207-25	17°57.01'	146°07.48'	Tully-Murray	19-Feb-07
KF 0207-26	17°58.28'	146°10.89'	Tully-Murray	19-Feb-07

3. New data (post 2008 – MTSRF program)

3.1. Flood plume data collected during 2008 – TropWater/JCU

Program: Spatial and temporal monitoring of flood plumes MTSFR 3.7.2

Duration: 2008

Organisation: TropWater

Individuals: M Devlin, Z Bainbridge, S. Lewis

Area: Fitzroy and Burdekin catchment

Events: 3

[Major flow event in January 2008, no cyclone associated]

Description:

For description of samples in each trip refer to the table below.

[Burdekin sampling trip 2]

[Burdekin sampling trip 3]

[Fitzroy sampling trip 1]

Data conversion:

Latitude and longitude converted to degrees and minutes.

Nutrient Laboratory results given in µg L^{-1} – converted to µM.

Comparison between *in situ* salinity and laboratory salinity.

Table: Sampling details for Burdekin and Fitzroy flood plumes taken by JCU.

Unique Id	Agency	Catchment	08 geographic da	8 geographic dat	Date	Cruise N0	Site description
FP01	JCU	Burdekin	-19.56355	147.58862	22-Jan-08	2008001	
FP02	JCU	Burdekin	-19.50687	147.54352	22-Jan-08	2008001	
FP03	JCU	Burdekin	-19.68697	147.62834	22-Jan-08	2008001	Burdekin mouth
FP04	JCU	Burdekin	-19.4568	147.50665	22-Jan-08	2008001	Ck
FP05	JCU	Burdekin	-19.61641	147.6238	22-Jan-08	2008001	
FP06	JCU	Burdekin	-19.36663	147.48798	22-Jan-08	2008001	
FP07	JCU	Burdekin	-19.58754	147.61167	22-Jan-08	2008001	
FP08	JCU	Burdekin	-19.64197	147.62457	22-Jan-08	2008001	
FP09	JCU	Burdekin	-19.66074	147.62412	22-Jan-08	2008001	
FP16	JCU	Burdekin	-19.24938	147.24872	23-Jan-08	2008001	
FP18	JCU	Burdekin	-19.1234	146.91307	23-Jan-08	2008001	Rocks
FP10	JCU	Burdekin	-19.71854	147.62259	22-Jan-08	2008001	Burdekin mouth
FP11	JCU	Burdekin	-19.22387	147.4162	23-Jan-08	2008001	plume edge
FP12	JCU	Burdekin	-19.22387	147.4162	23-Jan-08	2008001	
FP13	JCU	Burdekin	-19.15694	146.97378	23-Jan-08	2008001	
FP14	JCU	Burdekin	-19.18668	147.05028	23-Jan-08	2008001	rock and
FP15	JCU	Burdekin	-19.23247	147.208	23-Jan-08	2008001	
FP17	JCU	Burdekin			22-Jan-08	2008001	BLANK
FP19	JCU	Burdekin	-19.30541	147.44494	22-Jan-08	2008001	Bowling Green
FP20	JCU	Burdekin	-19.30278	147.61785	23-Jan-08	2008001	Yongala
FP25	JCU	Burdekin	-19.15305	146.87617	23-Jan-08	2008001	Bay
FPMP08	JCU	Fitzroy	-23.34425	150.81512	31-Jan-08	2008002	Near Girt Island
FPMP09	JCU	Fitzroy	-23.39691	150.9142	31-Jan-08	2008002	
FPMP10	JCU	Fitzroy	-23.44521	150.90679	31-Jan-08	2008002	
FPMP11	JCU	Fitzroy	-23.50563	150.91179	31-Jan-08	2008002	Fitzroy Mouth
FPMP12	JCU	Fitzroy	-23.33789	150.88849	31-Jan-08	2008002	
FPMP14	JCU	Fitzroy	-23.49767	150.91786	31-Jan-08	2008002	
FPMP18	JCU	Fitzroy	-23.31054	150.89497	31-Jan-08	2008002	Island
FPMP01	JCU	Fitzroy	-23.17101	150.88896	01-Feb-08	2008003	
FPMP02	JCU	Fitzroy			01-Feb-08	2008003	BLANK
FPMP03	JCU	Fitzroy	-22.96163	150.7995	01-Feb-08	2008003	Bay
FPMP04	JCU	Fitzroy	-23.17101	150.88896	01-Feb-08	2008003	
FPMP05	JCU	Fitzroy	-23.14868	150.80293	01-Feb-08	2008003	
FPMP06	JCU	Fitzroy	-23.20053	150.94788	01-Feb-08	2008003	
FPMP07	JCU	Fitzroy	-23.48771	150.92355	31-Jan-08	2008002	North of Mouth
FPMP13	JCU	Fitzroy	-23.184485	150.88812	01-Feb-08	2008003	Keppel
FPMP15	JCU	Fitzroy	-23.35713	150.88924	31-Jan-08	2008002	West of Peak
FPMP16	JCU	Fitzroy	-23.22835	150.86346	31-Jan-08	2008002	
FPMP17	JCU	Fitzroy	-23.37783	150.89907	31-Jan-08	2008002	
FPMP19	JCU	Fitzroy	-20.0556	150.79191	01-Feb-08	2008003	marina and
FPMP23	JCU	Fitzroy	-20.05621	150.82734	01-Feb-08	2008003	
FPMP31	JCU	Burdekin	-19.03832	146.61782	05-Feb-08	2008004	Island
FPMP32	JCU	Burdekin	-19.109505	146.88729	05-Feb-08	2008004	Orchard Rocks
FPMP33	JCU	Burdekin	-19.09023	146.56628	05-Feb-08	2008004	Paluma Shoals
FPMP35	JCU	Burdekin	-19.07545	146.85438	05-Feb-08	2008004	Bay
FPMP36	JCU	Burdekin	-19.12686	146.77656	05-Feb-08	2008004	Off West Point
FPMP37	JCU	Burdekin	-19.09594	146.84075	05-Feb-08	2008004	bay
FPMP38	JCU	Burdekin	-19.12683	146.71128	05-Feb-08	2008004	Burdekin Rock
FPMP39	JCU	Burdekin	-19.19566	146.80795	05-Feb-08	2008004	Middle Reef
FPMP70	JCU	Burdekin	-19.2098	146.84375	05-Feb-08	2008004	and MI
FPMP75	JCU	Burdekin	-19.170645	146.86373	05-Feb-08	2008004	Bay
FPMP34	JCU	Burdekin	-18.92696	146.60494	06-Feb-08	2008005	Nth Acaron Is
FPMP40	JCU	Burdekin	-19.19566	146.80795	06-Feb-08	2008005	BLANK
FPMP60	JCU	Burdekin	-18.84148	146.53289	06-Feb-08	2008005	Island
FPMP71	JCU	Burdekin	-18.56605	146.49194	06-Feb-08	2008005	Is.
FPMP72	JCU	Burdekin	-19.03132	146.62371	06-Feb-08	2008005	Rattlesnake
FPMP73	JCU	Burdekin	-18.96555	146.62817	06-Feb-08	2008005	Acaron Island
FPMP74	JCU	Burdekin	-18.81814	146.4371	06-Feb-08	2008005	Pandora Reef
FPMP76	JCU	Burdekin	-18.65961	146.50291	06-Feb-08	2008005	Phantom
FPMP77	JCU	Burdekin	-18.887	146.57062	06-Feb-08	2008005	Havannah and
FPMP78	JCU	Burdekin	-18.887	146.57062	06-Feb-08	2008005	Is.

2e. Flood plume data collected during 2008 – CSIRO (FITZROY)

Program: CSIRO

Duration: 2008

Organisation: CSIRO

Individuals: V. Brando

Area: Fitzroy catchment

Events: 1

[Major flow event in January 2008, no cyclone associated]

Data conversion

Latitude and longitude converted to degrees and minutes

Combined tables in ACCESS db

Sampling data for flood plume 1991- 2008

Mandatory fields

Field	Description
Unique Id	Id that links the sample and water quality data. Note all duplicates have been combined
Latitude	
Longitude	
Wind	
Clouds	

Appendix B4 TropWater Water Sampling Input Sheet

	Client/Business Name:	TROPWATER
	Billing Address:	TROPWATER
	Phone Number:	0:00
	E-mail Address:	
	Fax Number:	
	Contact Person/s:	Marine Monitoring Program
	Invoice to above?	**YES / NO**
	(If NO please specify)	

```
Laboratory Use Only
Client Code          _____
Quotation #          _____
Arrival Temperature  _____
Purchase Order       _____
Sampled by     Client ☐    Lab ☐
```

Note: Please indicate purchase order/cost centre & enter any comment about samples here:

							Physical					*Nutrients*												
N o	**Submitted Date**	**Sample Date**	**Sample Time**	**Sample No./Id**	**Sample Info**	**Site Info**	**TSS**	**Chl a**	**pH**	**EC**	**Sal**	**NOx**	**NO2**	**NO3**	**NH3**	**FRP**	**TDN**	**TDP**	**TN**	**TP**	**PN**	**PP**	**CDOM**	**Reactive Silica**	**Other**
1																									
2																									

Analysis Request Sheet
Water Quality Analyses

TropWater Laboratory Services
2nd Floor ATSIP Building, James Cook University, Qld. 4811.
Tel: 07-4781-552, Fax: 07-4781-5589
Email: actfr.laboratory@jcu.edu.au

TropWATER
Centre for Tropical Water and Aquatic Ecosystem Research

Appendix B5 TropWater Auto-analysis methods: Nitrogen – Ammonia

Nitrogen – Ammonia

1.0   SCOPE AND APPLICATION

This method provides a procedure for the determination of ammonia in drinking, surface, groundwater, saline and effluent wastewater samples.

The working range is 0.001 – 0.15 mg L^{-1} as N using the Alpkem segmented flow auto analyser.

The ammonium ion reacts with a basic media to form ammonia gas. The ammonia gas diffuses across a polypropylene membrane and is retained in a slightly acidic stream. The ammonia then reacts with phenol and alkaline hypochlorite to form indophenol blue in amounts proportional to the ammonia concentration present within the sample. This blue colour is then intensified with the addition of sodium nitroferricyanide and measured spectrophotometrically at 630 nm.

1.1   Interferences

1.1.1 Contamination of samples by ammonia is the most common interference. Be aware of potential sources of contamination such as laboratory atmosphere, tobacco smoke, sweat on the hands of workers, and take steps to minimise as much as possible (such as close laboratory door and uncap samples only minutes prior to analysis).

1.1.2 Maintain an awareness of biological organisms such as algae which can assimilate ammonia rapidly and deplete the content of ammonia within a sample.

1.1.3 Turbid samples can interfere and should be filtered be through a 0.45 μm polysuphone membrane filter

1.1.4 Metal ions such as calcium and magnesium interfere by causing precipitation at high levels. The addition of sodium citrate should minimise this problem.

1.1.5 Positive interference may be attributed to substances e.g. urea, which when present in samples may hydrolyse to form ammonia.

1.1.6 Amines and nitrates can cause positive interference at high concentrations.

1.1.7 Cyanide, thiocyanate and sulfide give positive interference at >10 mg L^{-1}.

1.2   Approximately 26 samples per hour can be analysed.

1.3   This method requires experience in the use of automated gas segmented flow colorimetric analyses. A minimum of 6 months training under the direct supervision of an experienced nutrient analyst is required before independent nutrient analyses can be performed.

2.0   DEFINITIONS

2.1   Calibration Standard (CAL) – A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

2.2   Reagent Water (RM) - Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing Materials (ASTN). Reverse osmosis system followed by a Reagent water System that produces water with 18 MΩohm resistance is used. To avoid contamination the reagent water should be used the day of preparation.

2.3   Laboratory Spike Blank (B+) - An aliquot of reagent water to which a known quantity of nitrogen is added. The B+ is analysed exactly like a sample, its purpose is to determine the amount of nitrogen added to samples and further used in the calculation on spike recoveries aiding confidence in the digestion procedure.

2.4   Laboratory Reagent Blank (LRB) - an aliquot of reagent water that is treated exactly as a sample including exposure to all lab ware, equipment, digestion mixture, digestion and reagents that are used with all other samples. The LRB is used to determine if any background interference is present and therefore needs to be taken into consideration when sample results are calculated.

2.5   Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

2.6   Stock Standard Solution (SSS) – A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

2.7   Certified Reference Material (CRM) - "Real' samples that have been purchased from an external reputable commercial source. The CRM's are used to match matrices and concentrations with the samples being analysed to determine the accuracy of results.

2.8   SYNC Peak Solution – A coloured solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by the data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

3.0   SAFETY

3.1   Water samples collected from the estuarine and coastal environments are generally not hazardous, however it is of paramount importance that the individual collecting the samples use the proper collection techniques and be aware of potential sources of contamination.

3.2 High standards of laboratory technique should be used when preparing reagents. Laboratory personnel should obtain material safety data sheets (MSDS) for all chemicals used in this method, and read and sign acknowledgement over the handling of these chemicals. A lab coat, safety glasses and gloves should be used when handling concentrated acid.

4.0 EQUIPMENT AND SUPPLIES

4.1 Gas Segmented Continuous Flow Autoanalyser consisting of:

4.1.1 Autosampler.

4.1.2 Analytical manifold with membrane module for ammonia analyses.

4.1.3 Peristaltic Pump and appropriate tubing.

4.1.4 Spectrophotometer equipped with a tungsten lamp (380-800 nm) or photometer with a 630 nm interference filter (2 nm bandwidth).

4.1.5 Computer based data acquisition system.

4.2 Glassware and Supplies

4.2.1 All labware used in the analysis must be low in residual ammonia to avoid sample or reagent contamination. Soaking with lab grade detergent, rinsing with reverse osmosis water, followed by rinsing with 10% HCl and thoroughly rinsing with reagent water is sufficient.

4.2.2 Automatic pipetters capable of delivering volumes ranging from 10 – 100 μL and 100 – 1000 μL with an assortment of high quality disposable pipette tips. It is standard protocol to check the accuracy of the pipetters at least once per week using a four decimal place analytical balance.

4.2.3 Analytical balance, with capability to measure 4 decimal places for preparing SSS. It is standard protocol to have this balanced serviced and calibrated once per year, then checked weekly with certified weights.

4.2.4 10 mL sterile high density polypropylene, glass volumetric flasks, and glass pipettes.

4.2.5 105° C drying oven.

4.2.6 Desiccator.

4.2.7 Polysulphone sterile membrane filters with 0.45 μm nominal pore size. Plastic sterile terumo syringe.

5.0 REAGENTS AND STANDARDS

5.1 Stock Reagent Solutions – all chemicals are analytical grade quality.

5.1.1 Stock Ammonia Solution (1000 mg N L^{-1}) – Quantitatively transfer 3.819 g of pre-dried (105°C for 1 hour minimum) ammonium chloride (NH$_4$CL FW 53.49) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is to be made daily.

5.1.2 Stock Ammonia Check Solution (100 mg N L^{-1}) - Quantitatively transfer 0.4721 g of pre-dried (105°C for 1 hour minimum) ammonium sulfate ((NH$_4$)$_2$SO$_4$ FW 132.14) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is to be made daily.

5.1.3 Spiking Stock Solution (10 mg N L^{-1}) - Pipette 1 mL of the stock ammonia solution into a 10 mL Sarstedt tube and fill to the marked line with reagent water. Mix well. This solution should be made daily.

5.1.4 Stock Phenol Colour Reagent– Quantitatively transfer 0.05 g sodium nitroprusside (Sodium nitrosopentacyanoferrate III Na$_2$[Fe(CN)$_5$NO]*2H$_2$0 FW 297.95) to an 80 mL glass volumetric containing approximately 80 mL of reagent water and dissolve. Fill to the mark with reagent water and then add 2 mL of phenol solution (phenol liquefied 80% ww/in water). Invert until phenol becomes well mixed. Transfer to the working phenol reagent glass amber bottle. This solution should be made daily.

5.1.5 Stock Hypochlorite Colour Reagent – Quantitatively transfer 0.5 g sodium hydroxide (NaOH FW 40.00) to an 80 mL glass volumetric containing approximately 80 mL of reagent water and dissolve. Fill to the mark with reagent water and then add 1 mL of 12% sodium hypochlorite solution (12% liquid chlorine). Invert until well mixed. Transfer to the working phenol reagent glass amber bottle. This solution should be made daily.

5.1.6 Stock Tri Sodium Citrate Colour Reagent – Quantitatively transfer 1.0g sodium hydroxide (NaOH FW 40.00) to a 250 mL glass volumetric containing approximately 180 mL of reagent water and dissolve. Then quantitatively transfer 28.0 g tri sodium citrate (Na$_3$C$_6$H$_5$O$_7$.2H$_2$0 FW 294.10) to the same 250 mL glass volumetric containing approximately 180 mL of reagent water and dissolve. Fill to the mark with reagent water and then add 1 mL of Brij - 35 solution (30% solution). Invert until well mixed. Transfer to the working tri sodium citrate reagent glass amber bottle. This solution should be made daily.

5.1.7 Stock 10% Hydrochloric acid solution – Measure 25 mL of concentrated hydrochloric acid into a plastic 250 mL bottle containing 200 mLs of reagent water. Fill to the mark with reagent water and then add 1 mL of Brij - 35 solution (30% solution). Invert until well mixed. This solution should be made monthly.

5.2 Working Reagents

5.2.1 10% Hydrochloric Start up Solution – add 1 mL of Brij - 35 surfactant to 250 mL of a 10% hydrochloric solution. Shake gently.

5.2.2 Brij – 35 Start up Solution – add 1 mL of Brij 28 surfactant to 500 mL of reagent water and mix gently.

5.2.3 Phenol Colour Reagent – Transfer reagent from volumetric flask to the 250 mL glass amber working reagent bottle.

5.2.4 Hypochlorite Colour Reagent – Transfer reagent from volumetric flask to the 250 mL glass amber working reagent bottle.

5.2.5 Tri Sodium Citrate Colour Reagent – Transfer reagent from volumetric flask to the 250 mL glass amber working reagent bottle.

5.2.6 Coloured SYNC Peak Solution – Use either a high ammonia standard that will obtain a peak between 25 to 100 percent full scale.

5.2.7 Calibration Standards – Prepare a series of calibration standards (CAL) by diluting a suitable volume of the stock standard solution to 100 mL volumetric flask using reagent water. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards should be used to construct the calibration curve.

6.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1   Sample Collection

6.1.1 Samples collected for nutrient analyses from estuarine, coastal waters, streams and lakes are usually collected by one of two methods: either a peristaltic pump or grab sample.

6.1.2 A peristaltic pump has a length of tube attached that is weighted down so that particular depths can be sampled if required. A grab sample is obtained using a hand held sampling pole with 1 L polypropylene bottle which is filled sub surface.

6.1.4 The filterable nutrient component is filtered immediately at the point of collection using a Terumo sterile 60 mL syringe and 0.45 µm polysulphone Mini-Sart filter purchased from Sartorius (Cat #165333K).

6.2   Sample Preservation

6.2.1 After collection and filtration the samples should either be analysed immediately or if this is not possible frozen at -18°C until analysis can be performed.

6.3   Sample Storage

6.3.1 Samples should be stored in a freezer that contains nutrient samples only. If possible samples should further be stored into their relevant matrix and concentration to further minimise any possible contamination e.g. store low level marine samples away from effluent samples.

7.0   QUALITY CONTROL

7.1   The minimum requirements of this program is to demonstrate performance using in house reference materials (IHRM), duplicate analyses, spiking samples, and the use of certified reference material (CRM's) purchased from an external source. Also, all vials used for sampling, the digestion procedure, and syringe filters (if used) are subject to rigorous quality control checks before being utilised.

Additionally, to participate in inter collaborative nutrient trials at least once per year e.g. National Low Level Nutrient Collaborative Trials (NLLNCT).

7.2   In House Reference Material (IHRM)

7.2.1 A 100 µg N L^{-1} ammonia check standard is made up from the stock standard solution (SSS) of the ammonium sulfate solution. This standard is used to assess the accuracy of the ammonia calibration standards.

Step 1: Make up a secondary ammonium sulfate stock solution

Stock Ammonium sulfate Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
1	100	1000

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as a check standard.

Stock Ammonium sulfate Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
1	100	100

7.3   Duplicate Analyses

7.3.1 Duplicate analyses of samples are performed to a minimum of 5% per TropWater job submission. The standard deviation of these results should fall between +/- 10%. Failure to achieve this standard will result in samples being re-analysed.

7.4 Spiked Samples

7.4.1 Samples are spiked to a minimum of 5% per TropWater job submission. The result of this addition should calculate to be between 90-110% recovery. Failure to achieve satisfactory recovery will results in samples being re- analysed. This may mean   using   a   different   series   of   dilutions.

7.4.2 CRM are purchased in 250 mL containers. After the first thawing the whole CRM is sub – sampled into 10 mL tubes for future usage. These 10 mL tubes are then thawed   as   required.

7.5 Consumable Quality Checks

7.5.1 10 mL Sarstedt vials are ordered in large batches of at least 10,000 units (Cat # 60.9921.820). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.2 The sterile minisart 0.45 μm polysulphone filters are ordered in large batches. These filters are divided into their manufacturer batches and 1% of the filters are randomly selected and analysed for back ground contamination.

7.6 Certified Reference Material (CRM)

7.6.1 CRM's are purchased from Queensland Health in both freshwater and saltwater. The CRM's are matched to the sample matrices being analysed and also concentration. The certified value range must be achieved for sample results to be deemed accurate or all samples must be re- analysed with the correct value for the CRM's obtained.

8.0 CALIBRATION AND STANDARDISATION

8.1 At least seven calibration standards should be prepared daily for system calibration.

8.1.1 The following dilutions are used for a working range of 0.001 – 0.15 mg N L^{-1}:

Step 1: Make up a secondary ammonium chloride stock solution

Stock Ammonium chloride Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (mg N L^{-1})
10	100	100

Step 2: Using the secondary stock solution made in step 1 make up the following tertiary stock solution.

Secondary Stock Ammonium chloride Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (μg N L^{-1})
1	100	1000

Step 3: Using the tertiary stock solution made in step 2 make up the following dilutions which are used as working calibration standards.

Tertiary Stock Ammonium chloride Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (μg N L^{-1})
1	100	10
2	100	20
4	100	40
5	100	50
8	100	80
10	100	100
15	100	150

8.2 A calibration curve should be constructed for each sample run.

8.3 The calibration standards should be placed at the start, middle and end of the run to allow for any sensitivity drift.

NOTE: For large amounts of samples it may be necessary to increase the amount of calibration standards digested to ensure that a ratio of one set of standards per 20 – 30 samples is achieved.

8.4   The CRM's should be placed at the start and the end of the run to ensure the accuracy of results is maintained throughout the whole run.

8.5 Spiked samples should be prepared as follows:

8.5.1 Ensure that the sample randomly chosen to be spiked is 10 mL exactly (marked line). Use a micro pipette to remove any excess sample of conversely to add a little more sample into the vial.

NOTE: Thoroughly mix the sample before remove or adding any sample

8.5.2 Make up the spiking stock mixture by adding 1 mL of the Secondary Stock Ammonium chloride solution to a Sarstedt 10 mL tube and fill with reagent water to the mark. Mix well.

8.5.3 Add 20µl of the spiking stock mixture (equivalent of 20 µg N L^{-1}) to the 10 mL sample and mix well.

8.5.4 A minimum of three spiked reagent water samples are to be prepared and analysed for every run.

9.0   SAMPLE, CALIBRATION STANDARDS, IHRM AND DRM PREPARATION PROCEDURE

9.1   If samples are frozen, thaw samples by running under a continuous flow of cold water.

9.2   Make up the seven calibration standards (refer 8.1.1), and prepare the CRM's (refer 7.4.2).

9.3   Choose and make up the spiked samples (refer 8.5).

9.4   Choose and make up the IHRM check standard (refer 7.2.1).

10.0 ANALYTICAL PROCEDURE

10.1 Turn on the auto analyser and peristaltic pump and run the 10% hydrochloric solution through the system which has the ammonia membrane module removed for a minimum of thirty minutes to let the system equilibrate.

NOTE: If ammonia membrane module is not removed then the 10% hydrochloric acid solution will destroy the membrane and a replacement will be required.

Remove 10% hydrochloric solution and run reagent water with Brij added for fifteen minutes then add the ammonia membrane module and continue to run for another fifteen minutes. Turn on the spectrophotometer and check that the baseline is stable and you have a consistent bubble pattern through the manifold.

NOTE: The absorbency on the spectrophotometer should read 630 nm and should be set at the required sensitivity level for the range of working standards.

10.2 Set up the autosampler software for the run. This will include the unique TropWater job number and sample identification number, along with any dilution data associated with samples.

10.3 Mix the samples by inverting three times, and place in the appropriate rack number and cup position as allocated by the autosampler software.

NOTE: Lids are to remain on samples, calibration standards and laboratory reagent blanks until the last moment before they are analysed to minimise potential ammonia contamination.

10.4 Remove the reagent water with Brij and add the working phenol solution, working hypochlorite solution, and working tri sodium citrate solution to the system. Allow to equilibrate for 5 minutes.

10.5 Inject a high level ammonia standard e.g. 100 µg N L^{-1} for 90 seconds and observe the peak absorbency through the spectrophotometer.

10.6 Start the autosampler run. Observe that the first set of standards peak heights are normal and then check run periodically to ensure no malfunctions have occurred.

10.8 When autosampler run is finished stop the software, remove the working phenol solution, working hypochlorite solution and the working tri sodium citrate solution then put the reagent water with Brij back on. Rinse the manifold for a minimum of one hour and then turn the auto-analyser and peristaltic pump off.

10.9 Print out a hard copy of the trace and calculate results.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Concentrations of ammonia in samples are calculated from both quadratic and cubic equations obtained from the calibration standards.

11.2 The Quality Control of the results (refer 7.0) is assessed and results are either accepted or rejected and re-run at a later date.

11.3 Results should be reported in µg N L^{-1} unless otherwise requested (e.g. high values > 5 mg N L^{-1}) with the replicate and spike values stated on the report.

11.3.1 To calculate spike values for undiluted samples:

$$\text{Spike Recovery (\%)} = \frac{\text{Sample + Average of Blanked Spiked Samples}}{\text{Spiked Sample}} \times 100$$

11.3.2 To calculate spike values for diluted samples:

$$\text{Spike Recovery (Dilutions) (\%)} = \frac{\text{(Sample/Dilution) + Average of Blanked Spiked Samples}}{\text{(Spiked Sample/dilution)}} \times 100$$

12.0   TABLES, DIAGRAMS, FLOWCHARTS

Appendix B6 TropWater Auto-analysis methods: Phosphorus (Filterable Reactive)

Phosphorus – Filterable Reactive

1.0   SCOPE AND APPLICATION

This method provides a procedure for the determination of 0.45 μm filterable reactive phosphorus (FRP) in drinking, surface, groundwater, saline and effluent wastewater samples.

Both filterable reactive phosphorus and nitrate or nitrite are auto analysed simultaneously.

The working range is 0.005 – 0.15 mg L^{-1} as P using the Alpkem segmented flow auto analyser.

This is an automated colorimetric method for the detection of 0.45 μm filterable reactive phosphorus. Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphate to form an antimony-phosphomolybdate complex. This complex is reduced to an intense blue coloured complex by the addition of ascorbic acid. The colour produced is proportional to the phosphate concentration present in the sample. This colour is read spectrophotometrically at 885nm.

1.2   Interferences

1.1.2   Samples with high levels of turbidity cause interference. Particulates should be removed by filtering through a Minisart sterile 0.45μm polysulphone membrane filter at the point of collection.

1.1.3   As phosphates are common in many detergents it is necessary that no phosphate detergents are used during the cleaning of any glassware or sample containers used in the laboratory. The technician must always be aware of potential contaminants and take the necessary steps to avoid this error, therefore ensuring the most accurate results are obtained.

1.1.4   Arsenate in concentrations above 0.1 mg As L^{-1} can interfere with the filterable reactive phosphorus results with arsenate forming a similar colour complex to phosphate with these colorimetric reagents.

1.1.5   Sulfides and nitrites at concentrations greater than 10 mg L^{-1} and 1 mg L^{-1} respectively can cause positive interference. Sulfide effects can be treated by simple dilution of the sample since high sulfide concentrations are most often associated with high phosphate values.

1.1.6   Chromium (VI) and chromium (III) at >2 mg L^{-1} and 30 mg L^{-1} respectively cause negative interference by reacting with phosphate.

1.1.7   Metal ions, Cu (II) and Fe (III) interfere at concentrations >10 mg L^{-1} causing negative interference by reacting with phosphate by precipitating out the phosphorus from the dissolved phase.

1.2   Samples should be between pH 5.0 and 9.0.

1.3   Approximately 30 samples per hour can be analysed.

1.4   This method requires experience in the use of automated gas segmented flow colorimetric analyses. A minimum of 6 months training under the direct supervision of an experienced nutrient analyst is required before independent nutrient analyses can be performed.

2.0   DEFINITIONS

2.1   Calibration Standard (CAL)

A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

2.2   Reagent Water (RM)

Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing Materials (ASTN). Reverse osmosis system followed by a Reagent Water System that produces water with 18 MΩohm resistance is used. To avoid contamination the reagent water should be used the day of preparation.

2.3   Laboratory Spike Blank (B+)

An aliquot of reagent water to which a known quantity of phosphorus is added. The B+ is analysed exactly like a sample, its purpose is to determine the amount of phosphorus added to samples and further used in the calculation on spike recoveries aiding confidence in the digestion procedure recovery.

2.4   Laboratory Reagent Blank (LRB)

An aliquot of reagent water that is treated exactly as a sample including exposure to all lab ware, equipment, digestion mixture, digestion and reagents that are used with all other samples. The LRB is used to determine if any background interference is present and therefore needs to be taken into consideration when sample results are calculated.

2.5   Method Detection Limit (MDL)

The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

2.6   Stock Standard Solution (SSS)

A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

2.7   Certified Reference Material (CRM)

"Real' samples that have been purchased from an external reputable commercial source. The CRM are used to match matrices and concentrations with the samples being analysed to determine the accuracy of results.

2.8 SYNC Peak Solution

A coloured solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by the data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

3.0 SAFETY

3.1 Water samples collected from the estuarine and coastal environments are generally not hazardous, however it is of paramount importance that the individual collecting the samples use the proper collection techniques and be aware of potential sources of contamination.

3.2 High standards of laboratory technique should be used when preparing reagents which should all be of analytical grade. Laboratory personnel should obtain material safety data sheets (MSDS) for all chemicals used in this method, and read and sign acknowledgement over the handling of these chemicals. A lab coat, safety glasses and gloves should be used when handling concentrated acid.

4.0 EQUIPMENT AND SUPPLIES

4.1 **Gas Segmented Continuous Flow Autoanalyser** consisting of :

  4.1.1 Autosampler.

  4.1.2 Analytical manifold with a cartridge equipped for heating set to 60°.

  4.1.3 Peristaltic Pump and appropriate tubing.

  4.1.4 Spectrophotometer equipped with a tungsten lamp (380-885 nm) or photometer with an 885 nm interference filter (2 nm bandwidth).

  4.1.6 Computer based data acquisition system.

4.2 **Glassware and Supplies**

  4.2.1 All labware used in the analysis must be low in residual phosphate to avoid sample or reagent contamination. Rinsing with reverse osmosis water, followed by rinsing with 10% HCl and thoroughly rinsing with reagent water is sufficient.

  4.2.2 Automatic pipetters capable of delivering volumes ranging from 10-100 µL and 100 – 1000 µL with an assortment of high quality disposable pipette tips. It is

standard protocol to check the accuracy of the pipetters at least once per week using a four decimal place analytical balance.

4.2.3 Analytical balance, with capability to measure 4 decimal places for preparing SSS. It is standard protocol to have this balanced serviced and calibrated once per year, then checked weekly with certified weights.

4.2.4 10 mL sterile high density polypropylene, glass volumetric flasks, and glass pipettes.

4.2.5 105°C drying oven.

4.2.6 Desiccator.

4.2.7 Polysulphone sterile membrane filters with 0.45 μm nominal pore size. Plastic sterile Terumo syringe.

5.0   REAGENTS AND STANDARDS

5.1   Stock Reagent Solutions – all chemicals are analytical grade quality.

5.1.1 Stock Phosphorus Solution (1000 mg P L^{-1}) – Quantitatively transfer 4.393 g of pre- dried (105°C for 1 hour minimum) potassium phosphate monobasic (KH_2PO_4 FW 136.09) to a 1000 mL glass volumetric flask containing 800 mL of reagent water and dissolve. Fill to mark with reagent water and refrigerate. This solution is stable for 2 – 3 weeks.

5.1.2 Spiking Stock Solution (250 mg P L^{-1}) – Pipette 1 mL of the stock phosphorus solution into a 250 mL glass volumetric containing 230 mL of reagent water and then fill to marked line and mix well. This solution should be made daily.

5.1.3 Sulphuric Acid Solution –Using a 25 mL glass measuring cylinder measure out 14 mL of concentrated sulphuric acid (H_2SO_4 98.08) into a 250 mL glass volumetric flask containing approximately 200 mL of reagent water and mix well. Fill to the mark with reagent water. This solution is stable for 1 week.

5.1.4 Ammonium Molybdate Solution - Quantitatively transfer 5.000 g of pre-dried (105°C for 1 hour minimum) ammonium molybdate (($NH_4)_6Mo_7O_{24}.4H_2O$ FW 1235.86) into a 250 mL glass volumetric flask containing approximately 180 mL of reagent water and mix well. Using a 25 mL glass measuring cylinder measure out 8 mL of concentrated sulphuric acid (H_2SO_4 98.08) and add to the molybdate mixture. Mix well and fill to the mark with reagent water. This solution is stable for 1 week.

5.1.5 Antimony Potassium Tartrate Solution – Quantitatively transfer 0.125 g of antimony potassium tartrate ($KSbO.C_4H_4O_6$ FW 324.92) and 5.000 g of L-ascorbic acid ($C_6H_8O_6$ FW 176.13) into a 250 mL glass volumetric flask

containing approximately 180 mL of reagent water and mix well until the antimony is dissolved. Using a 25mL glass measuring cylinder measure out 12.5 mL of concentrated sulphuric acid (H_2SO_4 98.08) and add to the antimony mixture. Mix well and fill to the mark with reagent water and cover with foil. This solution is stable for 1 day.

5.2   Working Reagents

5.2.1 Dowfax 2A1 Start up Solution – add 1 mL of Dowfax surfactant to 500 mL of reagent water and mix gently.

   NOTE: Dowfax contains levels of nitrate, therefore keep away from Triton X 100 start-up solution.

5.2.2 Working Sulphuric Solution – add 1 mL of Dowfax to the 250 mL glass volumetric flask of stock sulphuric solution and shake gently.

5.2.3 Working ammonium molybdate solution – 250 mL glass volumetric flask of stock molybdate solution.

5.2.4 Working antimony potassium tartrate solution – 250 mL glass volumetric flask of stock antimony solution (covered in foil).

5.2.5 Coloured SYNC Peak Solution – Use either a high phosphorus standard that will obtain a peak between 25 to 100 percent full scale. Or add 50 µL of blue food colouring solution to 1000 mL and mix thoroughly.

5.2.6 Calibration Standards – Prepare a series of calibration standards (CAL) by diluting a suitable volume of the stock standard solution into a 100 mL volumetric flask using reagent water. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards should be used to construct the calibration curve.

6.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1   Sample Collection

6.1.1 Samples collected for nutrient analyses from estuarine, coastal waters, streams and lakes are usually collected by one of two methods: either a peristaltic pump or grab sample.

6.1.2 A peristaltic pump has a length of tube attached that is weighted down so that particular depths can be sampled if required. A grab sample is obtained using a hand held sampling pole with 1 L polypropylene bottle which is filled sub surface.

6.1.3 The filterable nutrient component is filtered immediately at the point of collection using a Terumo sterile 60 mL syringe and 0.45 µm sterile polysulphone Mini-Sart filter purchased from Sartorius (Cat #165333K).

6.2 Sample Preservation

6.2.1 After collection and filtration the samples should either be analysed immediately or if this is not possible frozen at -18°C until analysis can be performed.

6.3 Sample Storage

6.3.1 Samples should be stored in a freezer that contains nutrient samples only. If possible samples should further be stored into their relevant matrix and concentration to further minimise any possible contamination e.g. Store low level marine samples away from effluent samples.

7.0   QUALITY CONTROL

7.1 The minimum requirements of this program is to demonstrate performance using duplicate analyses, spiked samples, and the use of certified reference material (CRM's) purchased from an external source. Also, all vials for sampling and the digestion procedure, and syringe filters are subject to rigorous quality control checks before being utilised.

Additionally, to participate in inter collaborative nutrient trials at least once per year e.g. National Low Level Nutrient Collaborative Trials (NLLNCT).

7.2 Duplicate analyses

7.3.1 Duplicate analyses of samples are performed to a minimum of 5% per TropWater job submission. The standard deviation of these results should fall between +/- 10%. Failure to achieve this standard will results in samples being re-analysed.

7.3 Spiked Samples

7.3.1 Samples are spiked to a minimum of 5% per TropWater job submission. The result of this addition should calculate to be between 90 – 110% recovery. Failure to achieve satisfactory recovery will result in samples being re-analysed. This may mean using a different series of dilutions if required.

7.4 Certified Reference Material (CRM)

7.4.1 CRM's are purchased from Queensland Health in both freshwater and saltwater. The CRM's are matched to the sample matrices being analysed and also concentration. The certified value range must be achieved for sample results to be deemed accurate or all samples must be re- analysed with the correct value for the CRM obtained.

7.4.2 CRM's are purchased in 250 mL containers. After the first thawing the whole CRM is sub – sampled into 10 mL tubes for future usage. These 10 mL tubes are then thawed as required.

7.5 Consumable Quality Checks

7.5.1 10 mL Sarstedt vials are ordered in large batches of at least 10,000 units (Cat # 60.9921.820). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.2 The sterile minisart 0.45 μm polysulphone filters are ordered in large batches. These filters are divided into their manufacturer batches and 1% of the filters are randomly selected and analysed for back ground contamination.

8.0   CALIBRATION AND STANDARDISATION

8.1 At least seven calibration standards should be prepared daily for system calibration. These calibration standards should undergo the same digestion procedure for samples being analysed.

8.1.1 The following dilutions are used for a working range of 0.005 – 0.200 µg P L^{-1}:

Step 1: Make up a secondary stock phosphorus solution

Stock Phosphorus Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg P L^{-1})
1	1000	1000

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as working calibration standards.

Secondary Stock Phosphorus Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg P L^{-1})
1	100	10

2	100	20
4	100	40
5	100	50
8	100	80
10	100	100
15	100	150

NOTE: The calibration standards are mixed phosphorus and either nitrate or nitrite standards (refer to Nitrate and Nitrite SOP 8.1.1 or 8.1.2 for direction)

8.2   A calibration curve should be constructed for each sample run.

8.3   The calibration standards should be placed at the start, middle and end of the run to allow for any sensitivity drift.

NOTE: For large amounts of samples it may be necessary to increase the amount of calibration standards to ensure that a ratio of one set of standards per 20 – 30 samples is achieved.

8.4   The CRM should be placed at the start and the end of the run to ensure the accuracy of results is maintained throughout the whole run.

8.5   Spiked samples should be prepared as follows:

8.5.1 Step 1: Ensure that the sample randomly chosen to be spiked is 10mLs exactly (marked line). Use a micro pipette to remove any excess sample or conversely to add a little more sample into the vial.

NOTE: Thoroughly mix the sample before remove or adding any sample.

8.5.2 Step 2: Make up the spiking stock mixture by adding 1mL of Stock Phosphorus Solution (SSS) to 230 mL of reagent water in a 250 mL glass volumetric flask. Mix well and fill to the marked line,

8.5.3 Step 3: Add 50µL of the spiking stock mixture (equivalent of 20 µg P L^{-1}) to the 10 mL sample and mix well.

8.5.4 A minimum of three spiked reagent water samples are to be prepared and analysed for every run.

9.0 SAMPLE, CALIBRATION STANDARDS, AND CRM PREPARATION PROCEDURE

9.1 If samples are frozen, thaw samples by running under a continuous flow of cold water.

9.2 Make up the seven calibration standards (refer 8.1.1), and prepare the CRM's (refer 7.4.2).

9.3 Choose and make up the spiked samples (refer 8.5).

10.0 ANALYTICAL PROCEDURE

10.1 Turn on the auto analyser and peristaltic pump, run reagent water with Dowfax added through the system for a minimum of thirty minutes to let the system equilibrate. Turn on the spectrophotometer and check that the baseline is stable and you have a consistent bubble pattern through the manifold.

NOTE: The absorbency on the spectrophotometer should read 885 nm and should be set at the required sensitivity level for the range of working standards.

10.2 Set up the autosampler software for the run. This will include the unique TropWater job number and sample identification number, along with any dilution data associated with samples.

10.3 Mix the samples by inverting three times, remove lid and place in the appropriate rack number and cup position as allocated by the autosampler software.

10.4 Remove the reagent water with Dowfax and add the working sulphuric solution, working ammonium molybdate solution, and working antimony potassium tartrate solution to the system. Allow to equilibrate for 5 minutes.

10.5 Inject a high level phosphorus standard e.g. 100 µg P L^{-1} for 40 seconds and observe the peak absorbency through the spectrophotometer.

10.6 Start the autosampler run. Observe that the first set of standards peak heights are normal and then check run periodically to ensure no malfunctions have occurred.

NOTE: If any samples are observed to exceed the upper working range standard then dilution of the sample and re-analysis should occur at the positions allocated for this purpose at the end of the run.

10.7 When the autosampler run is finished stop the software, remove the working sulphuric solution, working ammonium molybdate solution, and working antimony potassium tartrate solution then put the reagent water with Dowfax back on. Rinse the manifold for a minimum of one hour and then turn the auto analyser and peristaltic pump off.

10.8 Print out a hard copy of the trace and calculate results using the relevant Excel spreadsheets located in the JCU team drive (internal access only),

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Concentrations of filterable reactive phosphorus in samples are calculated from both quadratic and cubic equations obtained from the calibration standards.

11.2 The Quality Control of the results (refer 7.0) is assessed and results are either accepted or rejected and re-run at a later date.

11.3 Results should be reported in µg P L^{-1} unless otherwise requested (e.g. High values > 1 mg P L^{-1}) with the replicate and spike values stated on the report.

    11.3.1 To calculate spike values for undiluted samples:

$$\text{Spike Recovery (\%)} = \frac{\text{Sample + Average of blanked spiked samples}}{\text{Spiked Sample}} \times 100$$

    11.3.2 To calculate spike values for diluted samples:

$$\text{Spike Recovery (Dilutions) (\%)} = \frac{(\text{Sample/Dilution) + Average of Blanked Spiked Samples}}{(\text{Spiked Sample/Dilution})} \times 100$$

Appendix B7 TropWater Auto-analysis methods: Nitrate and Nitrite

Nitrogen – Nitrate and Nitrite

1.0   SCOPE AND APPLICATION

This method provides a procedure for the determination of 0.45 μm filtered nitrate and nitrite in drinking, surface, groundwater, saline and effluent wastewater samples.

Nitrate or nitrite are auto analysed simultaneously with filterable reactive phosphorus.

The working range for nitrite is 0.001 – 0.1 mg L^{-1} as N using the Alpkem segmented flow auto analyser.

The working range for nitrate is 0.002 – 2.0 mg L^{-1} as N using the Alpkem segmented flow auto analyser.

Determination of nitrate is made by calculation (i.e. Oxidised nitrogen – Nitrite).

This is an automated colorimetric method for the detection of 0.45 μm filterable nitrate and nitrite using an automated segmented flow analyser utilizing a copper coated cadmium reduction column. Samples are passed through the copper coated cadmium reduction column with the nitrate in the sample reducing to nitrite which is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a red colour azo dye which is read spectrophotometrically at 540 nm. The absorbance measured at 540 nm is linearly proportional to the concentration of nitrite + nitrate in the samples. Nitrate concentrations are obtained by subtracting the nitrite values which have been separately determined by analyzing the same sample minus the copper coated cadmium reduction column from the nitrate plus nitrite value obtained when using the cadmium column.

1.3   Interferences

1.1.1 Hydrogen sulfide at concentrations >0.1 mg S L^{-1} can interfere with the efficiency of the cadmium column causing a decrease in sensitivity.

1.1.2   Iron, Copper and other heavy metals at concentrations greater than 1 mg L^{-1} can reduce the efficiency of the cadmium column. The addition of EDTA in the buffer solution will complex these metal ions.

1.1.3   Oil and grease can interfere by coating the reactive surface of the cadmium column.

1.1.4 Residual chlorine oxidizes the cadmium column and should be removed by the addition of sodium thiosulfate to the sample.

1.1.5 Samples with high levels of turbidity cause interference. Particulates should be removed by filtering through a Minisart sterile 0.45 μm polysulphone membrane filter at the point of collection.

1.1.6 Highly coloured samples can bias the results. The effect of colour can be ascertained by removing the colour reagent and running the sample through the spectrophotometer.

1.1.7 Organic compounds such as phenols and aromatic amines, which couple with the diazonium ions can cause interference.

1.2 Approximately 30 samples per hour can be analysed.

1.3 This method requires experience in the use of automated gas segmented flow colorimetric analyses, and familiarity with the techniques of preparation, activation and maintenance of the cadmium reduction column. A minimum of 6 months training under the direct supervision of an experienced nutrient analyst is required before independent nutrient analyses can be performed.

2.0   DEFINITIONS

2.1   Calibration Standard (CAL) – A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

2.2   Reagent Water (RM) - Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing Materials (ASTN). Reverse osmosis system followed by a Reagent water System that produces water with 18 MΩohm resistance is used. To avoid contamination the reagent water should be used the day of preparation.

2.3   Laboratory Spike Blank (B+) - An aliquot of reagent water to which a known quantity of nitrogen is added. The B+ is analysed exactly like a sample, its purpose is to determine the amount of nitrogen added to samples and further used in the calculation on spike recoveries aiding confidence in the digestion procedure.

2.4   Laboratory Reagent Blank (LRB) - an aliquot of reagent water that is treated exactly as a sample including exposure to all lab ware, equipment, digestion mixture, digestion and reagents that are used with all other samples. The LRB is used to determine if any background interference is present and therefore needs to be taken into consideration when sample results are calculated.

2.5   Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

2.6   Stock Standard Solution (SSS) – A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

2.7 **Certified Reference Material (CRM) -** "Real' samples that have been purchased from an external reputable commercial source. The CRM's are used to match matrices and concentrations with the samples being analysed to determine the accuracy of results.

2.8 **SYNC Peak Solution –** A coloured solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by the data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

3.0 SAFETY

3.1 Water samples collected from the estuarine and coastal environments are generally not hazardous, however it is of paramount importance that the individual collecting the samples use the proper collection techniques and be aware of potential sources of contamination.

3.2 High standards of laboratory technique should be used when preparing reagents. Laboratory personnel should obtain material safety data sheets (MSDS) for all chemicals used in this method, and read and sign acknowledgement over the handling of these chemicals. A lab coat, safety glasses and gloves should be used when handling concentrated acid.

4.0 EQUIPMENT AND SUPPLIES

4.1 **Gas Segmented Continuous Flow Autoanalyser** consisting of:

   4.1.1 Autosampler.

   4.1.2 Analytical manifold with reaction coils for nitrate analyses.

   4.1.3 Open Tubular Cadmium Reactor (OTCR) is a laboratory prepared packed copper coated cadmium reduction column (prepared according to procedures in section 5.3.1)

   4.1.4 Peristaltic Pump and appropriate tubing.

   4.1.5 Spectrophotometer equipped with a tungsten lamp (380-800 nm) or photometer with a 540 nm interference filter (2 nm bandwidth).

   4.1.6 Computer based data acquisition system.

   4.1.7 Inert gas (high purity grade, 99.99%) e.g. argon, nitrogen.

4.2 **Glassware and Supplies**

   4.2.1 All labware used in the analysis must be low in residual nitrate to avoid sample or reagent contamination. Soaking with lab grade detergent, rinsing with reverse

osmosis water, followed by rinsing with 10% HCl and thoroughly rinsing with reagent water is sufficient.

4.2.2 Automatic pipettes capable of delivering volumes ranging from 10 – 100 µL and 100 – 1000 µL with an assortment of high quality disposable pipette tips. It is standard protocol to check the accuracy of the pipettes at least once per week using a four decimal place analytical balance.

4.2.3 Analytical balance, with capability to measure 4 decimal places for preparing SSS. It is standard protocol to have this balanced serviced and calibrated once per year, then checked weekly with certified weights.

4.2.4 10 mL sterile high density polypropylene, glass volumetric flasks, and glass pipettes.

4.2.5 105° C drying oven.

4.2.6 Desiccator.

4.2.7 Polysulphone sterile membrane filters with 0.45 µm nominal pore size. Plastic sterile terumo syringe.

4.2.8 A pH meter with glass electrode and a reference electrode. A set of standard buffer solutions for calibration of the pH meter.

5.0   REAGENTS AND STANDARDS

5.1   **Stock Reagent Solutions** – all chemicals are analytical grade quality.

5.1.1 Stock Sulfanilamide Solution – Dissolve 40g Sulfanilamide and 1g N-1-Naphthylethylenediamine dihydrochloride ($C_{12}H_{16}C_{l2}N_2$ (NEDD) FW 259.18) in 800 mL of reagent water. Add 100 mL ortho-phosphoric acid (H_3PO_4 85%) and make up to 1 L mark in a glass volumetric flask. Stir for one hour on a magnetic stirrer. Store refrigerated in an amber glass bottle. This solution is stable for 6 weeks.

5.1.2 Stock Nitrate Solution (200 mg N L^{-1}) – Quantitatively transfer 1.4436 g of pre-dried (105°C for 1 hour minimum) potassium nitrate (KNO_3 FW 101.099) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is stable for 2-3 weeks.

5.1.3 Spiking Stock Solution (20 mg N L^{-1}) - Pipette 1 mL of the stock nitrate solution into a 10 mL Sarstedt tube and fill to the marked line with reagent water. Mix well. This solution should be made daily.

5.1.4 Stock Nitrite Solution (50 mg N L^{-1}) - Quantitatively transfer 0.246 g of pre dried (105°C for 1 hour minimum) sodium nitrite ($NaNO_2$ FW 68.99) to a 1000 mL glass

volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is stable for 2-3 weeks.

5.1.5 Ammonium Chloride Buffer – Dissolved 85 g of ammonium chloride (NH$_4$CL FW 53.49) and 1 g of Ethylene-diamine – tetra-acetic acid (EDTA C$_{10}$H$_{13}$N$_2$O$_8$Na$_3$ FW 358.2) in 800 mL of reagent water. Using a 6 N NaOH solution buffer the pH up till pH 8.5 is reached. Add Reagent water to reach the 1000 mL fill line and then store refrigerated in an amber glass bottle.

5.2 Working Reagents

5.2.1 Triton X 100 Start-up Solution – add 1 mL of Triton X 100 surfactant to 500 mL of reagent water and mix gently.

5.2.2 Working Sulfanilamide Solution – add 1 mL of triton X 100 to 200 mL of the stock sulfanilamide solution in a glass amber bottle and shake gently.

5.2.3 Ammonium Chloride Buffer – Mix a 1:3 ratio of ammonium chloride buffer to Reagent water in a 1L amber glass bottle and add 2 mL of Triton X 100.

5.2.4 Copper Sulfate Solution (2%) – Dissolve 20 g of copper sulfate (CuSO$_4$.5H$_2$O, FW 249.61) in 1 L of reagent water.

5.2.5 Coloured SYNC Peak Solution – Use either a high nitrate standard that will obtain a peak between 25 to 100 percent full scale. Or add 50 µL of red food colouring solution to 1000 mL and mix thoroughly.

5.2.6 Calibration Standards – Prepare a series of calibration standards (CAL) by diluting a suitable volume of the stock standard solution to 100 mL volumetric flask using reagent water. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards should be used to construct the calibration curve.

5.3 Open Tubular Cadmium Reactor (OTCR)

5.3.1 Open Tubular Cadmium Reactor – nitrate in the samples is reduced to nitrite by either a commercial open tubular cadmium reactor or a laboratory packed copper coated cadmium reduction column. If an OTCR is being employed the following procedure should be used to activate it:

Prepare reagent water, 10% HCl solution and 2% CuSO$_4$ solution in three 60 mL syringes with unions. Firstly flush through 10 mL of reagent water, then flush with 10 mL of 10% HCl in 3 seconds and follow immediately with 30 mL of reagent water. Slowly flush 20 mL CuSO$_4$ solution until a large amount of black precipitated copper comes out of the OTCR then stop flushing. Finally flush the

OTCR with reagent water and then fill with ammonium chloride buffer for short term storage.

6.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1   Sample Collection

6.1.1 Samples collected for nutrient analyses from estuarine, coastal waters, streams and lakes are usually collected by one of two methods: either a peristaltic pump or grab sample.

6.1.2 A peristaltic pump has a length of tube attached that is weighted down so that particular depths can be sampled if required. A grab sample is obtained using a hand held sampling pole with 1 L polypropylene bottle which is filled sub surface.

6.1.4 The filterable nutrient component is filtered immediately at the point of collection using a Terumo sterile 60 mL syringe and 0.45 µm polysulphone Mini-Sart filter purchased from Sartorius (Cat #165333K).

6.2 Sample Preservation

6.2.1 After collection and filtration the samples should either be analysed immediately or if this is not possible frozen at -18°C until analysis can be performed.

6.3 Sample Storage

6.3.1 Samples should be stored in a freezer that contains nutrient samples only. If possible samples should further be stored into their relevant matrix and concentration to further minimise any possible contamination e.g. store low level marine samples away from effluent samples.

7.0 QUALITY CONTROL

7.1   The minimum requirements of this program is to demonstrate performance using in house reference materials (IHRM), duplicate analyses, spiking samples, and the use of certified reference material (CRM's) purchased from an external source. Also, all vials used for sampling, the digestion procedure, and syringe filters (if used) are subject to rigorous quality control checks before being utilised.

Additionally, to participate in inter collaborative nutrient trials at least once per year e.g. National Low Level Nutrient Collaborative Trials (NLLNCT).

7.2 In House Reference Material (IHRM)

7.2.1 A 1000 µg N L^{-1} nitrite standard is made up from the stock standard solution (SSS). This standard is used to assess the efficiency of the OTCR.

Stock Nitrite Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
2	100	1000

7.3 Duplicate Analyses

7.3.1 Duplicate analyses of samples are performed to a minimum of 5% per TropWater job submission. The standard deviation of these results should fall between +/- 10%. Failure to achieve this standard will result in samples being re-analysed.

7.4 Spiked Samples

7.5.1 Samples are spiked to a minimum of 5% per TropWater job submission. The result of this addition should calculate to be between 90-110% recovery. Failure to achieve satisfactory recovery will results in samples being re- analysed. This may mean using a different series of dilutions.

7.5.2 CRM are purchased in 250 mL containers. After the first thawing the whole CRM is sub – sampled into 10 mL tubes for future usage. These 10 mL tubes are then thawed as required.

7.6 Consumable Quality Checks

7.5.1 10 mL Sarstedt vials are ordered in large batches of at least 10,000 units (Cat # 60.9921.820). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.2 The sterile Mini-sart 0.45 µm polysulphone filters are ordered in large batches. These filters are divided into their manufacturer batches and 1% of the filters are randomly selected and analysed for back ground contamination.

7.6 Certified Reference Material (CRM)

7.6.1 CRM's are purchased from Queensland Health in both freshwater and saltwater. The CRM's are matched to the sample matrices being analysed and also concentration. The certified value range must be achieved for sample results to be deemed accurate or all samples must be re- analysed with the correct value for the CRM's obtained.

8.0 CALIBRATION AND STANDARDISATION

8.1 At least seven calibration standards should be prepared daily for system calibration. These calibration standards should undergo the same digestion procedure as the samples being analysed.

8.1.1 The following dilutions are used for a working range of 0.002 – 2.0 mg N L^{-1} as nitrate:

Step 1: Make up a secondary nitrate stock solution

Stock Nitrate Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (mg N L^{-1})
5	100	10

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as working calibration standards.

Secondary Stock Nitrate Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
1	100	100
2	100	200
4	100	400
5	100	500
8	100	800
10	100	1000
20	100	2000

NOTE: The calibration standards are mixed phosphorus and either nitrate or nitrite standards (refer to FRPSOP 8.1.1 for direction). Once the initial nitrogen specie has been analysed simultaneously with filterable reactive phosphorus the remaining specie is then analysed with new working calibration standards which has no phosphorus mixed in.

8.1.2 The following dilutions are used for a working range of 0.001 – 0.1 mg N L^{-1} as nitrite:

Step 1: Make up a secondary nitrite stock solution

Stock Nitrite Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (mg N L^{-1})
2	100	1

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as working calibration standards.

Secondary Stock Nitrate Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
1	100	10
2	100	20
4	100	40
5	100	50
8	100	80
10	100	100

8.2   A calibration curve should be constructed for each sample run.

8.3   The calibration standards should be placed at the start, middle and end of the run to allow for any sensitivity drift and or reduction in OTCR efficiency. A high nitrite standard should immediately follow the calibration standards to determine if any change in OTCR efficiency is occurring. The decline in efficiency should be less than 5% during a run. To correct for OTCR efficiency change, multiply the sample peak height by the corresponding sensitivity drift correlation.

NOTE: For large amounts of samples it may be necessary to increase the amount of calibration standards digested to ensure that a ratio of one set of standards per 20 – 30 samples is achieved.

8.4 The CRM's should be placed at the start and the end of the run to ensure the accuracy of results is maintained throughout the whole run.

8.5 Spiked samples should be prepared as follows:

8.5.1 Ensure that the sample randomly chosen to be spiked is 10 mL exactly (marked line). Use a micro pipette to remove any excess sample of conversely to add a little more sample into the vial.

NOTE: Thoroughly mix the sample before remove or adding any sample

8.5.2 Make up the spiking stock mixture by adding 1 mL of Stock Nitrate Solution (SSS) to a Sarstedt 10 mL tube and fill with reagent water to the mark. Mix well.

8.5.3 Add 50 µL of the spiking stock mixture (equivalent of 100 µg N L^{-1}) to the 10 mL sample and mix well.

8.5.4 A minimum of three spiked reagent water samples are to be prepared and analysed for every run.

9.0 SAMPLE, CALIBRATION STANDARDS, IHRM AND CRM PREPARATION PROCEDURE

9.1 If samples are frozen, thaw samples by running under a continuous flow of cold water.

9.2 Make up the seven calibration standards (refer 8.1.1 or 8.1.2, IHRM and prepare the CRM.

9.3 Choose and make up the spiked samples (refer 8.5).

10.0 ANALYTICAL PROCEDURE

10.1 Turn on the auto analyser and peristaltic pump and run reagent water with Triton X 100 added through the system for a minimum of thirty minutes to let the system equilibrate. Turn on the spectrophotometer and check that the baseline is stable and you have a consistent bubble pattern through the manifold.

NOTE: The absorbency on the spectrophotometer should read 540 nm and should be set at the required sensitivity level for the range of working standards.

10.2 Set up the autosampler software for the run. This will include the unique TropWater job number and sample identification number, along with any dilution data associated with samples.

10.3 Mix the samples by inverting three times, remove lid and place in the appropriate rack number and cup position as allocated by the autosampler software.

10.4 Remove the reagent water with Triton X-100 and add the working sulfanilamide solution, the working ammonium chloride buffer, and the inert gas to the system. Allow to equilibrate for 5 minutes.

10.5 If analysing for Nitrate and Nitrite then Regenerate the OTCR (refer 5.3.1) and place into the correct position on the manifold. Allow baseline to stabilize for 5 minutes. If analysing for nitrite only then ignore this step.

10.6 Inject a high level nitrate or nitrite standard e.g. 1000 µg N L^{-1} for 90 seconds and observe the peak absorbency through the spectrophotometer.

> **NOTE:** If the peak height it too low then remove OTCR and regenerate again. If this still does not fix peak height then use a new OTCR.

10.7 Start the autosampler run. Observe that the first set of standards peak heights are normal and then check run periodically to ensure no malfunctions have occurred.

> **NOTE:** If any samples are observed to exceed the upper working range standard then dilution of the sample and re-analysis should occur at the positions allocated for this purpose at the end of the run.

10.8 When autosampler run is finished stop the software, remove the OTCR (if used) followed by the working sulfanilamide solution and the working ammonium chloride buffer then put the reagent water with Triton X 100 back on. Rinse the manifold for a minimum of one hour and then turn the auto-analyser and peristaltic pump off.

10.9 Print out a hard copy of the trace and calculate results using the relevant excel spreadsheets located in the team drive (internal access only).

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Concentrations of nitrate and nitrite in samples are calculated from the linear regression obtained from the calibration standards.

11.4 The Quality Control of the results (refer 7.0) is assessed and results are either accepted or rejected and re-run at a later date.

11.5 Results should be reported in µg N L^{-1} unless otherwise requested (e.g. high values > 5 mg N L^{-1}) with the replicate and spike values stated on the report.

    11.5.1 To calculate spike values for undiluted samples:

$$\text{Spike Recovery (\%)} = \frac{\text{Sample + Average of Blanked Spiked Samples}}{\text{Spiked Sample}} \times 100$$

11.3.2 To calculate spike values for diluted samples:

$$\text{Spike Recovery (Dilutions) (\%)} = \frac{(\text{Sample/Dilution}) + \text{Average of Blanked Spiked Samples}}{(\text{Spiked Sample/dilution})} \times 100$$

Appendix B8 TropWater Auto-analysis methods: Nitrogen – Total Alkaline Persulfate (dissolved/filterable N)

Nitrogen – Total Alkaline Persulfate

1.0 SCOPE AND APPLICATION

This method provides a procedure for the determination on Total Nitrogen (TN) in drinking, surface, groundwater, saline and effluent wastewater samples.

Both Total Filterable Nitrogen and Total Filterable Phosphorus are digested simultaneously and also auto analysed simultaneously.

The working range is 0.025 – 2.0 mg L^{-1} as N.

Organically bound nitrogen in water samples is converted to free nitrate by digestion at elevated temperature in an alkaline solution of potassium persulfate. The resulting digest is then analysed using an automated segmented flow analyser utilizing a copper coated cadmium reduction column. The nitrate in the sample is reduced to nitrite which is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a red colour azo dye which is read spectrophotometrically at 540nm. The absorbance measured at 540nm is linearly proportional to the concentration of nitrite + nitrate in the samples.

1.4 Interferences

1.1.1 Samples containing high amounts of particulate matter (i.e. TSS mg L^{-1} value > 100) may give poor total nitrogen recoveries. These samples require dilution pre-digest that include a spike to check the nitrogen recoveries during the digestion procedure.

1.1.2 For saline samples a two stage digestion is required to ensure that the white precipitate formed with the addition of the digestion mixture is dissolved, therefore ensuring adequate nitrogen recoveries.

1.1.3 Contamination by ammonia can be a pre-digestion interference caused by the laboratory atmosphere, sweat on the technicians hand and tobacco smoke etc. To negate this contamination the technician must always be aware of any potential contaminants and take the necessary steps to minimise this error, therefore ensuring the most accurate results is obtained.

1.1.4 Samples which contain high ammonia may cause lower nitrogen recoveries due to the addition of the alkaline digestion mixture. Replicates using different dilutions and spike recoveries will ensure accurate results are obtained.

1.2 Approximately 30 samples per hour can be analysed.

1.3 This method requires experience in the use of automated gas segmented flow colorimetric analyses, and familiarity with the techniques of preparation, activation and

maintenance of the cadmium reduction column. A minimum of 6 months training under the direct supervision of an experienced nutrient analyst is required before independent nutrient analyses can be performed.

2.0   DEFINITIONS

2.1   Calibration Standard (CAL) – A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

2.2   Reagent Water (RM) - Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing Materials (ASTN). Reverse osmosis system followed by a Reagent water System that produces water with 18 MΩohm resistance is used. To avoid contamination the reagent water should be used the day of preparation.

2.3   Laboratory Spike Blank (B+) - An aliquot of reagent water to which a known quantity of nitrogen is added. The B+ is analysed exactly like a sample, its purpose is to determine the amount of nitrogen added to samples and further used in the calculation on spike recoveries aiding confidence in the digestion procedure.

2.4   Laboratory Reagent Blank (LRB) - an aliquot of reagent water that is treated exactly as a sample including exposure to all lab ware, equipment, digestion mixture, digestion and reagents that are used with all other samples. The LRB is used to determine if any background interference is present and therefore needs to be taken into consideration when sample results are calculated.

2.5   Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

2.6   Stock Standard Solution (SSS) – A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

2.7   Certified Reference Material (CRM) - "Real' samples that have been purchased from an external reputable commercial source. The CRM's are used to match matrices and concentrations with the samples being analysed to determine the accuracy of results.

2.8   SYNC Peak Solution – A coloured solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by the data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

3.0   SAFETY

3.1   Water samples collected from the estuarine and coastal environments are generally not hazardous, however it is of paramount importance that the individual collecting the samples use the proper collection techniques and be aware of potential sources of contamination.

3.2   High standards of laboratory technique should be used when preparing reagents. Laboratory personnel should obtain material safety data sheets (MSDS) for all chemicals used in this method, and read and sign acknowledgement over the handling of these chemicals. A lab coat, safety glasses and gloves should be used when handling concentrated acid.

4.0   EQUIPMENT AND SUPPLIES

4.1   **Gas Segmented Continuous Flow Autoanalyser** consisting of:

   4.1.1 Autosampler.

   4.1.2 Analytical manifold with reaction coils for nitrate analyses.

   4.1.3 Open Tubular Cadmium Reactor (OTCR) is a laboratory prepared packed copper coated cadmium reduction column (prepared according to procedures in section 5.3.1)

   4.1.4 Peristaltic Pump and appropriate tubing.

   4.1.5 Spectrophotometer equipped with a tungsten lamp (380-800 nm) or photometer with a 540 nm interference filter (2 nm bandwidth).

   4.1.6 Computer based data acquisition system.

   4.1.7 Inert gas (high purity grade, 99.99%) e.g. argon, nitrogen.

4.2   **Glassware and Supplies**

   4.2.1 All labware used in the analysis must be low in residual nitrate to avoid sample or reagent contamination. Soaking with lab grade detergent, rinsing with reverse osmosis water, followed by rinsing with 10% HCl and thoroughly rinsing with reagent water is sufficient.

   4.2.2 Automatic pipetters capable of delivering volumes ranging from 10 – 100 µL and 100 – 1000 µL with an assortment of high quality disposable pipette tips. It is standard protocol to check the accuracy of the pipetters at least once per week using a four decimal place analytical balance.

   4.2.3 Analytical balance, with capability to measure 4 decimal places for preparing SSS. It is standard protocol to have this balanced serviced and calibrated once per year, then checked weekly with certified weights.

4.2.4 10 mL and 60 mL sterile high density polypropylene, 30 mL polycarbonate vials, glass volumetric flasks, and glass pipettes.

4.2.5 105° C drying oven.

4.2.6 Desiccator.

4.2.7 Polysulphone sterile membrane filters with 0.45 μm nominal pore size. Plastic sterile terumo syringe.

4.2.8 A pH meter with glass electrode and a reference electrode. A set of standard buffer solutions for calibration of the pH meter.

4.3 Autoclave

5.0 REAGENTS AND STANDARDS

5.1 Stock Reagent Solutions – all chemicals are analytical grade quality.

5.1.1 Stock Sulfanilamide Solution – Dissolve 40g Sulfanilamide and 1g N-1-Naphthylethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$ (NEDD) FW 259.18) in 800 mL of reagent water. Add 100mL ortho-phosphoric acid (H_3PO_4 85%) and make up to 1 L mark in a glass volumetric flask. Stir for one hour on a magnetic stirrer. Store refrigerated in an amber glass bottle. This solution is stable for 6 weeks.

5.1.2 Stock Nitrate Solution (200 mg N L^{-1}) – Quantitatively transfer 1.4436 g of pre-dried (105°C for 1 hour minimum) potassium nitrate (KNO_3 FW 101.099) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is stable for 2-3 weeks.

5.1.3 Spiking Stock Solution (20 mg N L^{-1}) - Pipette 1 mL of the stock nitrate solution into a 10 mL Sarstedt tube and fill to the marked line with reagent water. Mix well. This solution should be made daily.

5.1.4 Stock Nitrite Solution (50 mg N L^{-1}) - Quantitatively transfer 0.246 g of pre dried (105°C for 1 hour minimum) sodium nitrite ($NaNO_2$ FW 68.99) to a 1000 mL glass volumetric flask containing approximately 800 mL of reagent water and dissolve. Fill to the mark with Reagent water and refrigerate. This solution is stable for 2-3 weeks.

5.1.5 Ammonium Chloride Buffer – Dissolved 85 g of ammonium chloride (NH_4CL FW 53.49) and 1 g of Ethylene-diamine – tetra-acetic acid (EDTA $C_{10}H_{13}N_2O_8Na_3$ FW 358.2) in 800 mL of reagent water. Using a 6 N NaOH solution buffer the pH up till pH 8.5 is reached. Add Reagent water to reach the 1000 mL fill line and then store refrigerated in a polypropylene bottle.

5.1.6 Digestive reagent – Mix 9 g of sodium hydroxide (NaOH FW 40.0) and 40 g of potassium persulfate ($K_2S_2O_8$ FW 270.33) in 800 mL of reagent water in a 1000 mL polypropylene bottle and then fill to the 1000 mL marked line. Magnetic stir for a minimum of 1 hour or until all reagents are dissolved. Make daily.

5.2 Working Reagents

5.2.1 Triton X 100 Start-up Solution – add 1 mL of Triton X 100 surfactant to 500 mL of reagent water and mix gently.

5.2.2 Working Sulfanilamide Solution – add 1 mL of triton X 100 to 200 mL of the stock sulfanilamide solution in a glass amber bottle and shake gently.

5.2.3 Ammonium Chloride Buffer – Mix a 1:3 ratio of ammonium chloride buffer to Reagent water in a 1L polypropylene bottle and add 2 mL of Triton X 100.

5.2.4 Copper Sulfate Solution (2%) – Dissolve 20 g of copper sulfate ($CuSO_4.5H_2O$, FW 249.61) in 1 L of reagent water.

5.2.5 Coloured SYNC Peak Solution – Use either a high nitrate standard that will obtain a peak between 25 to 100 percent full scale. Or add 50 µL of red food colouring solution to 1000mL and mix thoroughly.

5.2.6 Calibration Standards – Prepare a series of calibration standards (CAL) by diluting a suitable volume of the stock standard solution to 100 mL volumetric flask using reagent water. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of samples and not exceed two orders of magnitude. At least five calibration standards should be used to construct the calibration curve.

5.3 Open Tubular Cadmium Reactor (OTCR)

5.3.1 Open Tubular Cadmium Reactor – nitrate in the samples is reduced to nitrite by either a commercial open tubular cadmium reactor or a laboratory packed copper coated cadmium reduction column. If an OTCR is being employed the following procedure should be used to activate it:

Prepare reagent water, 10% HCl solution and 2% $CuSO_4$ solution in three 60 mL syringes with unions. Firstly flush through 10mL of reagent water, then flush with 10 mL of 10% HCl in 3 seconds and follow immediately with 30 mL of reagent water. Slowly flush 20 mL $CuSO_4$ solution until a large amount of black precipitated copper comes out of the OTCR, then stop flushing. Finally flush the OTCR with reagent water and then fill with ammonium chloride buffer for short term storage.

6.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1 Sample Collection

6.1.1 Samples collected for nutrient analyses from estuarine, coastal waters, streams and lakes are usually collected by one of two methods: either a peristaltic pump or grab sample.

6.1.2 A peristaltic pump has a length of tube attached that is weighted down so that particular depths can be sampled if required. A grab sample is obtained using a hand held sampling pole with 1 L polypropylene bottle which is filled sub surface.

6.1.3 The total nutrient component is sampled into a gamma sterile 60 mL Sarstedt polypropylene tube (Cat# 60.596.001).

6.1.4 The total filterable nutrient component is filtered immediately at the point of collection using a Terumo sterile 60 mL syringe and 0.45 µm polysulphone Mini-Sart filter purchased from Sartorius (Cat #165333K).

6.2 Sample Preservation

6.2.1 After collection and filtration the samples should either be analysed immediately or if this is not possible frozen at -18°C until analysis can be performed.

6.3 Sample Storage

6.3.1 Samples should be stored in a freezer that contains nutrient samples only. If possible samples should further be stored into their relevant matrix and concentration to further minimise any possible contamination e.g. store low level marine samples away from effluent samples.

7.0 QUALITY CONTROL

7.1 The minimum requirements of this program is to demonstrate performance using in house reference materials (IHRM), duplicate analyses, spiking samples, and the use of certified reference material (CRM's) purchased from an external source. Also, all vials used for sampling, the digestion procedure, and syringe filters (if used) are subject to rigorous quality control checks before being utilised.

Additionally, to participate in inter collaborative nutrient trials at least once per year e.g. National Low Level Nutrient Collaborative Trials (NLLNCT).

7.2 In House Reference Material (IHRM)

7.2.1 A 1000 µg N L^{-1} nitrite standards is made up from the stock standard solution (SSS). This standard is used to assess the efficiency of the OTCR.

Stock Nitrite Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
2	100	1000

7.3   Duplicate Analyses

7.3.1 Duplicate analyses of samples are performed to a minimum of 5% per TropWater job submission. The standard deviation of these results should fall between +/- 10%. Failure to achieve this standard will result in samples being re-analysed.

7.4   Spiked Samples

7.6.1 Samples are spiked to a minimum of 5% per TropWater job submission. The result of this addition should calculate to be between 90-110% recovery. Failure to achieve satisfactory recovery will results in samples being re- analysed. This may mean   using   a   different   series   of   dilutions.

7.6.2 CRM are purchased in 250 mL containers. After the first thawing the whole CRM is sub – sampled into 10 mL tubes for future usage. These 10 mL tubes are then thawed   as   required.

7.7   Consumable Quality Checks

7.5.1 10 mL Sarstedt vials are ordered in large batches of at least 10,000 units (Cat # 60.9921.820). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.2 60 mL Sarstedt vials are ordered in batches of 1,000 units (Cat #60.596.001).These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.3 30 mL Sarstedt vials are ordered in batches of 5,000 units (Cat # 60.9922.216 and 60.9922.210 (saltwater samples) with lids C3011 HDPE natural). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.6   Certified Reference Material (CRM)

7.6.1 CRM's are purchased from Queensland Health in both freshwater and saltwater. The CRM's are matched to the sample matrices being analysed and also concentration. The certified value range must be achieved for sample results to be deemed accurate or all samples must be re- analysed with the correct value for the CRM's obtained.

8.0   CALIBRATION AND STANDARDISATION

8.1   At least seven calibration standards should be prepared daily for system calibration. These calibration standards should undergo the same digestion procedure as the samples being analysed.

8.1.1 The following dilutions are used for a working range of 0.025 – 2.0 mg N L^{-1}:

Step 1: Make up a secondary nitrate stock solution

Stock Nitrate Solution (mLs)	Size Volumetric Flask (mL)	Calculated Concentration (mg N L^{-1})
5	100	10

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as working calibration standards.

Secondary Stock Nitrate Solution (mLs)	Size Volumetric Flask (mL)	Calculated Concentration (µg N L^{-1})
1	100	100
2	100	200
4	100	400
5	100	500
8	100	800
10	100	1000
20	100	2000

NOTE: The calibration standards are mixed phosphorus and nitrogen standards (refer to TPSOP 8.1.1 for direction).

8.2   A calibration curve should be constructed for each sample run.

8.4   The calibration standards should be placed at the start, middle and end of the run to allow for any sensitivity drift and or reduction in OTCR efficiency. A high nitrite standard should immediately follow the calibration standards to determine if any change in OTCR efficiency is occurring. The decline in efficiency should be less than 5% during a run. To correct for OTCR efficiency change, multiply the sample peak height by the corresponding sensitivity drift correlation.

> **NOTE:**   For large amounts of samples it may be necessary to increase the amount of calibration standards digested to ensure that a ratio of one set of standards per 20 – 30 samples is achieved.

8.6   The CRM's should be placed at the start and the end of the run to ensure the accuracy of results is maintained throughout the whole run.

8.7 Spiked samples should be prepared as follows:

> **8.5.1** Ensure that the sample randomly chosen to be spiked is 10 mL exactly (marked line). Use a micro pipette to remove any excess sample of conversely to add a little more sample into the vial.

> **NOTE:** Thoroughly mix the sample before remove or adding any sample

> **8.5.2** Make up the spiking stock mixture by adding 1 mL of Stock Nitrate Solution (SSS) to a Sarstedt 10 mL tube and fill with reagent water to the mark. Mix well.

> **8.5.3** Add 50µl of the spiking stock mixture (equivalent of 100 µg N L^{-1}) to the 10 mL sample and mix well.

> **8.5.4** A minimum of three spiked reagent water samples are to be prepared and analysed for every run.

9.0   SAMPLE, CALIBRATION STANDARDS, IHRM AND DRM PREPARATION PROCEDURE

9.1   If samples are frozen, thaw samples by running under a continuous flow of cold water.

9.2   Make up the seven calibration standards, IHRM and prepare the CRM.

9.3   Thoroughly mix the sample then decant 10 mL into a Sarstedt 10 mL sterile vial (if total filterable nitrogen is being analysed the sample will already be in the appropriate tube). If samples contain high amounts of particulates dilution series should be carried out at this point.

> **NOTE:**   Alternatively if samples fall outside of the working range for this method, samples should be diluted, re- digested, and re- analysed.

9.4   Choose and make up the spiked samples (refer 8.5).

9.5 Take the 10mL aliquot of samples, CRM, IHRM, and spiked samples and transfer after shaking sample to the Sarstedt 30 mL vial for digestion (ensure correct vial is used refer 9.7). Add 5 mL of digestive reagent and put the lid on tightly, mix well using vortex equipment and then loosen half a turn back and place vial into digestion rack for the autoclave.

9.6 Thoroughly mix and decant three lots of 10 mL for each working calibration standard and add 5mLs of digestion mixture. Put the lid on tightly, mix well using vortex equipment and then loosen half a turn back and place vial into digestion rack for the autoclave.

9.7 For freshwater digests use Sarstedt vials (Cat # 60.9922.216) and for saltwater digests use Sarstedt vials (Cat # 60.9922.210 with HDPE lids Cat # C3011).

9.8 For freshwater samples digest the samples, calibrations standards, IHRM and CRM in an autoclave at 121°C for a period of seventy minutes. For saltwater samples, also digest the calibration standards, IHRM and CRM autoclave at 121°C for a period of 35 minutes, remove samples and vortex stir then autoclave for another period of 35 minutes.

9.9 Let samples sit overnight to cool before analysing.

10.0 ANALYTICAL PROCEDURE

10.1 Turn on the auto analyser and peristaltic pump and run reagent water with Triton X 100 added through the system for a minimum of thirty minutes to let the system equilibrate. Turn on the spectrophotometer and check that the baseline is stable and you have a consistent bubble pattern through the manifold.

>   **NOTE:**   The absorbency on the spectrophotometer should read 540 nm and should be set at the required sensitivity level for the range of working standards.

10.2 Set up the autosampler software for the run. This will include the unique TropWater job number and sample identification number, along with any dilution data associated with samples.

10.3 Mix the digested sample using a vortex and decant into a 10 mL Sarstedt tubes (Cat # 60.9921.820) and place into the correct cup position as identified by the autosampler software.

10.4 Remove the reagent water with Triton X-100 and add the working sulfanilamide solution, the working ammonium chloride buffer, and the inert gas to the system. Allow to equilibrate for 5 minutes.

10.5 Regenerate the OTCR (refer 5.3.1) and place into the correct position on the manifold. Allow baseline to stabilize for 5 minutes.

10.6 Inject a high level nitrate standard e.g. 1000 µg N L^{-1} for 90 seconds and observe the peak absorbency through the spectrophotometer.

> **NOTE:** If the peak height it too low then remove OTCR and regenerate again. If this still does not fix peak height then use a new OTCR.

10.7 Start the autosampler run. Observe that the first set of standards peak heights are normal and then check run periodically to ensure no malfunctions have occurred.

10.8 When autosampler run is finished stop the software, remove the OTCR followed by the working sulfanilamide solution and the working ammonium chloride buffer then put the reagent water with Triton X 100 back on. Rinse the manifold for a minimum of one hour and then turn the auto-analyser and peristaltic pump off.

10.9 Print out a hard copy of the trace and calculate results.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Concentrations of total nitrogen in samples are calculated from the linear regression obtained from the calibration standards.

11.6 The Quality Control of the results (refer 7.0) is assessed and results are either accepted or rejected and re-run at a later date.

11.7 Results should be reported in µg N L^{-1} unless otherwise requested (e.g. high values > 5 mg N L^{-1}) with the replicate and spike values stated on the report.

11.7.1 To calculate spike values for undiluted samples:

$$\text{Spike Recovery (\%)} = \frac{\text{Sample + Average of Blanked Spiked Samples}}{\text{Spiked Sample}} \times 100$$

11.7.2 To calculate spike values for diluted samples:

$$\text{Spike Recovery (Dilutions) (\%)} = \frac{\text{(Sample/Dilution) + Average of Blanked Spiked Samples}}{\text{(Spiked Sample/dilution)}} \times 100$$

12.0   TABLES, DIAGRAMS, FLOWCHARTS

540 nm Detector

Debubbler

TN Manifold – Nitrogen
Total Alkaline Persulfate

Waste

9

10

7

8

5

6

3

4

2

Colour Reagent

Sample

Inert Gas (Ar)

NH$_4$Cl Buffer
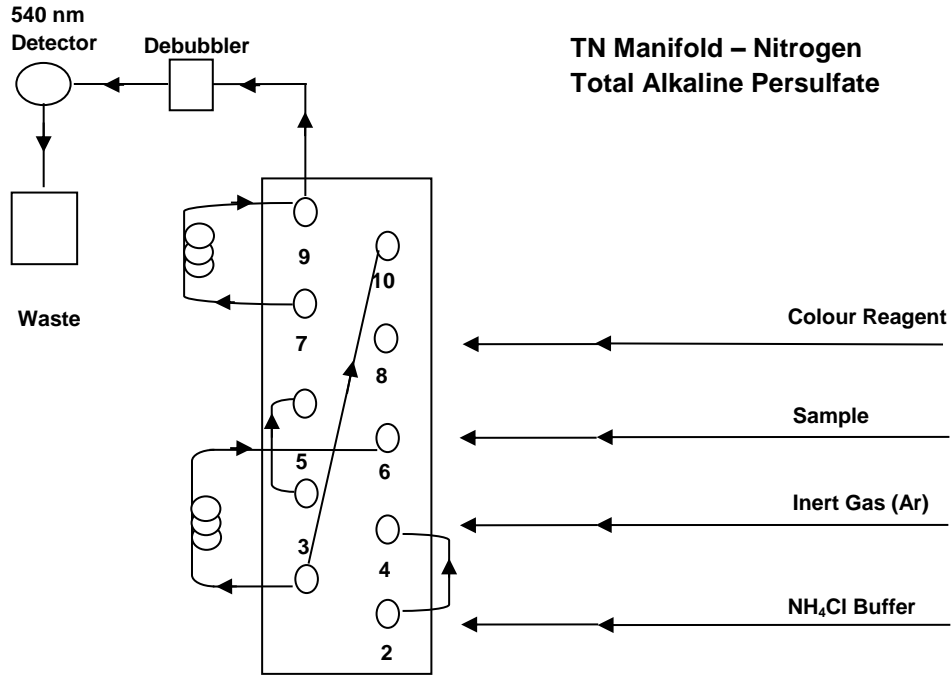
Appendix B9 TropWater Auto-analysis methods: Phosphorus – Total Alkaline Persulfate (total filterable/dissolved P)

Phosphorus – Total Alkaline Persulfate

1.0   SCOPE AND APPLICATION

This method provides a procedure for the determination of Total Filterable Phosphorus (TFP) in drinking, surface, groundwater, saline and effluent wastewater samples using persulfate digestion.

Both Total Filterable Phosphorus and Total Filterable Nitrogen are digested simultaneously and also auto analysed simultaneously.

The working range is 0.005 – 0.2 mg L^{-1} as P.

This is an automated colorimetric method for the detection of Total Phosphorus. Organic phosphorus in water samples is converted to ortho-phosphate by digestion at 121°C with persulfate in an acidic medium. The resulting digest is then analysed using an automated segmented flow analyser. Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphate to form an antimony-phosphomolybdate complex. This complex is reduced to an intense blue coloured complex by the addition of ascorbic acid. The colour produced is proportional to the phosphate concentration present in the sample. This colour is read spectrophotometrically at 885nm.

1.5   Interferences

1.1.2   Samples containing high amounts of particulate matter (i.e. TSS mg L^{-1} value > 100) may give poor total phosphorus recoveries. The dilution of these samples will derive nutrient concentrations that are considered to best reflect the 'total' phosphorus that is potentially bioavailable and limited to the non-geochemical phosphorus that is unlikely to be bioavailable, at least within a relative short term period e.g. months to years. This issue confronts some laboratories with respect to the most reliable nutrient digest procedure, but the focus of the TropWater Water Quality Laboratory is primarily to utilise the most appropriate method to determine the total bioavailable pool of phosphorus to the aquatic system.

1.1.3   For saline samples a two stage digestion process is required to ensure that the white precipitate formed with the addition of the digestion mixture is dissolved, therefore ensuring adequate phosphorus recoveries.

1.1.4   Phosphorus is commonly found in many detergents, therefore it is extremely important that phosphate based detergents are not used to clean any glassware or sample containers. The technician must always be aware of any potential contaminants and take the necessary steps to minimise this error, therefore ensuring the most accurate results are obtained.

1.1.5   Arsenate in concentrations above 0.1 mg As L^{-1} can interfere with the total phosphorus results with arsenate forming a similar colour complex to phosphate with these colorimetric reagents.

1.1.6 Sulfides and nitrites at concentrations greater than 10 mg L^{-1} and 1 mg L^{-1}, respectively, can cause positive interference.

1.1.7 Chromium (VI) and chromium (III) at >2 mg L^{-1} and 30 mg L^{-1}, respectively, cause negative interference by reacting with phosphate.

1.1.8 Metal ions, Cu (II) and Fe (III) interfere at concentrations >10 mg L^{-1} causing negative interference by reacting with phosphate.

1.2 Approximately 30 samples per hour can be analysed.

1.3 This method requires experience in the use of automated gas segmented flow colorimetric analyses. A minimum of 6 months training under the direct supervision of an experienced nutrient analyst is required before independent nutrient analyses can be performed.

2.0   DEFINITIONS

2.1   Calibration Standard (CAL)

A solution prepared from the primary dilution standard solution or stock standard solution containing analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

2.2   Reagent Water (RM)

Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing Materials (ASTN). Reverse osmosis system followed by a Reagent Water System that produces water with 18 MΩohm resistance is used. To avoid contamination the reagent water should be used the day of preparation.

2.3   Laboratory Spike Blank (B+)

An aliquot of reagent water to which a known quantity of phosphorus is added. The B+ is analysed exactly like a sample, its purpose is to determine the amount of phosphorus added to samples and further used in the calculation on spike recoveries aiding confidence in the digestion procedure recovery.

2.4   Laboratory Reagent Blank (LRB)

An aliquot of reagent water that is treated exactly as a sample including exposure to all lab ware, equipment, digestion mixture, digestion and reagents that are used with all other samples. The LRB is used to determine if any background interference is present and therefore needs to be taken into consideration when sample results are calculated.

2.5   Method Detection Limit (MDL)

The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

2.6 Stock Standard Solution (SSS)

A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

2.7 Certified Reference Material (CRM)

"Real' samples that have been purchased from an external reputable commercial source. The CRM are used to match matrices and concentrations with the samples being analysed to determine the accuracy of results.

2.8   SYNC Peak Solution

A coloured solution used to produce a synchronization peak in the refractive index measurement. A synchronization peak is required by the data acquisition programs to initialize the peak finding parameters. The first cup in every run must always be identified as a SYNC sample. The SYNC sample is usually a high standard, but can be any sample that generates a peak at least 25% of full scale.

3.0 SAFETY

3.1 Water samples collected from the estuarine and coastal environments are generally not hazardous, however it is of paramount importance that the individual collecting the samples use the proper collection techniques and be aware of potential sources of contamination.

3.2 High standards of laboratory technique should be used when preparing reagents which should all be of analytical grade. Laboratory personnel should obtain material safety data sheets (MSDS) for all chemicals used in this method, and read and sign acknowledgement over the handling of these chemicals. A lab coat, safety glasses and gloves should be used when handling concentrated acid.

4.0 EQUIPMENT AND SUPPLIES

4.1 **Gas Segmented Continuous Flow Autoanalyser** consisting of:

  4.1.1 Autosampler.

  4.1.2 Analytical manifold with a cartridge equipped for heating set to 60°.

  4.1.3 Peristaltic Pump and appropriate tubing.

  4.1.4 Spectrophotometer equipped with a tungsten lamp (380-885 nm) or photometer with an 885 nm interference filter (2 nm bandwidth).

4.1.6 Computer based data acquisition system.

4.2 Glassware and Supplies

4.2.1 All labware used in the analysis must be low in residual phosphate to avoid sample or reagent contamination. Rinsing with reverse osmosis water, followed by rinsing with 10% HCl and thoroughly rinsing with reagent water is sufficient.

4.2.2 Automatic pipetters capable of delivering volumes ranging from 10-100 µL and 100 – 1000 µL with an assortment of high quality disposable pipette tips. It is standard protocol to check the accuracy of the pipetters at least once per week using a four decimal place analytical balance.

4.2.3 Analytical balance, with capability to measure 4 decimal places for preparing SSS. It is standard protocol to have this balanced serviced and calibrated once per year, then checked weekly with certified weights.

4.2.4 10 mL and 60 mL sterile high density polypropylene, 30 mL polycarbonate vials, glass volumetric flasks, and glass pipettes.

4.2.5 105°C drying oven.

4.2.6 Desiccator.

4.2.7 Polysulphone sterile membrane filters with 0.45 µm nominal pore size. Plastic sterile Terumo syringe.

4.3 Autoclave

5.0 REAGENTS AND STANDARDS

5.1 Stock Reagent Solutions – all chemicals are analytical grade quality.

5.1.1 Stock Phosphorus Solution (1000 mg P L^{-1}) – Quantitatively transfer 4.393 g of pre- dried (105°C for 1 hour minimum) potassium phosphate monobasic (KH_2PO_4 FW 136.09) to a 1000 mL glass volumetric flask containing 800 mL of reagent water and dissolve. Fill to mark with reagent water and refrigerate. This solution is stable for 2 – 3 weeks.

5.1.2 Spiking Stock Solution (250 mg P L^{-1}) – Pipette 1 mL of the stock phosphorus solution into a 250 mL glass volumetric containing 230 mL of reagent water and then fill to marked line and mix well. This solution should be made daily.

5.1.3 Sulphuric Acid Solution –Using a 25 mL glass measuring cylinder measure out 14 mL of concentrated sulphuric acid (H_2SO_4 98.08) into a 250 mL glass volumetric flask containing approximately 200 mL of reagent water and mix well. Fill to the mark with reagent water. This solution is stable for 1 week.

5.1.4 Ammonium Molybdate Solution - Quantitatively transfer 5.000 g of pre-dried (105°C for 1 hour minimum) ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$ FW 1235.86) into a 250 mL glass volumetric flask containing approximately 180 mL of reagent water and mix well. Using a 25 mL glass measuring cylinder measure out 8 mL of concentrated sulphuric acid (H_2SO_4 98.08) and add to the molybdate mixture. Mix well and fill to the mark with reagent water. This solution is stable for 1 week.

5.1.5 Antimony Potassium Tartrate Solution – Quantitatively transfer 0.125 g of antimony potassium tartrate ($KSbO.C_4H_4O_6$ FW 324.92) and 5.000 g of L-ascorbic acid ($C_6H_8O_6$ FW 176.13) into a 250 mL glass volumetric flask containing approximately 180 mL of reagent water and mix well until the antimony is dissolved. Using a 25mL glass measuring cylinder measure out 12.5mLs of concentrated sulphuric acid (H_2SO_4 98.08) and add to the antimony mixture. Mix well and fill to the mark with reagent water and cover with foil. This solution is stable for 1 day.

5.1.6 Digestive reagent – Mix 9 g of sodium hydroxide (NaOH FW 40.0) and 40 g of potassium persulfate ($K_2S_2O_8$ FW 270.33) in 800 mL of reagent water in a 1000 mL polypropylene bottle and then fill to the 1000 mL marked line. Magnetic stir for a minimum of 1 hour or until all reagents are dissolved. Make daily.

5.2   Working Reagents

5.2.1 Dowfax 2A1 Start up Solution – add 1 mL of Dowfax surfactant to 500 mL of reagent water and mix gently.

> **NOTE:**   Dowfax contains levels of nitrate, therefore keep away from Triton X 100 start-up solution.

5.2.2 Working Sulphuric Solution – add 1 mL of Dowfax to the 250 mL glass volumetric flask of stock sulphuric solution and shake gently.

5.2.3 Working ammonium molybdate solution – 250 mL glass volumetric flask of stock molybdate solution.

5.2.4 Working antimony potassium tartrate solution – 250 mL glass volumetric flask of stock antimony solution (covered in foil).

5.2.5 Coloured SYNC Peak Solution – Use either a high phosphorus standard that will obtain a peak between 25 to 100 percent full scale. Or add 50 µL of blue food colouring solution to 1000 mL and mix thoroughly.

5.2.6 Calibration Standards – Prepare a series of calibration standards (CAL) by diluting a suitable volume of the stock standard solution into a 100 mL volumetric flask using reagent water. Prepare these standards daily. The concentration range of calibration standards should bracket the expected concentrations of

samples and not exceed two orders of magnitude. At least five calibration standards should be used to construct the calibration curve.

6.0   SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1   Sample Collection

6.1.1 Samples collected for nutrient analyses from estuarine, coastal waters, streams and lakes are usually collected by one of two methods: either a peristaltic pump or grab sample.

6.1.2 A peristaltic pump has a length of tube attached that is weighted down so that particular depths can be sampled if required. A grab sample is obtained using a hand held sampling pole with 1L polypropylene bottle which is filled sub surface.

6.1.3 The total nutrient component is sampled into a gamma sterile 60mL Sarstedt polypropylene tube (Cat# 60.596.001).

6.1.4 The total filterable nutrient component is filtered immediately at the point of collection using a Terumo sterile 60 mL syringe and 0.45 µm sterile polysulphone Mini-Sart filter purchased from Sartorius (Cat #165333K).

6.2   Sample Preservation

6.2.1 After collection and filtration the samples should either be digested immediately or if this is not possible frozen at -18°C until analysis can be performed.

6.3   Sample Storage

6.3.1 Samples should be stored in a freezer that contains nutrient samples only. If possible samples should further be stored into their relevant matrix and concentration to further minimise any possible contamination e.g. Store low level marine samples away from effluent samples.

7.0   QUALITY CONTROL

7.1 The minimum requirements of this program is to demonstrate performance using duplicate analyses, spiked samples, and the use of certified reference material (CRM's) purchased from an external source. Also, all vials for sampling and the digestion procedure, and syringe filters (if used) are subject to rigorous quality control checks before being utilised.

Additionally, to participate in inter collaborative nutrient trials at least once per year e.g. National Low Level Nutrient Collaborative Trials (NLLNCT).

7.2   Duplicate analyses

7.3.1 Duplicate analyses of samples are performed to a minimum of 5% per TropWater job submission. The standard deviation of these results should fall between +/- 10%. Failure to achieve this standard will results in samples being re-analysed.

7.3 Spiked Samples

7.4.1 Samples are spiked to a minimum of 5% per TropWater job submission. The result of this addition should calculate to be between 90 – 110% recovery. Failure to achieve satisfactory recovery will result in samples being re-analysed. This may mean using a different series of dilutions if required.

7.4 Certified Reference Material (CRM)

7.4.1 CRM's are purchased from Queensland Health in both freshwater and saltwater. The CRM's are matched to the sample matrices being analysed and also concentration. The certified value range must be achieved for sample results to be deemed accurate or all samples must be re- analysed with the correct value for the CRM obtained.

7.4.2 CRM's are purchased in 250 mL containers. After the first thawing the whole CRM is sub – sampled into 10 mL tubes for future usage. These 10mL tubes are then thawed as required.

7.5 Consumable Quality Checks

7.5.1 10 mL Sarstedt vials are ordered in large batches of at least 10,000 units (Cat # 60.9921.820). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.2 60 mL Sarstedt vials are ordered in batches of 1,000 units (Cat # 60.596.001). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

7.5.3 30 mL Sarstedt vials are ordered in batches of 5,000 units (Cat # 60.9922.216 and 60.9922.210 (saltwater samples) with lids C3011 HDPE natural). These vials are divided into their manufacturer batches and 1% of the vials are randomly selected and analysed for back ground contamination.

8.0 CALIBRATION AND STANDARDISATION

8.1 At least seven calibration standards should be prepared daily for system calibration. These calibration standards should undergo the same digestion procedure for samples being analysed.

8.1.1 The following dilutions are used for a working range of 0.005 – 0.200 µg P L^{-1}:

Step 1: Make up a secondary stock phosphorus solution

Stock Phosphorus Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg P L^{-1})
1	1000	1000

Step 2: Using the secondary stock solution made in step 1 make up the following dilutions which are used as working calibration standards.

Secondary Stock Phosphorus Solution (mL)	Size Volumetric Flask (mL)	Calculated Concentration (µg P L^{-1})
1	100	10
2	100	20
4	100	40
5	100	50
8	100	80
10	100	100
20	100	200

NOTE: The calibration standards are mixed phosphorus and nitrogen standards (refer to TNSOP 8.1.1 for direction)

8.2   A calibration curve should be constructed for each sample run.

8.3   The calibration standards should be placed at the start, middle and end of the run to allow for any sensitivity drift.

NOTE: For large amounts of samples it may be necessary to increase the amount of calibration standards digested to ensure that a ratio of one set of standards per 20 – 30 samples is achieved.

8.4 The CRM should be placed at the start and the end of the run to ensure the accuracy of results is maintained throughout the whole run.

8.5 Spiked samples should be prepared as follows:

    8.5.1 Step 1: Ensure that the sample randomly chosen to be spiked is 10mLs exactly (marked line). Use a micro pipette to remove any excess sample or conversely to add a little more sample into the vial.

    NOTE: Thoroughly mix the sample before remove or adding any sample.

    8.5.2 Step 2: Make up the spiking stock mixture by adding 1mL of Stock Phosphorus Solution (SSS) to 230 mL of reagent water in a 250 mL glass volumetric flask. Mix well and fill to the marked line,

    8.5.3 Step 3: Add 50µL of the spiking stock mixture (equivalent of 20 µg P L^{-1}) to the 10 mL sample and mix well.

    8.5.4 A minimum of three spiked reagent water samples are to be prepared and analysed for every run.

9.0 SAMPLE, CALIBRATION STANDARDS, AND CRM PREPARATION PROCEDURE

9.1 If samples are frozen, thaw samples by running under a continuous flow of cold water.

9.2 Make up the seven calibration standards (refer 8.1.1), and prepare the CRM's (refer 7.4.2).

9.3 Thoroughly mix the sample then decant 10 mL into a Sarstedt 10mL sterile vial (if total filterable phosphorus is being analysed the sample will already be in the appropriate tube). If samples contain high amounts of particulates dilution series should be carried out at this point.

    NOTE: Alternatively if samples fall outside of the working range for this method, samples should be diluted, re- digested, and re- analysed.

9.4 Choose and make up the spiked samples (refer 8.5).

9.5 Take the 10 mL aliquot of samples, CRM, or spiked samples and transfer after shaking sample to the Sarstedt 30 mL vial for digestion (ensure correct vial is used refer 9.7). Add 5 mL of digestive reagent and put the lid on tightly, mix well using vortex equipment and then loosen half a turn back and place vial into digestion rack for the autoclave.

9.6   Thoroughly mix and decant three lots of 10 mL for each working calibration standard and add 5 mL of digestion mixture. Put the lid on tightly, mix well using vortex equipment and then loosen half a turn back and place vial into digestion rack for the autoclave.

9.7   For freshwater digests use Sarstedt vials (Cat # 60.9922.216) and for saltwater digests use Sarstedt vials (Cat # 60.9922.210 with HDPE lids Cat # C3011).

9.8   For freshwater samples digest the samples, calibrations standards, and CRM's in an autoclave at 121°C for a period of seventy minutes. For saltwater samples, calibration standards, and CRM autoclave at 121°C for a period of 35 minutes, remove samples and vortex stir then autoclave for another period of 35 minutes.

9.9   Let samples sit overnight to cool before analysing.

10.0 ANALYTICAL PROCEDURE

10.1 Turn on the auto analyser and peristaltic pump, run reagent water with Dowfax added through the system for a minimum of thirty minutes to let the system equilibrate. Turn on the spectrophotometer and check that the baseline is stable and you have a consistent bubble pattern through the manifold.

> **NOTE:**   The absorbency on the spectrophotometer should read 885 nm and should be set at the required sensitivity level for the range of working standards.

10.2 Set up the autosampler software for the run. This will include the unique TropWater job number and sample identification number, along with any dilution data associated with samples.

10.3 Mix the digested sample using a vortex and decant into 10 mL Sarstedt tubes (Cat # 60.9921.820) and place into the correct cup position as identified by the autosampler software.

10.4 Remove the reagent water with Dowfax and add the working sulphuric solution, working ammonium molybdate solution, and working antimony potassium tartrate solution to the system. Allow to equilibrate for 5 minutes.

10.5 Inject a high level phosphorus standard e.g. 100 µg P L^{-1} for 40 seconds and observe the peak absorbency through the spectrophotometer.

10.6 Start the autosampler run. Observe that the first set of standards peak heights are normal and then check run periodically to ensure no malfunctions have occurred.

10.7 When the autosampler run is finished stop the software, remove the working sulphuric solution, working ammonium molybdate solution, and working antimony potassium tartrate solution then put the reagent water with Dowfax back on. Rinse the manifold for a minimum of one hour and then turn the auto analyser and peristaltic pump off.

10.8 Print out a hard copy of the trace and calculate results.

11.0 DATA ANALYSIS AND CALCULATIONS

11.1 Concentrations of total phosphorus in samples are calculated from both quadratic and cubic equations obtained from the calibration standards.

11.2 The Quality Control of the results (refer 7.0) is assessed and results are either accepted or rejected and re-run at a later date.

11.3 Results should be reported in µg P L^{-1} unless otherwise requested (e.g. High values > 1 mg P L^{-1}) with the replicate and spike values stated on the report.

11.3.1 To calculate spike values for undiluted samples:

$$\text{Spike Recovery (\%)} = \frac{\text{Sample + Average of blanked spiked samples}}{\text{Spiked Sample}} \times 100$$

11.3.2 To calculate spike values for diluted samples:

$$\text{Spike Recovery (Dilutions) (\%)} = \frac{\text{(Sample/Dilution) + Average of Blanked Spiked Samples}}{\text{(Spiked Sample/Dilution)}} \times 100$$

Appendix B10 TropWater Auto-analysis methods: Chlorophyll

Chlorophyll: Analysis of chlorophyll a and phaeophytin a in marine and fresh waters

DEFINITION

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass. All green plants contain chlorophyll *a* which constitutes approximately 1 to 2 % of the dry weight of planktonic algae. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins and carotens. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides and phaeophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups.

MSf.1 Objective

To measure the concentration of chlorophyll *a* and phaeophytin *a* present in seawater to obtain an estimation of phytoplankton biomass.

MSf.2 Principle of Analysis

This method utilises the natural fluorescence of chlorophyll *a* to derive the concentration of the pigment present in an extract of material filtered from seawater. The fluorescence of decomposition products (phaeophytin) can cause significant interference. This method compensates by taking an additional fluorescence reading after acidification of the original sample (by which all pigments present are decomposed) and using the decreased fluorescence reading to derive the original concentration of live pigment.

This method follows the basic fluorometric method set out in Strickland and Parsons (1972). The concentration of the primary standard used for calibration is determined spectrophotometrically using the equations laid out in Jeffrey and Humphrey (1975).

MSf.3 Sample Collection Procedure and Storage

MSf.3.1 Equipment

- 25 mm Whatman GF/F glass fibre filters.
- Millipore filter forceps (or equivalent).
- Squares of aluminium foil (8 x 8 cm)
- Black, fine-tipped, waterproof labelling pen (eg Staedtler Lumocolour).
- 250 ml measuring cylinders.
- 25 mm filter funnels.
- Vacuum manifold, water reservoir, vacuum tubing and vacuum pump.

MSf.3.2 Method

1. Filter 100 ml of seawater sample through a 25 mm Whatman GF/F glass fibre filter. Duplicate samples are recommended.

2. The filter is then wrapped in alfoil, labelled with site, depth, date collected and volume filtered. When sampled in duplicate, there should be 2 filters per alfoil packet.
3. Freeze the filters immediately.

MSf.4 Sample Analysis

MSf.4.1 Equipment

- Turner Designs Model 10-Au or TD700 digital fluorometer.
- 10ml quartz cuvette.
- High speed tissue grinder comprising motor, pestle and tube (Potter Elversham No. 23).
- 12ml centrifuge tubes with caps.
- Centrifuge with 12ml holders.
- Filter forceps.
- Thin spatula.
- Chlorophyll laboratory log sheets.
- Fume cupboard (see notes).

Note 1: Chlorophyll, especially when extracted, is extremely light sensitive. If possible, carry out all extractions in the dimmest light possible. When not using extracted solutions, keep them wrapped to exclude light and store in a refrigerator. Keep extracted samples in a closed light box or cover with a black cloth.

Note: Chlorophyll is rapidly and irreversibly degraded by acid, converting active chlorophyll to phaeophytin. Do all chlorophyll sample grinding, extractions and analyses in the designated chlorophyll fume cupboard. Do not use acids especially HCl in this fume cupboard. Keep all equipment used for chlorophyll analysis free of acid.

MSf.4.2 Reagents

1. 90% Acetone – 900ml of analytical grade acetone and 100ml of deionised water.
2. 10% hydrochloric acid – 10ml of analytical grade hydrochloric acid and 95ml of deionised water.

MSf.4.3 Procedure

1. Grind the filter in 90% acetone in a tissue grinding tube.
2. Pour suspension into a graduated or marked centrifuge tube and make up to 10ml.
3. Cap tube and shake.
4. Leave stand in the dark for 2 hours. We either cover the box of extracted samples or cover the tubes with a thick black cloth.
5. Centrifuge for 10 minutes at 3500 rev/min and read the fluorescence of the supernatant on the Turner Designs (Model 10-AU or TD700) Digital Fluorometer. Make note of reading on log sheet (Appendix 1).
6. Add 2 drops of 1N HCl. Allow the acid to react and take another reading. Make note of reading on log sheet.
7. An unused filter should also be processed in the same manner to give a blank value.

MSf.4.4 Calibration

a. Machine Calibration

Primary Standard:

1. Filter an aliquot from an exponentially-growing phytoplankton culture through a Whatman GF/F filter (or equivalent). It is best to use diatom (e.g. *Chaetoceros*) or chrysophyte (e.g. *Isochrysis*, *Phaeodactylum*) cultures. Do not use a of a chlorophyte (e.g. *Dunaliella*, *Tetraselmis*) culture as they contain chlorophyll b which distorts the fluorometric analysis of chlorophyll a.
2. Grind the filter in 90% acetone in a tissue grinding tube. If you have filtered enough culture, a light green solution will be obtained. Do the grinding and extraction of the culture in the dark to prevent photo-degradation of the pigments. Keep all extracts in the dark as much as possible (wrap flasks in foil, etc…).
3. Clear the extract by centrifugation.
4. Read extinctions of the extract on a UV/VIS spectrometer (using a 1cm quartz cell) at the following wavelengths; 750nm, 664nm, 647nm and 630nm.

The concentration of chlorophyll *a* (C_a) in µg/l can then be calculated using the formulae:

$$C_a = (11.85E_{664} - 1.54E_{647} - 0.08E_{630}) \times 1000$$

Where:

E_x is the absorbance at wavelength X nm minus the absorbance at wavelength 750nm.

Calibration Curve:

Using this solution as a primary standard solution make up a diluted pigment solution of about 4µg/l. This should read about 0.400 mV on the fluorometer. If it does not the fluorometer may need to be adjusted. The routine for adjusting the Turner Designs Fluorometer Model 10Au to suit low level marine samples is shown in Appendix 2. Make up a series of dilutions ranging from 1 to 25 µg/l and measure the original and acidified fluorescence values.

Calculating Door Factor (F_D):

$$F_D = C_a / (1830(R_B - R_A))$$

where:

F_D = door value

R_A = reading before acid

R_B = reading after acid

C_a = concentration of chlorophyll *a* in diluted sample

Calculate a working F_D value $(F_D{}'')$ for the Excel spreadsheet that includes normal volumes

$$F_D{}'' = (1.83 \times v \times F_D)/V$$

where:

   v = 10 ml acetone extract

   V = 0.100 litres of seawater filtered per sample (100 ml)

therefore:

$$F_D{}'' = 183 \times F_D$$

The calculations for this calibration can be done automatically using the EXCEL spreadsheet template "Calib. fluoro template". An example work form derived from this spreadsheet is shown in Appendix 3.

The new calibration should then be assigned a number and recorded against the date of the calibration on the EXCEL spreadsheet "Chl a calibration equations" as shown in Appendix 4. All measurements taken by the machine after this date must use this calibration equation.

b. Calibration Control

Each fluorometer has a manufactured fluorescence standard that can be used for a daily working calibration or to calculate drift. Notes from Turner Designs web page on use of the Solid Secondary Standard can be found in Appendix 5.   Insert the standard at the beginning and end of each set of samples read and at regular intervals throughout the set. Make sure the "L" marking on the barrel is placed toward the left hand side of the holder. A record is kept of the Solid Standard measured at the time of machine calibration and drift should be corrected making reference to this original measurement.

   Drift corrected C_a = (Calculated C_a x Original Solid Standard) / (Measured Solid
      Standard)

If at the beginning of the day the reading shows greater than 5% variation from the original, the emission and excitation filters of the fluorometer may need to be cleaned with an ethanol dampened tissue.

MSf.4.5 Calculations

Chlorophyll *a* and phaeophytin *a* may then be calculated using the following equations. The equation assumes that the filtered sample volume is 100 ml and that there is 10 ml of acetone extract. The calculation spreadsheet allows for variation of these numbers for individual samples.

   $C_a = F_D{}''(R_B - R_A)$
   $Phaeo = F_D{}''(2.2R_A - R_B)$

where:

C_a = concentration of chlorophyll a in µg/l

Phaeo = concentration of phaeophytin a in µg/l

$F_D"$ = door factor (see appendix 1.)

R_B = reading before acid addition-blank

R_A = reading after acid addition-blank

MSf.5 Method Capabilities and Limits of detection.

Chlorophyll a concentrations ranging from 0.5 to 50 µg/l in the acetone extract (ie a 100ml sample ranging in concentration between 0.05 to 5 µg/l) may be measured by this method without recalibration of the instrument. Detection capability for water samples with very low concentrations can be improved by increasing the volume of water filtered. Likewise, in high concentration samples, either smaller volumes may be filtered or the extracted sample may be diluted.

MSf.6 Data Management

Each filter is assigned a unique identifier which is usually a combination of sampling site reference, (or site, date, time), depth in the water column from which the sample was taken and the replicate number.

The fluorescence reading before and after the acid addition is noted on a laboratory worksheet against the sample identifier on a laboratory worksheet. A proforma of this log sheet can be found in Appendix 1. These readings are then enter onto an Excel spreadsheet which then acts as feeder sheets for the Oracle Database (http://hydatina.aims.gov.au:8080/wqfdesimport). Utilising the import tool, all the analytical results will be inputted and a copy of the related excel file complete with QAQA data will be embedded within the database. It will automatically perform the all calculations required. All laboratory work forms are archived. All Excel calculation files are archived after the results have been entered in an Microsoft Access database.

MSf.7 Quality Control

- Solid secondary standard calibration run daily at beginning, middle and end of sample run.
- Filter blanks run daily.
- Reagents AR grade.

x.y Quality Control

QAQC tests undertaken and reporting as part of the data reporting include:

Assessment of the limit of detection (LOD)

NB: the Limit of Detection (LOD) or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence).

- Minimum detection limits were calculated for this method using repetition of blanks.

Assessment of accuracy of the analysis

NB: This is generally achieved by using reference materials to assess recovery of known amount of analyte.

- The Solid secondary standard is run daily at beginning, middle and end of sample run and for each sample set an average of the readings as a percentage of the true value is used to determine accuracy.

Assessment of precision of the analysis

NB: This is generally achieved by the repeated analyses of the same concentration of analyte to check for reproducibility.

- The coefficient of variance between repeated readings of the secondary solid standard associated with a set of samples is calculated to assess precision.

Procedural blanks:

- Filter blanks are run with every batch (generally daily). These are used as blank readings in the calculation of analytical results.
- Wet filter blanks (filter placed on filtration unit and wetted with filtered seawater, then further handled like samples) were prepared during the on-board sample preparation to measure contamination during the preparation procedure

Reproducibility of duplicate analytical units

NB: The variation between results for sample duplicates indicates the reproducibility of the analysis and also the effects of various sources of contamination and analytical error during collection, sample preparation and analyses. Before data analysis, results are generally averaged over duplicates.

- Coefficients of variance are calculated from analytical results of sample duplicates. Duplicates with CV>30% are discarded from the analysis and the analysis repeated using extra duplicates that are generally collected as a backup.

Other:

- The linear range of this method was established utilising increasing concentrations of primary standard. Samples are diluted if above this range.
- Calibrations cross referenced by computer to analysis date.
- External calibration run periodically or, if solid, standard detects machine drift greater than 5%.

MSf.8 References

Jeffrey, S.W. and G.F. Humphrey, (1975). New spectrophotometric equations for determining chlorophylls a,b,c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol.Pflanzen, 167: 191-194

Strickland, J.D.H. and T.R. Parsons, (1972). A Practical Handbook of Seawater Analysis, Bull. Fish. Res. Board Can.167.

USEPA METHOD 445.0: In vitro Determination of chlorophyll *a* and phaeophytin *a* in marine and freshwater phytoplankton by fluorescence, United States Environmental Protection Agency Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples, EPA/600/R-92/121.

Appendix B11 TropWater Auto-analysis methods: Total Suspended Solids
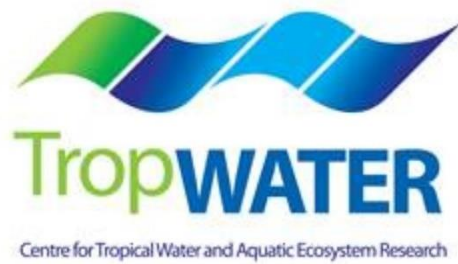
Total Suspended Solids (TSS)

DEFINITION

Suspended solids refers to any matter suspended in water or wastewater. Solids may affect water or effluent quality adversely in a number of ways. Waters high in suspended solids may be aesthetically unsatisfactory for such purposes as drinking or bathing. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

"Total suspended solids" or TSS comprise the portion of total solids retained by a filter.

Sources of error and variability

Sampling, sub-sampling, and pipetting may introduce serious errors. Make and keep samples homogeneous during transfer. If part of a sample adheres to the sample container, consider this in evaluating and reporting results.

The temperature at which the residue is dried has an important bearing on results, because weight losses due to volatilization of organic matter, mechanically occluded water, water crystallization, and gases from heat-induced chemical decomposition, as well as weight gains due to oxidization, depend on temperature and time of heating. Each sample requires close attention to desiccation after drying; note the characteristic of both the filtered material and dried material through the analysis. Minimize opening desiccator because moist air enters. Some solids may be stronger desiccants than those used in the desiccator and may take on water.

Residues dried at 103 to 105ºC may retain not only water of crystallization but also some mechanically occluded water. Because removal of occluded water is marginal at this temperature, attainment of constant weight may be very slow.

Results for residues high in oil or grease may be questionable because of the difficulty of drying to constant weight in a reasonable time.

To aid in quality assurance, analyse a proportion of the samples in duplicate and dry samples to a constant weight. This entails including multiple drying-cooling-weighing cycles for one batch of samples per week.

PRINCIPLE – TSS$_{GF/C}$

A well-mixed sample is filtered through a weighed standard polycarbonate filter and the residue retained on the filter is dried to a constant weight at 103 to 105ºC. The increase in weight of the filter represents the total suspended solids.

Interferences and Control Measures

Exclude large, floating particles from the sample if it is determined that their inclusion is not desired in the final result. Because excessive residue in the filter may form a water-trapping crust and adhere to the filtration vessel wall, limit sample size to no more than 200 mg residue. For samples high in dissolved solids (e.g. marine and brackish waters) thoroughly wash the

filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter. When filtering less than 100 ml of sample perform replicates where possible. Measure volumes less than 250 ml using a measuring cylinder. For marine water samples and samples of known low TSS filter no less than 3 L if possible.

SAMPLING AND STORAGE

Use resistant glass or plastic bottles provided that the material in suspension does not adhere to the container walls. Begin analysis as soon as possible because of the impracticality of preserving the sample. Refrigerate sample at 0 to 4 ºC up to the time of analysis to minimize microbiological decomposition of solids. It is preferable to analyse the samples within 24 hours of collection. In this holding period cannot be met due to field work commitments or otherwise, the sample should not be held for more than 7 days. Bring samples to room temperature before analysis.

PROCEDURE

Laboratory Apparatus

- 250 ml measuring cylinder
- Filtration apparatus
- Pre-weighed Polycarbonate filter papers (47 mm diameter, pore size - 0.4µm)
- Labelled dishes
- Measuring cylinder
- Rinse bottles
- Drying Oven for operation at 103 to 105ºC
- Fine forceps (light weight, broad ended to prevent spiking the filter (e.g. those used for macro-invertebrate sorting))
- Analytical balance, capable of weighing to 0.1 mg

Method

Determining dry filter weight:

a)   Heat clean dish carrying filter paper to 103 to 105ºC for at least 3 hours.
b)   Store and cool dish in desiccator until needed.
c)   Once in balance room, open desiccator, remove a filter dish and close desiccator.
d)   Remove filter using the forceps and place on the balance tray.
e)   Weigh filter paper.
f)   For one batch a week repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weight or 0.5 mg whichever is less.
g)   Record weight and clean dish label in TSS analyses log book.
h)   Place filter back in dish and return to the desiccator.
i)   For one batch per week determine filter paper purity by placing pre-weighed filter on clean filtration apparatus and washing with three successive 20 ml portions of deionized water. Remove all traces of water and repeat steps a. to h. Compare results with unwashed filter papers.

Sample filtration

a.   Run samples in numerical order.
b.   Choose a sample volume that will yield a residue between 2.5 and 200mg. Residues generally greater than 200mg are likely to adhere to the side of the filtration vessel and will result in an underestimate of suspended solids. Use a 100 ml measuring cylinder to measure volumes between 250mL & 25mL. For volumes less than 25mL, use a pipette (5mL, 10mL & 20mL).
c.   Use the filter papers in numerical order. Place pre-weighed filter on filtration apparatus and wet filter with a small volume of RO water and apply vacuum to seal it and check for leaks.
d.   Ensure vacuum is turned off, even when adding sample from a cylinder or pipette.
e.   Pour a measured volume of well mixed sample into filter. Apply sample evenly and quickly (if water samples with a high amount of fine suspended solids are added slowly to the filter a proportion of fine material (<1μm) will pass through the filter – it is best to flood the filter). Be sure to shake sample well each time before pouring or before extracting each aliquot if using a pipette.
f.   Apply vacuum.
g.   Filter as much sample as possible; however, if more than 10 minutes is required to complete filtration repeat with a lower volume. If there is insufficient sample to repeat, the allow sample to filter completely and mark record with '10+'.
h.   Record volume of sample filtered in TSS analyses log book.
i.   After complete filtration, rinse filter and sides of manifold (and measuring cylinder if used) with a total of 50mL RO water for fresh water samples and 500mL RO water for salt water samples.
j.   Ensure filter paper is almost dry via suction, remove and place on labelled dish.

Determining dry TSS weight:

a.   Dry filter for at least 3 hours in 103 to 105⁰C oven.
b.   Cool dish carrying filter in desiccator to balance temperature, and weigh filter paper and record weight.
c.   For one batch a week repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less.

Note: When weighing dried filter paper, be alert to change in weight due to air exposure and/or sample degradation. Keep filter in the desiccator until weighing, carefully removing the filters and re-sealing the desiccator between each filter weighing. Also check that silicate beads in the desiccator are still bright blue. If pale, change beads before use.

Refrigerate remaining sample after filtration and retain for one week if possible.

Blanks

Analyse at least one blank filter per week. The blank filter should be carried through the filtration process using 250mL deionized water and oven drying. The average of the blank filter should be accounted for in the TSS calculation.

Calculations

mg TSS$_{GF/C}$ L^{-1} = ((A – B) – filter blank) x 1000 / sample volume, ml

where:

A = dry weight of dried residue + filter paper, mg, and

B = dry weight of filter, mg

RESULTS DOCUMENTATION

Report:

Values in the range of 0 to 10 mg L^{-1}:   to one decimal place e.g. 6.7 mg L^{-1}

Values in the range of 10-99 mg L^{-1}:   to unit place e.g. 54 mg L^{-1}

Values 100 mg L^{-1} or over: to 2 significant figures e.g. 340 mg L^{-1}, 2500 mg L^{-1}

Record results on data sheets:

Results sheet # Total Suspended Solids Analysis

Results sheet # Total Suspended Solids Batch Blanks

Results sheet # Total Suspended Solids RO Blank

ACCEPTABLE LIMITS

Report values above 0.2 mg L^{-1} on a 1 L filtered volume basis

VERIFICATION

Analyse at least 10% of all samples in duplicate. Duplicate determinations should agree within 10% of their average weight (as per APHA (2000) methods). When filtering less than 50 ml of sample perform replicates 50% of the samples.

Sample duplicates should be marked with the appropriate symbol to indicate reason for performing repeat:

R - Replicate for QC purposes

RL - Repeated due to leak

RV - Repeated with a lower volume

Perform replicates between analysts once per month. Duplicate determinations should agree within 10% of their average weight (as per APHA (2000) methods).

Where possible continue quality assurance practices with purchased standards and inter-laboratory standards.

QA STATISTICS

REFERENCES

1. Standard Methods for the Examination of Water and Wastewater APHA AWWA WEF 20th Ed 1998

Appendix B12 TropWater Auto-analysis methods: Coloured Dissolved Organic Matter

CDOM

Samples are allowed to come to room temperature before placement into a 10 cm path-length quartz cell. The CDOM absorption coefficient (m-1) of each filtrate is measured from 250-800 nm using a Shimadzu UV1700 spectrophotometer. Milli-Q water (Millipore) is used as a reference.

CDOM absorption is measured using a dual beam Shimadzu UV1700 spectrophotometer.

The instrument is baselined with a Milli-Q water reference cuvette and a 0.2 μm filtered Milli-Q water as the sample. After baselining the instrument the reference remains in the machine and a field sample is then placed into the sample cuvette. The optical density (OD) of the sample is then measured over 250-800 nm at 0.5 nm resolution. To obtain the absorption spectrum, the mean value from 680 nm where absorption is deemed to be zero is subtracted from spectrum.

For a complete description of the method refer to 'Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 4, Volume IV' (NASA/TM-2003-211621/Rev4-Vol.IV).

The resultant is multiplied by 2.303/L (where L is the path-length of the cuvettes, ca. 0.1 m) to give the absorption in units of inverse metres (m-1). CDOM absorption spectra are fitted to an exponential function over the range 370-600 nm. Not only the nominal value for CDOM at 443 nm is stored in the data base, but also the fitting coefficients, allowing the CDOM determination at any wave-length between 370-600 nm as required.

Appendix B13 TropWater methods: Laboratory Salinity
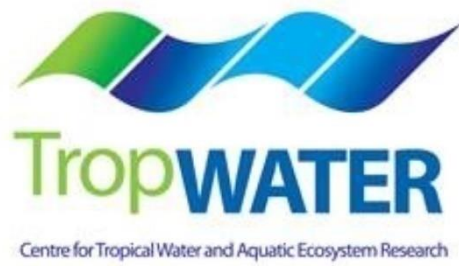
EC/SALINITY

Samples are allowed to come to room temperature before using a Metrohm 912 Lab Conductometer to read the Electrical Conductivity (µS/cm) at 25°C and Salinity(PSU) is calculated using the formula on page 36 of the"USGS Guidelines and Standard Procedures for Continuous Water Quality Monitors: Operation, Record Computation and Data"

The Conductometer is calibrated with the 0.5M KCl (58670 µS/cm) at 25°C and the OSIL/IAPSO salinity standard is used as a QC check after calibration.

Appendix C Waypoints for Coral Monitoring Transects

Appendix C1 Waypoints for the start of Transect 1 of each coral site reported by the MMP. Reefs in Italics are monitored by the AIMS Long-term Monitoring Program

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
Wet Tropics		*Low Isles*	1	6	145.571000	-16.384000
			2	6	145.572600	-16.386483
			3	6	145.573450	-16.390217
		Snapper North	1	2	145.496883	-16.292400
			1	5	145.496517	-16.291883
			2	2	145.500583	-16.295833
			2	5	145.500367	-16.294867
			3	2	145.506967	-16.301833
		Snapper South	1	2	145.496000	-16.299750
			1	5	145.495950	-16.299867
			2	2	145.499367	-16.300767
			2	5	145.499367	-16.301067
			3	2	145.501967	-16.302117
	Russell / Mulgrave Johnstone	*Green Island*	1	6	145.980000	-16.775000
			2	6	145.976683	-16.772867
			3	6	145.973567	-16.769950
		Fitzroy East	1	2	146.006217	-16.923833
			1	5	146.006867	-16.924100
			2	2	145.991170	-16.943290
			2	5	145.991133	-16.943533
		Fitzroy West	1	2	145.996267	-16.923317
			1	5	145.996250	-16.923150
			2	2	145.999633	-16.922100
			2	5	145.999633	-16.922100
		Fitzroy West	1	6	145.993000	-16.923000
			2	6	145.996083	-16.923067
			3	6	145.999317	-16.921950
		Frankland East	1	2	146.076750	-17.203783

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			1	5	146.077000	-17.203250
			2	2	146.090250	-17.219550
			2	5	146.091433	-17.219100
		Frankland West	1	2	146.090367	-17.226967
			1	5	146.090367	-17.226967
			2	2	146.075967	-17.212467
			2	5	146.075733	-17.212583
		High East	1	2	146.014700	-17.158150
			1	5	146.014817	-17.158433
			2	2	146.012450	-17.163217
			2	5	146.012833	-17.163683
		High West	1	2	146.006983	-17.162150
			1	5	146.006983	-17.162150
			2	2	146.005750	-17.160050
			2	5	146.005750	-17.160050
		Barnards	1	2	146.174200	-17.672233
			1	5	146.174583	-17.671750
			2	2	146.179500	-17.673900
			2	5	146.179500	-17.673900
		Bedarra	1	2	146.148040	-18.009370
			1	5	146.148080	-18.009700
			2	2	146.146440	-18.008690
			2	5	146.146380	-18.009020
	Herbert Tully	Dunk North	1	2	146.136800	-17.923700
			1	5	146.137300	-17.923317
			2	2	146.146133	-17.926283
			2	5	146.146133	-17.926283
		Dunk South	1	2	146.154333	-17.958850
			1	5	146.154350	-17.959083
			2	2	146.143867	-17.957000
			2	5	146.143850	-17.957317
		Havannah Island	1	2	146.542833	-18.847217
			1	5	146.542700	-18.847550

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			2	2	146.540683	-18.846367
			2	5	146.540583	-18.846683
		Havannah Island (North)	1	6	146.537000	-18.832000
			2	6	146.541367	-18.834000
			3	6	146.545083	-18.835683
		Lady Elliot	1	2	146.332467	-18.679850
			1	5	146.333033	-18.680167
			2	2	146.331767	-18.681833
			2	5	146.332067	-18.682350
		Magnetic	1	2	146.868517	-19.154833
			1	5	146.868483	-19.154950
			2	2	146.862150	-19.156217
			2	5	146.862200	-19.156783
Burdekin	Burdekin	Palms East	1	2	146.494683	-18.571783
			1	5	146.495067	-18.571733
			2	2	146.496240	-18.574330
			2	5	146.497150	-18.574450
		Palms West	1	2	146.482317	-18.569117
			1	5	146.482017	-18.569067
			2	2	146.488767	-18.540583
			2	5	146.488550	-18.540583
		Pandora	1	2	146.435983	-18.814033
			1	5	146.436317	-18.814050
			2	2	146.434067	-18.816400
			2	5	146.434567	-18.816800
		Pandora (North)	1	6	146.427000	-18.813000
			2	6	146.430050	-18.811567
			3	6	146.432650	-18.810700
Mackay Whitsunday	Proserpine	*Border*	1	6	149.036000	-20.175000
			2	6	149.037717	-20.181900
			3	6	149.031967	-20.170967
		Daydream	1	2	148.812417	-20.255617
			1	5	148.812150	-20.255750

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			2	2	148.812450	-20.253433
			2	5	148.812283	-20.253450
		Dent	1	2	148.938217	-20.344983
			1	5	148.938217	-20.344983
			2	2	148.937050	-20.347433
			2	5	148.937050	-20.347433
		Double Cone	1	2	148.721750	-20.104817
			1	5	148.721750	-20.104917
			2	2	148.718850	-20.105733
			2	5	148.719017	-20.105950
		Hayman	1	6	148.899000	-20.057000
			2	6	148.901650	-20.059667
			3	6	148.903933	-20.062033
		Hook	1	2	148.887133	-20.168117
			1	5	148.887133	-20.168117
			2	2	148.883933	-20.165717
			2	5	148.883933	-20.165717
		Langford	1	6	148.874000	-20.078000
			2	6	148.876900	-20.079667
			3	6	148.879667	-20.081750
		Pine	1	2	148.888367	-20.377983
			1	5	148.888367	-20.377983
			2	2	148.886400	-20.375450
			2	5	148.886400	-20.375450
		Seaforth	1	2	149.038950	-20.468450
			1	5	149.038950	-20.468450
			2	2	149.037150	-20.471900
			2	5	149.037150	-20.471900
		Shute	1	2	148.803217	-20.301733
			1	5	148.803217	-20.301733
			2	2	148.798283	-20.300850
			2	5	148.798283	-20.300850
		Barren	1	2	151.070467	-23.157483

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			1	5	151.070467	-23.157483
			2	2	151.072900	-23.156583
			2	5	151.072900	-23.156583
		Keppels South	1	2	150.963783	-23.216150
			1	5	150.963400	-23.216267
			2	2	150.969783	-23.202467
			2	5	150.969783	-23.202683
		Middle	1	2	150.920550	-23.161533
			1	5	150.920633	-23.161467
			2	2	150.923967	-23.163900
Fitzroy	Fitzroy	North Keppel	1	2	150.905183	-23.086450
			1	5	150.905183	-23.086450
			2	2	150.901733	-23.085217
			2	5	150.901733	-23.085217
		Peak	1	2	150.936867	-23.341267
			1	5	150.937000	-23.340950
			2	2	150.938833	-23.341683
			2	5	150.938933	-23.341400
		Pelican	1	2	150.874183	-23.239667
			1	5	150.874483	-23.238917
			2	2	150.878083	-23.242050
			2	5	150.878500	-23.241783