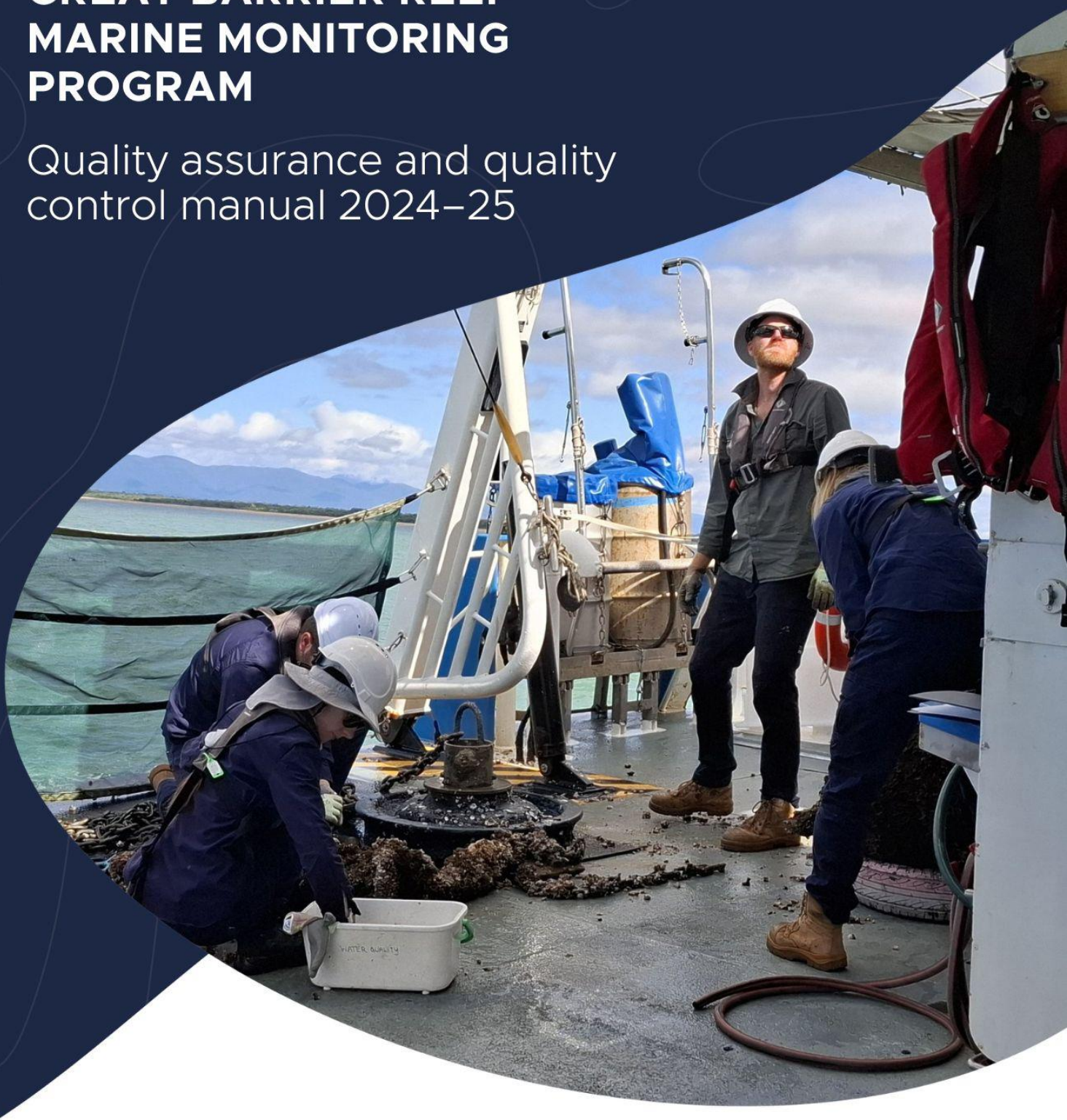
Australian Government
Great Barrier Reef Marine Park Authority

Reef Authority

GREAT BARRIER REEF MARINE MONITORING PROGRAM

Quality assurance and quality control manual 2024–25



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Front cover image: AIMS water quality and oceanographic instrument technicians servicing the Burdekin River mooring following mooring exchange in February 2025. ©Australian Institute of Marine Science. Photo credit: Renee Gruber.

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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COMMONLY USED ABBREVIATIONS, ACRONYMS, DEFINITIONS AND UNITS

Abbreviations, acronyms, and definitions

AIMS	Australian Institute of Marine Science
AODN	Australian Ocean Data Network
ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
BoM	Bureau of Meteorology
CDOM	coloured dissolved organic matter
Chl-*a*	chlorophyll *a*
CTD	Conductivity Temperature Depth profiler
CYWP	Cape York Water Partnership
DIN	dissolved inorganic nitrogen
DOC	dissolved organic carbon
GV	guideline value
HPLC	High-performance liquid chromatography
JCU	James Cook University
LC-MS	Liquid chromatography-mass spectrometry
LOD	limit of detection
MMP	Marine Monitoring Program
Marine Park	Great Barrier Reef Marine Park
MODIS	Moderate Resolution Imaging Spectroradiometer
MS	Mass spectrometry
NATA	National Association of Testing Authorities
NH_3	ammonia
NO_2	nitrite
NO_3	nitrate
NO_x	Nitrate/nitrite (NO_3 + NO_2)
NRM	natural resource management
PN	particulate nitrogen
PO_4	phosphate (dissolved inorganic phosphorus)
POC	particulate organic carbon
PP	particulate phosphorus
PSII herbicide	photosystem II inhibiting herbicide
QA/QC	quality assurance/quality control
QAEHS	Queensland Alliance for Environmental Health Sciences
QLUMP	Queensland Land Use Mapping Program
Reef	Great Barrier Reef
Reef Authority	Great Barrier Reef Marine Park Authority
Reef 2050 WQIP	*Reef 2050 Water Quality Improvement Plan*
Reef 2050 Plan	*Reef 2050 Long-Term Sustainability Plan*
$Si(OH)_4$	silicate
SDD	Secchi disk depth
SOP	Standard Operating Procedure
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus

TropWATER	Centre for Tropical Water and Aquatic Ecosystem Research
TSS	total suspended solids
UQ	University of Queensland
water year	1 October to 30 September (e.g., 2010–11)
WS colour scale	wet season colour scale
WQ Index	Water Quality Index

Units

GL	gigalitre
m	metre
mm d^{-1}	millimetres per day
mg L^{-1}	milligram per litre
ML	megalitre
km	kilometre
km h^{-1}	kilometres per hour
kt	kilotonne
t	tonne
µg L^{-1}	microgram per litre

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 2024–25 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 with partners including:

- Australian Institute of Marine Science (AIMS)
- James Cook University (JCU)
- University of Queensland (UQ)
- Cape York Water Partnership (CYWP)

We work together to deliver the sub-programs of the MMP which monitor the inshore marine environment including water quality, seagrass, coral and pesticides 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; and
- **Inshore pesticide monitoring:** To assess the presence of pesticides in inshore areas of the Reef lagoon and identify any temporal and spatial trends.

Each monitoring provider has a different responsibility in the delivery of the sub-programs that make up the MMP (Table 1-1).

Table 1-1: Current monitoring sub-programs, projects, and providers.

Monitoring sub-program	Component project	Monitoring provider
Inshore marine water quality	Routine water quality monitoring (ambient conditions)	AIMS, JCU, and CYWP
	Event-based water quality monitoring (flood events)	JCU and CYWP
	Mapping (satellite imagery) and modelling (reefs model outputs) of water quality	i-Sea, JCU
Inshore seagrass condition	Inshore seagrass monitoring	JCU
Inshore coral reef condition	Inshore coral monitoring	AIMS
Inshore pesticide presence	Pesticide monitoring	UQ (deployment by several providers including JCU and 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 independently reviewed in 2014, and 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 each focus region. The design included four focus regions: the Russell-Mulgrave, Tully, Burdekin, and Mackay-Whitsunday. Extra reporting for the Barron-Daintree focus region of the Wet Tropics was also included due to the continued collection of data along AIMS' long-term 'Cairns transect' where sampling started in 1989. In 2016–17, four focus regions were added in Cape York around the Pascoe, Normanby-Kennedy, Annan-Endeavour and Stewart Rivers.

In 2020–21, monitoring was reinstated in the Fitzroy region with funding from the Reef Trust Partnership (RTP) between the Australian Government and the Great Barrier Reef Foundation and co-investment by AIMS. Water quality monitoring in this region was re-incorporated into the MMP starting in the 2024–25 water year and has been conducted at the same sites and using the same sampling design principles of the MMP.

The sites in each focus region are located to capture water masses along cross-shelf and alongshore gradients. The site selection in the focus regions 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*), dissolved silica [$Si(OH)_4$], salinity, turbidity, coloured dissolved organic matter (CDOM) and temperature.

Techniques used are a combination of:

- continuous measurement of salinity and temperature using *in situ* loggers,
- continuous measurement of Chl-*a* and turbidity using *in situ* loggers,
- ambient water quality sampling during the year with more frequent sampling during the wet season, and
- event-based water quality sampling during flood conditions.

1.2 Remote sensing and modelling of water quality

The use of satellite information in combination with *in situ* water quality measurements provide 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 ocean colour satellite images reclassified to optical water type maps have been used to map and characterise the spatial and temporal distribution of river plumes, and document the impact of these river plumes on Reef ecosystems.

To define and map wet-season water-quality conditions and track the movement, composition, and frequency of occurrence of flood plumes across the Reef, current remote-sensing methods use ocean colour retrieved from optical satellite imagery to classify Reef waters into Optical Water Types. These Optical Water Types group water bodies that share similar optical properties and are typically found in the Reef during the wet season (Hereafter Reef water types). Catchment run-off in sediment-laden river discharge appears in satellite images as brownish floodwaters, while productive waters appear with a greenish colour and ambient (clear) marine waters with a bluish colour. The resulting water-type maps describe the typical colour and water-quality gradients encountered in the Reef during the wet season, including the extent and behaviour of river plumes.

Methods have been historically based on the extraction and analysis of Moderate-Resolution Imaging Spectroradiometer (MODIS) true colour data. However, MODIS sensors are ageing (MODIS-Aqua was launched in 2002) and the quality of the MODIS imagery is declining. The use of Sentinel-3 Ocean Land Colour Instrument (OLCI) satellite imagery was proposed for the continuous mapping of Reef waters in Petus *et al.* (2019) and was implemented in the MMP in 2020.

To understand wet season marine water quality conditions and identify where seagrass and coral reefs may be at risk from water quality influences, several products derived from Sentinel-3 satellite imagery are produced. These include Reef-wide maps representing the frequency of occurrence of wet season Reef water types, as well as maps that estimate exposure to potential risk – based on the mapped wet season water-quality conditions. Exposure is assessed in terms of the area (km^2) and percentage (%) of coral reefs and seagrass meadows exposed to different categories of potential risk. Weekly panels of maps showing regional environmental and marine wet season conditions are also prepared for full annual reports.

River discharge and end-of-catchment loads are reported annually providing environmental context and inputs to maps representing the dispersion of end-of-catchment loads of DIN, TSS, and PN loads to the Reef.

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 8 fixed sites in 2024–25) 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 considering mixtures of herbicides that may have additive effects.
- 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 annually at 30 inshore coral reefs in the Wet Tropics, Burdekin, Mackay Whitsunday and Fitzroy NRM regions along gradients of exposure to run-off from regionally important rivers.

- at each reef, two sites are monitored at two depths (two metres and five metres) across five replicate transects. An exception is Middle Island site 2 where monitoring occurs at 2 m depth only due to insufficient water depth for deeper 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 Seagrass-Watch Global Seagrass Observing Network (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. 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 late-wet seasons; extra sampling is conducted at more accessible locations in the dry and wet seasons.
- monitoring includes *in situ* within canopy temperature and light levels.
- data are also collected annually from an additional 33 sites across 19 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; and
- 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, and
- improvement of passive sampling techniques for pesticides.

The monitoring partners of the MMP have a long-standing culture of QA/QC in their sub-programs. Common elements across the partner organisations 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; and
- procedures for data entry, storage, validation, and reporting.

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

2 Inshore *in-situ* water quality monitoring

Renee Gruber[2], Jane Waterhouse[1], Daniel Moran[2], Stephen Lewis[1], Christina Howley[3], Jack Massuger[2], and Jane Mellors [1]

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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. The MMP inshore water quality monitoring program informs the Paddock to Reef Integrated Monitoring, Modelling and Reporting Program (Paddock to Reef Program) by:

- Describing spatial patterns and temporal trends in inshore concentrations of sediment, Chl-*a*, and nutrients as assessed against water quality guideline values;
- Monitoring local water quality using autonomous instruments for high-frequency measurements at selected inshore locations;
- 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; and
- 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, JCU, and CYWP sampling program comprises routine sampling sites visited throughout the year, with sampling at additional sites during flood events (Table 2-1, Figure 2-1). The sampling sites are located across focus regions in five Natural Resource Management (NRM) regions: Cape York (Pascoe, Stewart, Normanby-Kennedy, and Annan-Endeavour); Wet Tropics (Barron-Daintree, Russell-Mulgrave, and Tully); Burdekin; Mackay Whitsunday; and Fitzroy. 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 CYWP during the 2024–25 water year. The proposed number of visits is shown.

Site Location		Logger Deployment		Ambient sampling at fixed sites		Event-based sampling
NRM Region and Focus Area	**Short Name**	**Turbidity and chlorophyll**	**Salinity**	**Number of times (season) site is visited/year by AIMS**	**Number of times (season) site is visited/year by JCU/CYWP**	**Additional sampling/year by JCU/CYWP**
Cape York						
Normanby-Kennedy Focus Area						
Kennedy River mouth	KR01					✓
Kennedy inshore	KR02					✓
Bizant River mouth	BR01					✓
Normanby River mouth	NR01					✓
Normanby inshore	NR02				4 (4 wet)	
NR-03	NR03				4 (4 wet)	
NR-04	NR04				4 (4 wet)	
NR-05	NR05				4 (4 wet)	
Corbett Reef	NR06				4 (4 wet)	
Pascoe Focus Area						
Pascoe mouth north	PRN01					✓
Pascoe River mouth south	PRS01				5 (5 wet or 4 wet and 1 dry) (Surface sample only)	
PR-North 02	PRN02				5 (5 wet or 4 wet and 1 dry) (Surface sample only)	
Eel Reef-North	PRN04				5 (5 wet or 4 wet and 1 dry)	
Eel Reef	PRN05					✓
Eel Reef North	PRN06					✓
PR-South 2.5	PRS2.5				5 (5 wet or 4 wet and 1 dry)	
Middle Reef	PRBB				5 (5 wet or 4 wet and 1 dry)	
Eel Reef South	PRS05				5 (5 wet or 4 wet and 1 dry)	
Annan-Endeavour Focus Area						
Annan mouth	AR01					✓
Walker Bay	AR02b				5 (5 wet or 4 wet and 1 dry)	
Dawson Reef	AR03b				5 (5 wet or 4 wet and 1 dry)	
Dawson FLNTU	Dawson-FLNTU	✓			4 (4 wet) (Surface sampling only)	
Endeavour mouth	ER01					✓
Endeavour north shore	ER02b				5 (5 wet or 4 wet and 1 dry)	
Endeavour offshore	ER03				5 (5 wet or 4 wet and 1 dry)	
Egret and Boulder Reef	AE04				5 (5 wet or 4 wet and 1 dry)	
Forrester Reef FLNTU	FR-FLNTU	✓			4 (4 wet) (Surface sampling only)	
Stewart Focus Area						
Stewart mouth	SR01					✓

Stewart River	SR02				5 (5 wet or 4 wet and 1 dry)	
S3	SR03				5 (5 wet or 4 wet and 1 dry)	
Burkitt Island	SR04				5 (5 wet or 4 wet and 1 dry)	
Hannah Island	SR05				5 (5 wet or 4 wet and 1 dry)	
Cliff Islands	CI01				4 (4 wet)	
Wet Tropics						
Barron-Daintree Focus Area						
Cape Tribulation*	C1			3 (1 wet and 2 dry)		
Port Douglas*	C4			3 (1 wet and 2 dry)		
Double Island*	C5			3 (1 wet and 2 dry)		
Yorkey's Knob*	C6			3 (1 wet and 2 dry)		
Fairlead Buoy*	C8			3 (1 wet and 2 dry)		
Green Island*	C11			3 (1 wet and 2 dry)		
Russell-Mulgrave Focus Area						
Fitzroy Island West*	RM1	✓		5 (3 wet and 2 dry)		
RM2	RM2					✓
RM3	RM3			5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
RM4	RM4					✓
High Island East	RM5					✓
Normanby Island	RM6					✓
Frankland Group West (Russell Isl.)*	RM7	✓		5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
High Island West*	RM8	✓	✓	5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Palmer Point	RM9					✓
Russell-Mulgrave River mouth mooring	RM10	✓	✓	5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Russell-Mulgrave River mouth	RM11					✓
Russell-Mulgrave junction [River]	RM12					✓
Tully Focus Area						
King Reef	TUL1					✓
East Clump Point	TUL2			5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Dunk Island North*	TUL3	✓	✓	5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
South Mission Beach	TUL4					✓
Dunk Island South East	TUL5			5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Between Tam O'Shanter and Timana	TUL6			5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Hull River mouth	TUL7					✓
Bedarra Island	TUL8			5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Triplets	TUL9					✓
Tully River mouth mooring	TUL10	✓	✓	5 (3 wet and 2 dry)	5 (4 wet and 1 dry)	
Tully River	TUL11					✓
Burdekin						
Burdekin Focus Area						
Pelorus and Orpheus Island West*	BUR1	✓		4 (2 wet and 2 dry)	5 (5 wet and 0 dry)	
Pandora Reef*	BUR2	✓		4 (2 wet and 2 dry)	5 (5 wet and 0 dry)	
Cordelia Rocks	BUR3					✓
Magnetic Island (Geoffrey Bay)*	BUR4	✓		4 (2 wet and 2 dry)	5 (5 wet and 0 dry)	

Inner Cleveland Bay	BUR5					✓
Cape Cleveland	BUR6					✓
Haughton 2	BUR7			4 (2 wet and 2 dry)	5 (5 wet and 0 dry)	
Haughton River mouth	BUR8					✓
Barratta Creek	BUR9					✓
Yongala IMOS NRS	BUR10			4 (2 wet and 2 dry)		
Cape Bowling Green	BUR11					✓
Plantation Creek	BUR12					✓
Burdekin River mouth mooring	BUR13	✓	✓	4 (2 wet and 2 dry)	5 (5 wet and 0 dry)	
Burdekin Mouth 2	BUR14					✓
Burdekin Mouth 3	BUR15					✓
Mackay-Whitsunday						
O'Connell Focus Area						
Double Cone Island*	WHI1	✓		5 (3 wet and 2 dry)		
Hook Island W	WHI2					✓
North Molle Island	WHI3					✓
Pine Island*	WHI4	✓	✓	5 (3 wet and 2 dry)		
Seaforth Island	WHI5	✓		5 (3 wet and 2 dry)		
O'Connell River mouth	WHI6	✓	✓	5 (3 wet and 2 dry)		
Repulse Islands dive mooring	WHI7			5 (3 wet and 2 dry)		
Rabbit Island NE	WHI8					✓
Brampton Island	WHI9					✓
Sand Bay	WHI10					✓
Pioneer River mouth	WHI11					✓
Fitzroy						
Fitzroy Focus Area						
Barren Island*	FTZ1	✓		10 (7 wet and 3 dry)		
Humpy Island*	FTZ2	✓	✓	10 (7 wet and 3 dry)		
Pelican Island*	FTZ3			10 (7 wet and 3 dry)		
North Keppel	FTZ4			10 (7 wet and 3 dry)		
Peak West	FTZ5			10 (7 wet and 3 dry)		
Fitzroy River Mouth	FTZ6	✓	✓	10 (7 wet and 3 dry)		

*Sites which were part of the MMP sampling design from 2005–2015

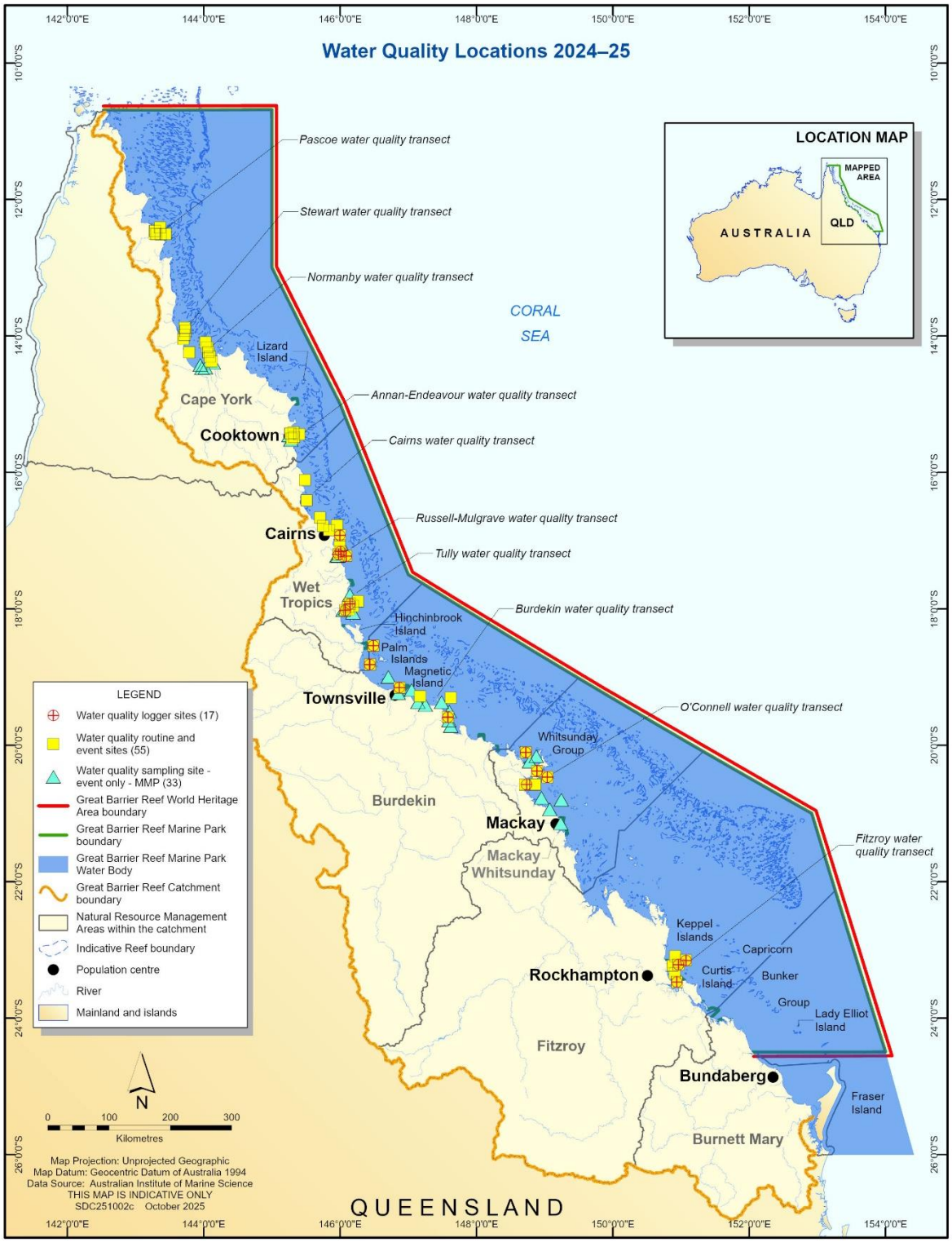

Figure 2-1: Site locations of the MMP inshore water quality sub-program sampled from 2015 onwards.

2.2.2 Sample collection, preparation, and analysis (AIMS)

The following standard quality assurance protocols are followed for all water chemistry sampling performed by AIMS:

- Before sampling, staff clean their hands thoroughly with fresh water. Grease, oils, soap, fertilisers, sunscreen, hand creams and smoking can all contribute to contamination.
- Clean sampling paraphernalia are used at all times for the handling and transfer of sample water.
- Uncoloured and powder-free PVC disposable gloves are worn by staff at all times during sample collection and manipulation of samples for low level nutrient analyses.
- The sampling container and other sampling paraphernalia are well-rinsed with site water before sampling.
- The filling instructions and storage requirements (described in the following sections) are adhered to when conducting chemistry sampling.
- On each sampling run, the date, time and unique sample identification are recorded on the field datasheet. Each sampling kit for each site contains sets of sampling bottles and vials labelled with a unique alpha-numeric station name (the convention is for a three letter-three number code (e.g., WQQ001).
- Any significant change of sampling conditions (i.e. weather and other factors which may influence the ability to collect samples) is noted in the comments section of the record sheet.

Vertical profiles of the water column are conducted using a Conductivity Temperature Depth (CTD) profiler. AIMS uses several Sea-Bird Scientific CTD profilers (SBE 19plus), which are fitted with additional sensors including fluorometers, nephelometers, transmissometers and Photosynthetically Active Radiation (PAR) sensors. Annual calibrations of the profiler instrumentation are carried out in Sea-Bird Scientific laboratories in the USA or CSIRO Oceans and Atmosphere Oceanographic Calibration Facility in Hobart, Tasmania. 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 and battery status.

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 ~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 CTD is then raised to ~0.2 below the surface and the profile is commenced at a rate of 0.5 – 1 m s^{-1}. 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 further details on CTD cast procedures and details of data post-processing and storage. 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. Secchi depth is determined at each station by a person not wearing sunglasses.

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 NML) into acid-washed, screw-cap plastic test tubes, which are pre-rinsed twice with filtered site water. Filter cartridges are purged with site water to remove loose carbon and other potential contaminants before sample tube rinsing and sample collection. 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 at the AIMS Analytical Technology Laboratory. 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 (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.

DOC (i.e., non-purgeable organic carbon) concentrations are measured by high temperature catalytic combustion (720°C) using a Shimadzu TOC-L carbon analyser. Before analysis, the samples are acidified to remove inorganic carbon, and CO_2 remaining in the sample water is then removed by sparging with O_2 carrier gas. The acidification and sparging process leave behind only the (non-purgeable) organic carbon in the water sample at the time of analysis. This portion is then combusted at temperature to form CO_2 and quantified by infrared detection (Appendix A4).

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 A5).

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). Before subsampling, the sampling containers are thoroughly agitated to ensure particulates do not settle and skew results. 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 A6). The method is standardised using pure chemicals (orthophosphoric acid salt) as the primary standards, with oxidisable QCs for digest monitoring and quality control.

The POC content of material collected on filters is determined by high temperature catalytic combustion (950°C) using a Shimadzu TOC-V carbon analyser fitted with a SSM-5000A solid sample module (Appendix A7). 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 boat is then introduced into the sample chamber where it is first purged of atmospheric CO_2 in an oxygen stream before being placed into the analyser's furnace at 950°C. At this temperature, the organic carbon is combusted, forming carbon dioxide, which is then detected 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 A7). 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 A8) (Parsons, Maita, and Lalli 1984). The fluorometer is calibrated using a Chl-*a* standard from *A. nidulans* algae (Sigma-Aldrich C1644). 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 for 72 hours at 60° C (Appendix A9).

2.2.3 Autonomous environmental water quality loggers (AIMS)

In situ autonomous water quality monitoring is undertaken using both WET Labs Environmental Characterisation Optics (ECO) FLNTUSB Combination Fluorometer and Turbidity Sensors, and Sea Bird SBE37 Microcat Conductivity and Temperature loggers. (Appendix A10).

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*’.

FLNTU instruments are used in ‘logging’ mode and record a data point every 10 minutes for each of the parameters; each data point is calculated as the mean of 50 instantaneous burst readings. Pre-deployment checks of each instrument include measurements of ‘dark counts’ (instrument response with no external fluorescence, essentially the ‘zero’ point) as well as noise assessment and instrument condition. Factory servicing and calibration checks are performed at a WET Labs-approved facility after 12–18 months of in-water deployment time (Appendix A10).

SBE37 instruments record a data point every 10 minutes for temperature, conductivity and (if available) pressure. Where possible, pumped units are deployed to increase flow over sensors, though on occasion unpumped units are deployed at sites with higher current speeds depending on instrument availability.

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, ºC for temperature, and S m^{-1} for conductivity) according to the standard procedures of the manufacturer.

Time-series instrument data from 2023 onwards have been through an updated QC workflow and have been made available through the Australian Ocean Data Network (AODN) in NetCDF file formats. Deployment metadata are stored in a protected MS Access “OceanDB” database, which is used in conjunction with “IMOS Toolbox”, a Matlab-based UI toolset for data QC and NetCDF output. Using IMOS Toolbox, data undergo a range of pre-processing routines to standardise time zone and correct instrument clock variation, as well as a suite of automated QC routines which flag out data based on global ranges, in/out of water times and impossible values. Data then undergo manual QC and are assigned a flag value from 0–9

based on their reliability and QC status. Files are exported in NetCDF format alongside deployment and calibration metadata meeting Climate and Forecast 1.8 conventions (Appendix A11).

2.2.4 Sample collection, preparation, and analysis (JCU and CYWP)

Sampling kits containing all sampling consumables (i.e. containers, filters, etc.) are prepared by AIMS and provided to partner organisations. Following sample collection, all samples and data sheets are returned to AIMS for analysis and databasing.

Methods of sampling are consistent with AIMS processes; however, some modifications have been made to sampling and storage procedures to allow for the equipment availability and transport of samples back to AIMS for analysis. Field processing procedures and sample storage requirements are listed in Table 2-2.

At each sampling station in the Wet Tropics and Burdekin regions, vertical profiles of water depth, temperature, salinity, dissolved oxygen, turbidity, and photosynthetically available radiation 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.

Water samples are collected from the same strata of the water column as AIMS samples (~0.5 m below the water surface and ~1 m above the seabed). Depending on the sampling vessel, partners organisations use 5 L Niskin bottles or may collect surface samples using an extended sampling pole with swing sampler and clean 500 mL bottle or 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. Sub-samples are taken for different water quality parameters (see Appendix A1). All water quality parameters are collected for all stations.

Dissolved nutrient, silica, and CDOM samples are collected from the sampling container with sterile 60 mL syringes using the same methods outlines above. 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 AIMS laboratory for analysis. DOC samples are frozen and the acid added prior to laboratory analysis at AIMS. The analysis methods are described in Appendix A3 and Appendix A5.

POC, PN and PP samples are collected in sterile 2 L containers and stored on ice before being filtered on Whatman GF/F filter papers on return to shore-based facilities. The target filter volumes for POC and PN are 500 mL and PP are 250 mL. These volumes are varied for very turbid samples so as not to run off scale during analysis (i.e., if the Secchi depth is <3 m, the filter volumes for POC/PN are reduced by half). The filters are frozen and transported to the AIMS laboratory for analysis. The analysis methods are described in Appendix A7 (POC/PN) and Appendix A6 (PP).

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 must be filtered within 24 hours of collection. Following filtration, frozen filters are transported to AIMS for analysis. The analysis methods are described in Appendix A9. For salinity samples, unfiltered water is stored in 250 mL bottles and stored on ice on the sampling vessel then transported to AIMS for analysis (see Appendix A2 for analysis methods). Chl-*a* samples are filtered through a 25 mm diameter GF/F filter paper held with a plastic syringe filter holder attached to the syringe. 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, then

transported to AIMS for analysis. The analysis method for the Chl-*a* samples is provided in Appendix A8.

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

Water quality parameter	Field processing	Laboratory container	Storage following field processing	Laboratory method
Dissolved Organic Carbon (DOC)	Filtered water sample (0.45 µm)	10 mL plastic tube	Acidification and stored at 4 °C (AIMS) or un-acidified and frozen (JCU/CYWP)	Appendix A4
Oxidised nitrogen (nitrate + nitrite: NO_x)	Filtered water sample (0.45 µm)	10 mL plastic tube	Frozen	Appendix A3
Ammonium (NH_3)	Filtered water sample (0.45 µm)	10 mL plastic tube	Frozen	Appendix A3
Total dissolved nitrogen (TDN)	Filtered water sample (0.45 µm)	10 mL plastic tube	Frozen	Appendix A3
Filterable reactive phosphorus (PO_4)	Filtered water sample (0.45 µm)	10 mL plastic tube	Frozen	Appendix A3
Total dissolved phosphorus (TDP)	Filtered water sample (0.45 µm)	10 mL plastic tube	Frozen	Appendix A3
Silica	Filtered water sample (0.45 µm)	10 mL plastic tube	Stored at 4 °C	Appendix A3
Coloured Dissolved Organic Matter (CDOM)	Filtered water sample (0.2 µm)	60 mL amber glass bottle	Stored at 4 °C	Appendix A5
Particulate Organic Carbon (POC)	500 mL filtered sample (0.7 µm GF/F filter)	Filter wrapped in aluminium foil	Frozen	Appendix A7
Particulate nitrogen (PN)	500 mL filtered sample (0.7 µm GF/F filter)	Filter wrapped in aluminium foil	Frozen	Appendix A7
Particulate phosphorus (PP)	250 mL filtered sample (0.7 µm GF/F filter)	Filter wrapped in aluminium foil	Frozen	Appendix A6
Chlorophyll-*a* (Chl-*a)*	100 mL filtered sample (0.7 µm GF/F paper)	Filter wrapped in aluminium foil	Frozen	Appendix A8
Total suspended solids (TSS)	1000 mL filtered onto pre-weighed (0.4 µm) 47 mm polycarbonate filter	Filter in glass scintillation vial	Stored at 4 °C	Appendix A9
Salinity	Unfiltered sample (250 mL)	250 mL plastic bottle	Stored at 4 °C	Appendix A2

2.2.5 Sample collection, preparation, and analysis: wet season flood events (JCU and CYWP)

Event response sampling is conducted in addition to the routine wet season sampling. The JCU and AIMS laboratories organises a flood sampling kit for each site with unique identifiers for all samples. During an event, samples are collected at both the fixed routine sites and the reactive flood plume sampling sites. The parameters measured and the procedures for sample collection and analysis are identical to those described for the routine program. Analysis is undertaken jointly by JCU and AIMS.

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. While there is no single statistical definition of a flood event, event-based sampling is typically considered when the river level is classified as 'Minor' or above by the Bureau of Meteorology but may vary depending on the local conditions and the river itself. For the analysis of the water quality data, data collected in conditions within or following a 'Moderate' event is identified as 'flood' data. 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), 'Pioneer R at Dumbleton TW' (Pioneer River), Annan R at Beesbike, Normanby R at Kalpowar, Stewart R at Telegraph Road, and Pascoe R at Garroway Creek (Table 2-1).

Extra samples may also be collected depending on the extent of the plume depending on *in situ* salinity measurements. 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. Flood event 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 A11). Data from the AIMS MMP inshore water quality monitoring and monitoring by JCU, CYWP and supplementary sampling by external contractors 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 QC and validation processes, they are consolidated via Oracle views (see Appendix A11).

It is AIMS policy that all datasets collected have an associated metadata record 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. The parent metadata record for the MMP is located here: https://apps.aims.gov.au/metadata/view/2b2aa4e4-1368-49e0-8b25-1559ee297854.

Specific child metadata records for MMPWQ datasets are listed below:

- Physico-chemical and nutrient database (containing all dissolved and particulate nutrient data, including data from JCU and CYWP): https://apps.aims.gov.au/metadata/view/a5a02dc8-16b4-4b50-abad-af4a1c1e9c49
- FLNTU time-series database (containing data from 2007-2022): https://apps.aims.gov.au/metadata/view/8a698de1-3fbf-48a5-b068-358b07aad35c
- FLNTU time-series NetCDF catalogue on AODN (containing data from 2023 onwards): https://thredds.aodn.org.au/thredds/catalog/AIMS/Marine_Monitoring_Program/FLNTU_timeseries/catalog.html
- Salinity/temperature time-series database (containing data from 2007-2022): https://apps.aims.gov.au/metadata/view/351d44f5-6888-45fc-ac46-44ddbd623bf1
- Salinity/temperature NetCDF catalogue on AODN (containing data from 2023 onwards): https://thredds.aodn.org.au/thredds/catalog/AIMS/Marine_Monitoring_Program/SBE37_timeseries/catalog.html
- CTD database (containing all AIMS' vertical cast data from CTD instruments): https://apps.aims.gov.au/metadata/view/acad78d1-e235-45e6-8f27-0a00184e2ca9

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

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3.1 Introduction

Remote sensing imagery is a useful assessment tool in the monitoring of turbid water masses and river flood plumes (hereafter river plumes). Ocean colour imagery provides synoptic-scale information regarding the movement, frequency of occurrence and composition of turbid waters. Combined with in situ water quality sampling and modelling, the use of remote sensing is a valid and practical way to estimate wet season marine conditions in the Reef lagoon.

This component of the MMP provides remotely sensed information and modelled outputs of the dispersion of pollutant loads during the wet season (December to April), capturing river flood plumes and resuspension events. It assesses frequencies and composition of Reef optical water 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.

Maps showing the distribution of end-of-catchment pollutant provide additional insight into how pollutants move through the marine environment in river discharge. These products combine eReefs tracer maps and end-of-catchment pollutant loads to represent the wet season distribution of fine sediments and nutrients across the Reef. They allow comparison of the intensity of exposure to pollutant loads between years, and comparison of pre-development and current loads to illustrate anthropogenic surface loads. The maps draw on annual river flow and end-of-catchment load data, as described below.

3.2 Methods

3.2.1 Remote sensing products

There have been several methods within the flood plume program to characterise, map and monitor flood events in the Reef over the last 20 years (e.g., 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 ocean colour products and optical water type maps 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). Greater integration with the eReefs marine model is also becoming increasingly important.

Until 2020, trends in Reef marine water composition during the wet season were monitored using a combination of Moderate-Resolution Imaging Spectroradiometer (MODIS) satellite imagery and a “wet season” colour scale specifically developed for the Reef (Alvarez-Romero *et al.* 2013). MODIS satellite pixels were reclassified into 6 colour classes, then into 4 broad groups of water type characteristics: the primary (corresponding to colour classes 1 to 4), secondary (colour class 5), and tertiary (colour class 6) wet season water types and the marine water type (**Error! Reference source not found.**a). This method is extensively presented in Álvarez-Romero *et al.* (2013) and used in, for example, Devlin *et al.* (2013) and Petus *et al.* (2014b, 2016, 2018 and 2019).

These water types represent typical colour and water quality gradients encountered in the Reef during the wet season (December to April), including river plumes; and are characterised

by different concentrations of optically active components (TSS, CDOM and Chl-*a*). Catchment runoff in sediment-laden river discharge appears in satellite images as brownish flood plumes, while productive chlorophyll-rich waters appear with a greenish colour, and ambient (clear) marine waters are a blueish colour. Brownish-green waters also appear when sediments are re-suspended by wind or tide, and it is impossible to fully separate the direct influence of riverine plume from wind- and wave-driven sediment resuspension (some of which may have been originally derived from river discharge in previous events) in optical satellite images. Therefore, the term “wet season waters” referred collectively to flood river plumes, associated resuspension, and oceanographic processes occurring in the Reef during the wet season.A transition to Sentinel-3 Ocean Land Colour Instrument (OLCI) satellite imagery and another colour scale (the Forel-Ule [FU] colour scale) adopted in 2020 (Petus *et al.* 2019). The FU colour scale is a historical colour scale global standard to determine the colour and classifies worldwide bodies of water (Novoa *et al.* 2013). It is composed of 21 colours; going from indigo blue to cola brown, and is applicable for all natural waters (inland, estuarine, inshore and offshore) and all environmental conditions, including wet and dry season conditions (Wernand et al. 2012, 2013; Van der Woerd *et al.* 2016; Van der Woerd and Wernand, 2018). In a case study focusing on Wet Tropics and Burdekin regions of the Reef over the 2017–18 wet season, the MODIS-Aqua WS and Sentinel-3 FU colour class maps showed very similar patterns (Petus *et al.* 2019 and Figure 3-1**Error! Reference source not found.**b). This suggested that Sentinel-3 FU water colour products can be used to assure continuity in the monitoring of Reef water quality trends.

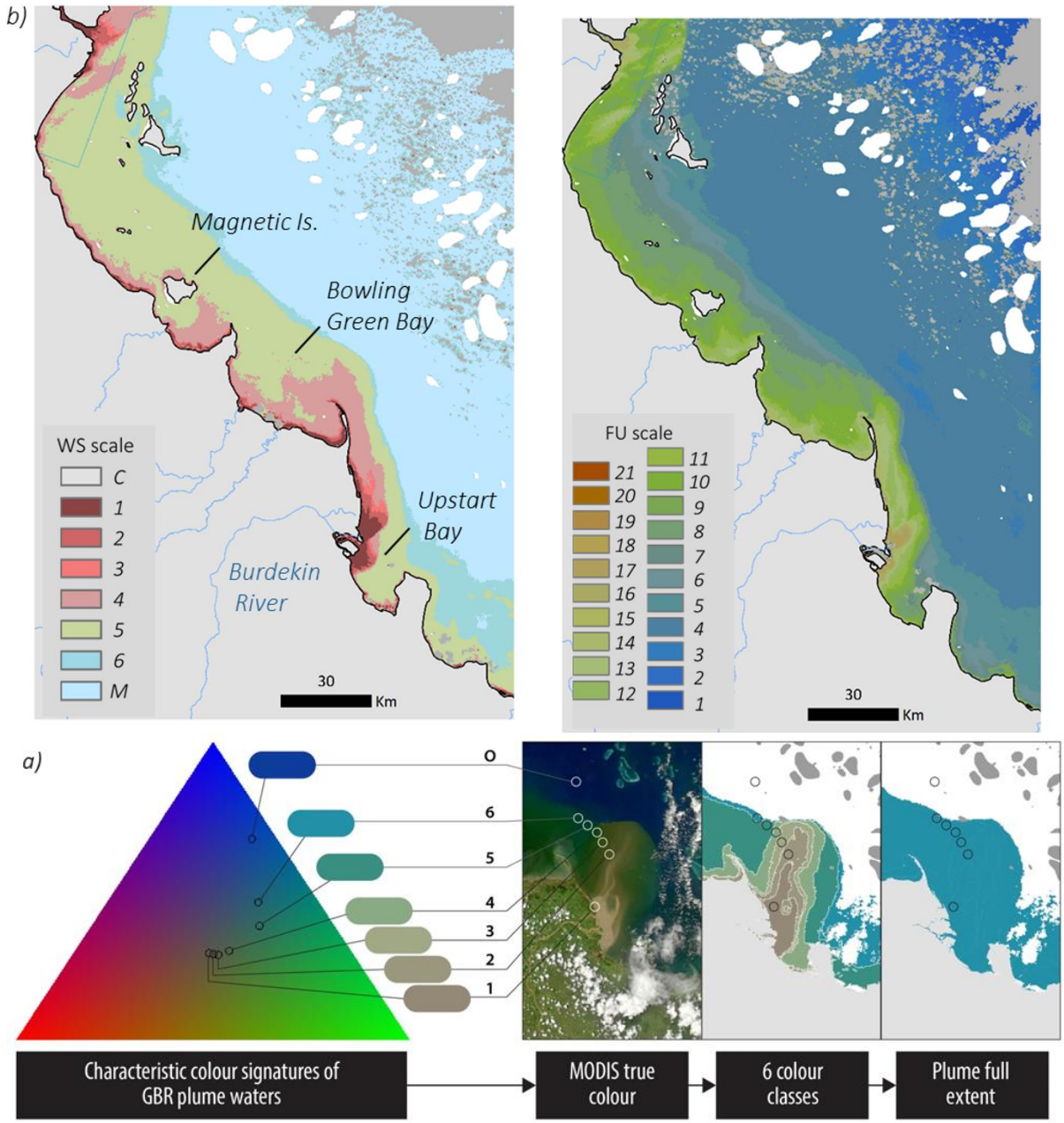

Figure 3-1: Triangular colour plot showing the characteristic colour signatures of the wet season water types in the Red-Green-Blue (RGB or true colour) space. Álvarez-Romero *et al.* (2013) developed a method to map these characteristic coastal water masses in the Reef using a supervised classification of MODIS true colour data (modified from Devlin *et al.* 2015). b) Illustration of the very similar colour patterns between the (left) MODIS wet season and (right) Sentinel-3 Forel-Ule colour class maps: Burdekin River plume, 14 March 2018.

Several monitoring products are derived from the water type maps and water quality data. They are summarised in Figure 3-2 and aim to:

- Map water types and water quality gradients during the wet season and assess the extent and movement of river flood plumes during high flow conditions;
- Characterise the composition of the Reef wet season water types (boxplot of long-term TSS, Chl-*a,* CDOM, DIN, DIP, PP and PN concentrations and Secchi values);
- Identify where mean long-term concentrations of TSS, Chl-*a,* PP, and PN are likely to 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); and

- Assess the exposure of coral reefs and seagrass ecosystems to potential risk from exposure 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 l with baseline reference trends in water composition, including long-term conditions, typical wet year and dry year conditions and conditions over a documented recovery period for coral reefs. Available satellite data are biased toward non-cloudy days and may underrepresent poor water quality in regions of higher rainfall and cloudiness like the Wet Tropics and Cape York. However, they provide a unique large-scale and long-term view of the Reef that is not available using water quality data only.

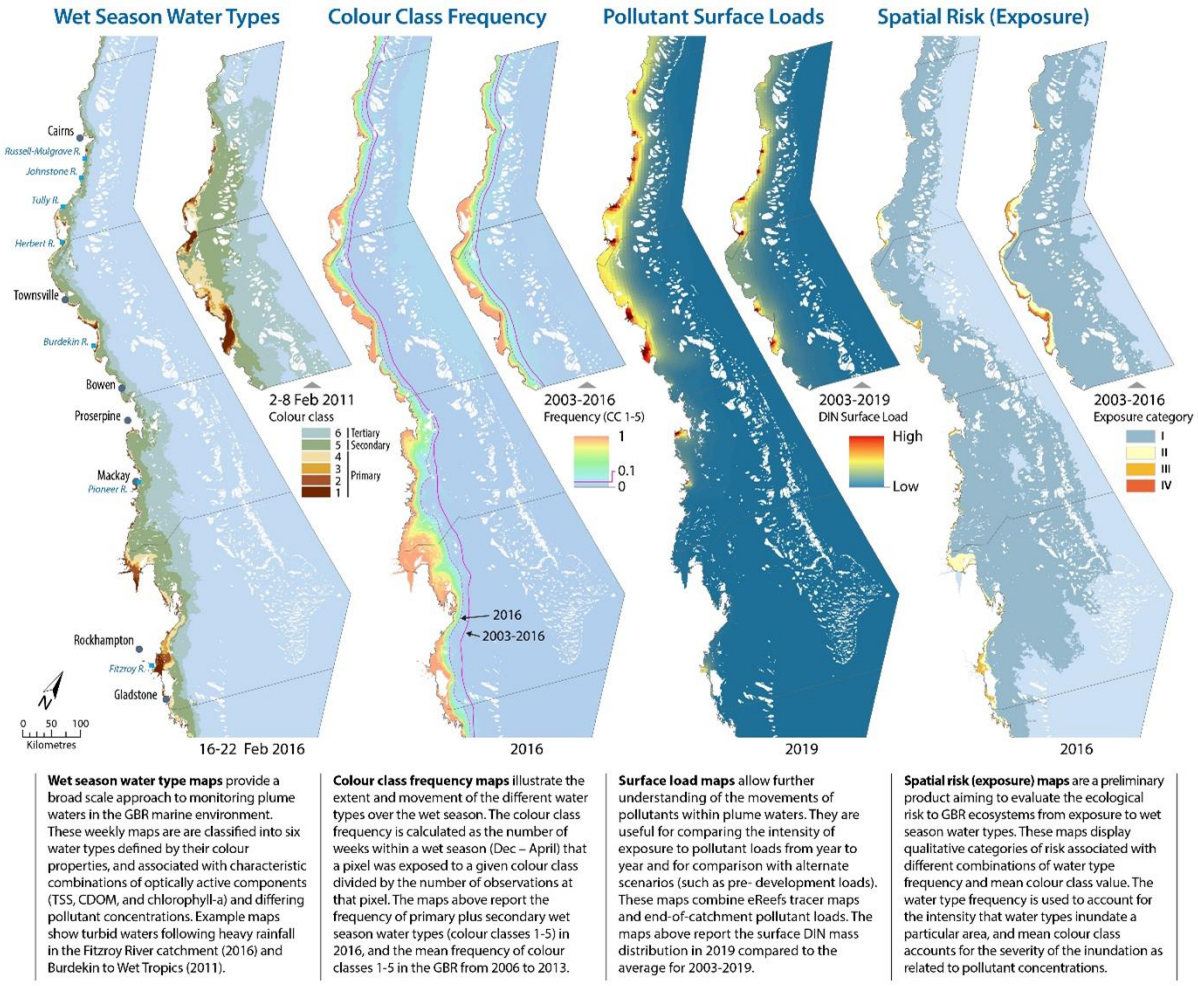

Figure 3-2: Summary description of the wet season water quality products.

3.2.1.1 Optical satellite data

- **MODIS true colour imagery (2002–2020)**

The Terra and Aqua satellite platforms were launched in 1999 and 2002 respectively, by the National Aeronautics and Space Administration (NASA). They carry the MODIS instrument, a visible imaging radiometer which provides regular coverage (twice per day) of the GBR coastal waters. MODIS-Aqua data were operationally used in the MMP from 2002–2020 (Figure 3-2), and MODIS Terra only occasionally, as the radiometric quality of the MODIS-Terra ocean colour bands is significantly worse and cross-calibrated to MODIS Aqua.

Previous methods used Daily MODIS (Moderate Resolution Imaging Spectroradiometer) Level-0 data acquired from the NASA Ocean Colour website, spectrally enhanced (from red-

green-blue to hue-saturation-intensity colour system) and classified to 6 colour categories through a supervised classification using spectral signatures from typical wet season water masses types (including river plumes) in the Reef lagoon (Álvarez-Romero *et al.* 2013).

The methods are described in detail in the previous QA/QC reports (e.g. Gruber *et al.* 2025). MODIS true-colour images for 2011 (very wet), 2016 and 2017 (dry) years, and from 2018 to 2020 were processed by the Bureau of Meteorology, while all other years were processed in-house by TropWATER JCU.

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 2002–April 2003 up to December 2019–April 2020 and were processed with the wet season Satellite Toolbox to produce MODIS-Aqua wet season water type maps of the study area following the processes outlined below. Processed MODIS data were stored in external media and also at the national Research Data Services, which is part of Research Data Australia (https://researchdata.ands.org.au/).

- **Sentinel-3 OLCI imagery (2020–present)**

The Sentinel-3A and 3B platforms were launched in February 2016 and April 2018 respectively by the European Space Agency (ESA). They carry the OLCI, a visible imaging radiometer which provides a daily coverage of the GBR coastal waters since 2018 (two to three times a week coverage since 2016).

Sentinel-3 OLCI Level-2 imagery (hereafter, Sentinel-3) of the study area is downloaded on the EUMETSAT data centre (URL: https://user.eumetsat.int/data-access/data-centre) which provide OLCI Level-2 data for the entire earth. Sentinel-3 data are atmospherically corrected and are processed with the FU Satellite Toolbox implemented in SNAP (Van der Woerd *et al.* 2016; Van der Woerd and Wernand, 2018) to produce S3-FU maps of the study area following steps described below. Since 2024, the data has been processed by i-Sea in collaboration with TropWATER JCU.

3.2.1.2 Colour classifications

Supervised classification of MODIS-Aqua imagery using the Wet Season Colour Scale (2002–2020)

A set of spatial analyses (Python, ArcGIS) based on supervised classification of spectrally enhanced true colour images was 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. The six colour classes are further reclassified into three wet season water types (Primary/WT1, Secondary/WT2 and Tertiary/WT3) 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 was used to classify daily MODIS images and create an historical database of daily MODIS wet season water type maps for wet season between 2002 and 2020.

Classification of Sentinel-3 OLCI imagery using the Forel-Ule Colour Scale (2020–present)

Since the 2021-22 wet season, Reef water type maps were produced using daily Sentinel-3 imagery reclassified to 21 distinct colour classes defined by their colour properties and using the FU colour classification scale. The FU colour scale comparator is a 21-level colour classification system based on human visual comparison with glass encased colour

standards. It was developed in the late 19th century and can be used worldwide, with any natural water body (marine, coastal, estuarine and lake). A remote sensing toolbox has been developed to classify water bodies into FU categories from satellite ocean colour imagery through European ECFP7 funding and the Citclops project (URL: http://www.citclops.eu/home). It has been implemented in the Sentinel-3 Toolbox, which is built on the Sentinel Application Platform (SNAP, URL: https://step.esa.int/main/toolboxes/snap/). The FU satellite algorithm converts satellite normalised multi-band reflectance information into a discrete set of FU numbers using uniform colourimetric functions. The derivation of the colour of natural waters is based on the calculation of Tristimulus values of the three primaries (X, Y, Z) that specify the colour stimulus of the human eye. The algorithm is validated by a set of hyperspectral measurements from inland, coastal and marine. Technical details about the FU scale algorithm, including detailed mathematical descriptions, are presented in Novoa *et al*. (2013), Van der Woerd and Wernand (2016, 2018), and Wernand *et al.* (2013).

Since 2020, Reef water type maps are defined in the MMP by grouping the FU colour classes to align with the previous classification. In addition, the water type (WT) terminology was furthermore modified to: Reef WT1, WT2, WT3, and WT4 instead of Primary, Secondary, Tertiary, and marine wet season water types in the 2021–22 Annual Report (Moran *et al.* 2023). This change was made in response to recognition that the previous terminology may be misleading and systematically implied the presence of flood plume waters, while the Reef WT1 (Primary waters) may also represent sediment resuspension in shallower parts of the Reef lagoon, and the Reef WT3 (tertiary waters) may represent oceanographic processes such as upwelling or sediment resuspension around reefs and islands. Importantly, while names of the water types changed, the definition of the water types essentially remained the same.

The characteristics of the four Reef water types are defined in Table 3-1. This classification links each water type to its typical colour, average concentrations of optically active components (TSS, CDOM, and Chl-a), water-quality indicators such as nutrient levels (Devlin et al. 2015; Petus et al., 2019), and light-attenuation conditions (Petus *et al.* 2018) measured in the Reef during the wet season. These characteristics vary the potential impact on the underlying ecological systems. In summary:

- The brownish Reef WT1 (FU ≥ 10, equivalent to Primary water type) represents turbid waters from river flood plumes, and also sediment resuspension in the shallower parts of the Reef;
- The greenish Reef WT2 (FU 6–9, equivalent to the Secondary water type) represents the less turbid parts of flood plumes enriched in Chl-*a* and fine sediment. It is usually found in the inshore to mid-shelf regions of the Reef;
- The greenish-blue Reef WT3 (FU 4–5, equivalent to the Tertiary water type) represents waters with suspended sediment concentrations slightly above ambient conditions and high light penetration typically found in the outer areas of river flood plumes. It can also represent oceanographic processes such as upwelling or the fine sediment resuspension around reefs and islands; and
- The blueish Reef WT4 (FU1–3) represents ambient waters with high light penetration and negligible concentrations of optically active water quality constituents.

Table 3-1: Description of the Sentinel-3 Reef water types (WT) and corresponding Forel-Ule (FU) colour classes (and comparison with MODIS wet season water types). Mean long-term (2004–23) concentrations of water quality parameters (± standard deviation) across the Reef water types were updated in 2023 and are indicated in the right column (modified from Petus et al., 2019).

Reef water types	FU and wet season colour classes	Description	Mean long-term (2004–23) concentrations of water quality parameters
WT1 (prev Primary)	FU ≥ 10 (WS1–4)	Brownish to brownish-green turbid waters typical of inshore regions of the Reef that receive land-based discharge and/or have high concentrations of resuspended sediments during the wet season. In flood conditions, this water type typically contains high sediment and dissolved organic matter concentrations resulting in reduced light levels. It is also enriched in coloured dissolved organic matter and phytoplankton concentrations and has elevated nutrient levels.	Secchi: 2.1 ± 2.1 m TSS: 17.4 ± 44.1 mg L^{-1} Chl-*a*: 1.5 ± 2.2 µg L^{-1}
WT2 (prev Secondary)	FU6–9 (WS5)	Greenish to greenish-blue turbid water typical of coastal waters with colour dominated by phytoplankton (Chl-*a*), but also containing dissolved organic matter and fine sediment. This water body is often found in open coastal waters of the Reef as well as in the mid-shelf where relatively high nutrient availability and increased light levels due to sedimentation favour coastal productivity (Bainbridge *et al*., 2012).	Secchi: 4.6 ± 2.8 m TSS: 4.6 ± 7.0 mg L^{-1} Chl-*a*: 0.6 ± 0.7 µg L^{-1}
WT3 (prev Tertiary)	FU4–5 (WS6)	Greenish-blue waters corresponding to waters with slightly above-ambient suspended sediment concentrations and high light penetration typical of areas towards the open ocean. This water type includes the outer areas of river flood plumes, fine sediment resuspension around reefs and islands and oceanographic processes such as upwelling. Reef WT3 waters are associated with low land-based contaminant concentrations and the ecological relevance of these waters is likely to be minimal although not well researched. The Type III areas have a low magnitude score in the Reef exposure assessment.	Secchi: 8.2 ± 4.1 m TSS: 2.3 ± 3.9 mg L^{-1} Chl-*a*: 0.4 ± 0.4 µg L^{-1}
WT4 (previously marine)	FU1–3 No number	Blueish marine waters with high light penetration.	Secchi: 11.0 ± 4.8 m TSS: 1.8 ± 3.5 mg L^{-1} Chl-*a*: 0.5 ± 0.9 µg L^{-1}

A set of scripts and automated toolboxes (Python, ArcGIS) was developed for the MMP and is used to run the FU Satellite Toolbox and process daily Sentinel-3 FU maps in a GeoTIFF format. Where multiple granules are required to cover the region of interest, the true colour GeoTIFF images are then combined using a Python script in ArcMAP 10.6.1. The Forel Ule images have a spatial resolution of 300 m × 300 m and a daily temporal resolution. They cover the entire Reef area (extreme coordinates: -10.5, -27.0, 142.3 and 154.0) and the wet season period (i.e. December to April, inclusive) since December 2020.

3.2.1.3 *Production of weekly wet season water type maps*

Weekly wet season water type composites are created to minimise the image area contaminated by dense cloud cover and intense sun glint (Álvarez-Romero *et al.* 2013). The minimum wet season or maximum FU value of each pixel/week is used to keep the colour class with the highest turbidity level for each wet season week (i.e., assuming the colour classes represented a gradient in turbidity):

- WS CC1 > CC2 > ... > CC6 or (for the MODIS imagery)
- FU22 > FU21 > FU20 > ... > FU1 (for the Sentinel imagery).

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/WT1 waters (CC 1–4 or FU ≥ 10). 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 the Marine (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, using Con (Spatial Analyst). 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 (MODIS imagery), and
- FU classes ≥ 10 in the cleaned raster are replaced with pixels of FU classes ≥ 10 in the original raster (Sentinel imagery).

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 been 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.

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

Seasonal frequency map: Weekly wet season water type composites are 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.

Reference maps: Annual frequency maps are then overlaid in ArcGIS to create multi-annual normalised frequency composites of occurrence of Reef water types. Multi-annual composites are calculated over different time frames, using the archive of MODIS-Aqua (2002–03 to 2019–20) and Sentinel-3 (2020–21 to 2022–23) water type maps. In order to combine the MODIS-Aqua and Sentinel-3 frequency composites, the MODIS frequency rasters were resampled to the same spatial resolution as the Sentinel imagery (0.00329 decimal degrees) using the Nearest Interpolation methods in ArcMAP 10.6 (Resample tool, Data Management)

Multi-annual frequency composites include: (i) a long-term period (2002–03 to 2021–22: 20 wet seasons) 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 four wettest and driest years in each NRM region. Wet and dry years are defined using the total catchment discharge in the NRM region (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. All years are in the top/bottom quartiles, total catchment discharge in the NRM region except 2005 and 2007 for Cape York which are under the long-term median.

Region	Wet years				Dry years			
Cape York	2021	2006	2011	2019	2003	2016	2005	2007
Wet Tropics	2018	2009	2019	2011	2003	2020	2015	2005
Burdekin	2019	2008	2009	2011	2015	2016	2004	2003
Mackay-Whitsunday	2012	2008	2010	2011	2004	2015	2003	2006
Fitzroy	2008	2010	2013	2011	2006	2005	2007	2004
Burnett-Mary	2012	2022	2013	2011	2021	2006	2007	2005

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) were all updated in 2023 (covering 20 years: 2002–03 to 2021–22) to ensure they remain valid as a representative period and to improve their accuracy as more satellite data becomes available. The previous 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. Weekly maps are not presented in summary Reports but are presented in full reports.

Surface exposure assessment: Frequency maps are compared with ecological health information collected through the coral reef and seagrass components of the MMP (e.g., McKenzie *et al.* 2026, Thompson *et al.* 2026) to better understand the exposure of the seagrass meadow and coral reef ecosystems to water quality conditions.

3.2.1.5 Composition of Reef water types

The classification of four Reef water types allows mapping of large Reef waterbodies with different colour characteristics and concentrations of optically active components (TSS, CDOM, and Chl-*a*), water quality indicators (e.g. nutrients levels; Devlin *et al*., 2015; Petus *et al*., 2019), and light attenuation levels (Petus *et al*., 2018) typically found in the Reef during the wet season (Table 3-1**Error! Reference source not found.**). Match-up of *in situ* concentrations of water quality parameters and the colour classes and four Reef water types are performed to validate this concept and quantify the range and average of water quality concentrations found in each Reef water type. Several water quality components are investigated including DIN, PO_4, PP, PN, TSS, Chl-*a*, CDOM, and Secchi depth.

All mean concentrations of water quality parameters were reviewed in 2023 to ensure that the water type characterisation remained appropriate, and to improve its accuracy building on the

field data that are collected every wet season. The colour class category and water type corresponding to the location and week of acquisition of each water quality sample were extracted from the archive of MODIS-Aqua (wet seasons 2003–2020) and Sentinel-3 (wet seasons 2020–2023) weekly colour class maps at the site location using the PointGeometry and/or Extract Values to Points tools in ArcGIS 10.7 (ESRI, 2019) . The R tool interpolates from the values of the 4 nearest raster cells (R Core Team, 2019) while the ArcMap tool extract cell values at the exact location (used from 2020). Weekly composites were used rather than daily colour class/water type data to minimise data loss due to the periodic dense cloud cover in the Reef. This approach maximises the incorporation of water quality parameters measured during each wet season since 2003–04 that can be associated with a Reef water type (and colour class) category.

Ideally, match-ups between satellite and in situ water quality information should be performed using field data collected ±2 hours from the satellite overpass. This is very complicated to achieve in the MMP, which is in part focused on responsive monitoring of flood events and in areas of the Reef where the cloud cover has a major influence during the wet season. The methodology above was thus selected to maximise the number of data points used to assess the water quality characteristics of each Reef water type. The limitations are considered acceptable as the mean concentrations of water quality parameters are used as a relative measure to assign a potential risk grading for each Reef water type (see below). However, the long-term average concentration values should not be used as an exact value *per se*.

The long-term concentrations of water quality parameters were calculated using all surface data (<0.2 m) collected between December and April by JCU since the 2003–04 water year, and up to April 2023. It included data collected by AIMS and the CYWP since the 2016–17 water year and covered all regions and waterbodies of the Reef, and all Reef water types. TSS and Chl-*a* data collected in mid-shelf and offshore areas as part of a Reef Trust Partnership project with the locations via the Crown of Thorns Starfish Control Program between December 2021 and April 2023 (Waterhouse *et al*., 2023) were also included. In previous assessments, long-term mean DIN, PP, and PN concentrations were calculated as: DIN = nitrite + nitrate + ammonia, PP = total phosphorus – total dissolved phosphorus, and PN = total nitrogen – DIN, respectively. In the current assessment, mean long-term direct measurements of PP and PN were used (rather than indirectly estimated values as above), and NO_x was used instead of DIN and calculated as NO_x = nitrite + nitrate due to its greater robustness than ammonia as an indicator of N availability in marine waters.

Boxplots of water quality concentration and Secchi depth are plotted against their water types and the mean long-term water quality concentrations across the three wet season water types in and across all focus regions are presented (Figure 3-3 and Figure 3-4). Additional boxplots showing water quality concentrations and Secchi depth across the former MODIS wet-season colour classes are available from previous analyses, although these have not been updated since the transition from MODIS to Sentinel-3 imagery in 2019 (Figure 3-5). Further work is needed to subdivide the current turbid WT1 category into a greater number of water types and to identify Sentinel-3 Forel–Ule colour classes that correspond to the former MODIS colour classes 1 through 4.

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. The mean long-term TSS, Chl-*a*, PP, and PN concentrations are then assessed against wet season GVs as a relative measure to assign potential risk grading for each Reef water type. Reef-wide wet season GVs were derived from De'ath and Fabricius (2008).

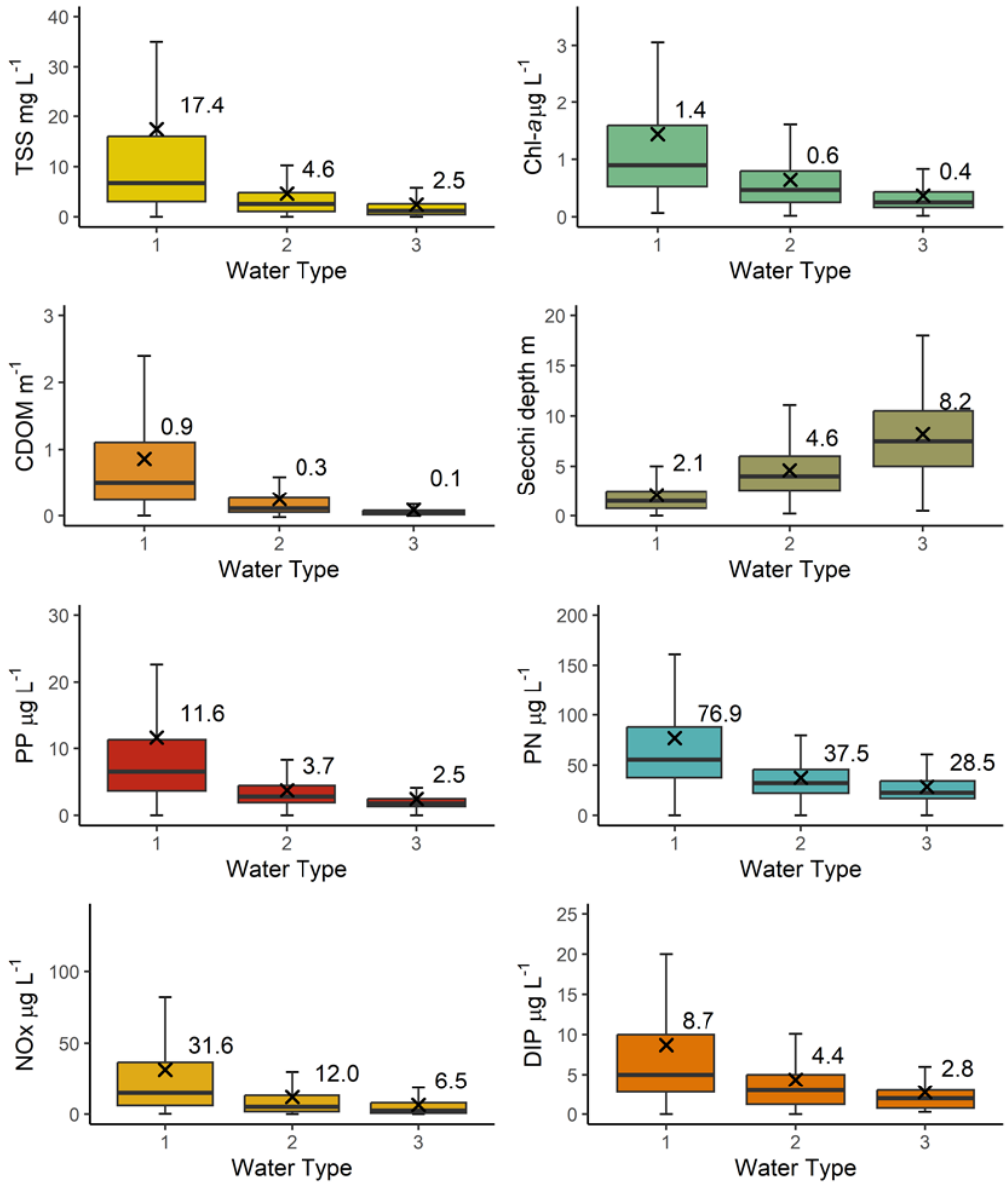

Figure 3-3: Long-term (2004–2023) concentrations of water quality parameters and Secchi depth boxplots for each Reef water type

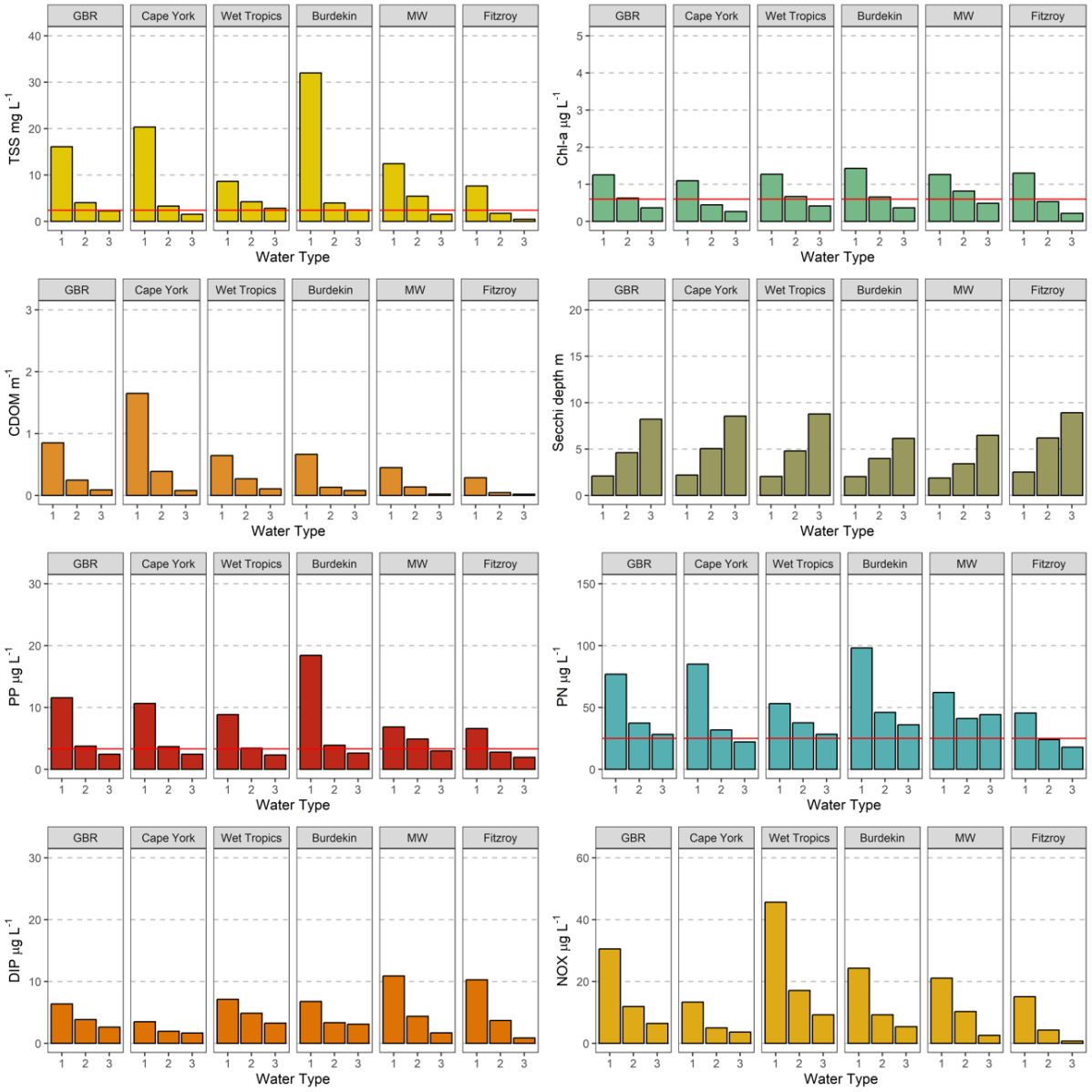

Figure 3-4: Mean long-term (2004–2023) concentrations of water quality parameters across the 3 wet season water types in all focus regions. Red lines show the Reef-wide wet season GVs. The Burdekin region has the greatest average TSS, PP, and PN concentrations in the Reef Water Type 1, which exceeded the long-term Reef-scale average. The greatest mean NOx and CDOM concentrations are measured in the Reef Water Type 1 of the Wet Tropics and Cape York regions, respectively. The greatest mean Chl-a concentrations are measured in the Reef water type 1 of the Burdekin region, but concentrations are more uniform across region. Mean long-term concentrations of water quality parameters include samples collected from the enclosed coastal water body, where high TSS, PN, and PP concentrations are likely to contribute to exceedances of the Reef-wide GVs.

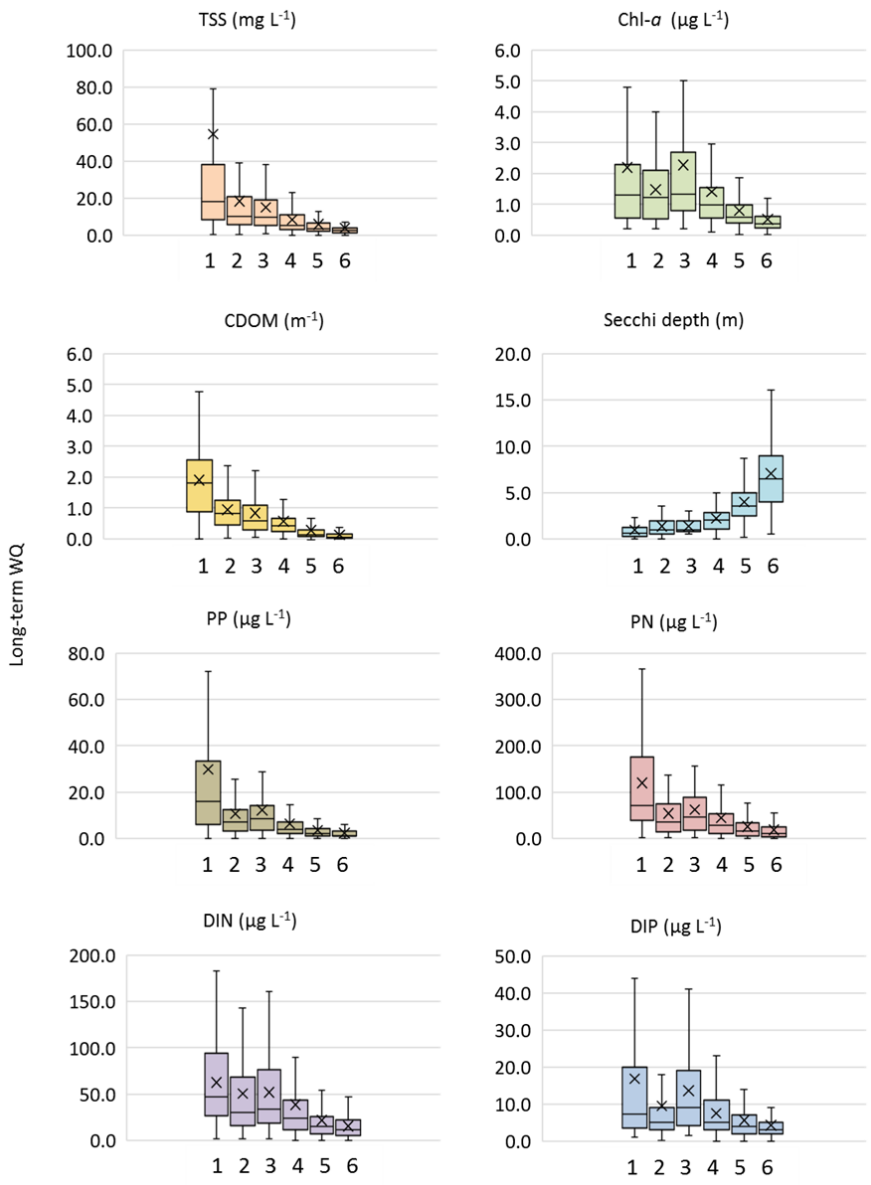

Figure 3-5: Long-term water quality concentrations and Secchi depth boxplots for each wet season colour class. The mean is plotted as a cross (x) and its numerical value is indicated. The interquartile range is delimited by the box and the median by the line inside the box. Whiskers indicate variability outside the upper and lower quartiles. Data beyond the whiskers range are considered outliers and are not plotted. Long-term 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. The last update was in 2019, using all field data available (from 2004–2019). Work is underway to update this figure.

3.2.1.6 Potential risk maps and exposure assessment

Information on the long-term water chemistry concentrations measured in the wet season colour classes (Figure 3-6Table 3-5) are compared to published water quality guideline values (Table 3-3) and, combined with frequency maps of occurrence of wet season water types, are used in a *"magnitude x likelihood"* risk management framework to develop potential risk maps (also referred to as surface exposure maps in some Reef studies). Exposure maps are produced for the whole of the Reef, for all focus regions, and over the same timeframes as those reported for the frequency maps above. Long-term exposure composites were also reviewed for this report to produce 20-year composite maps.

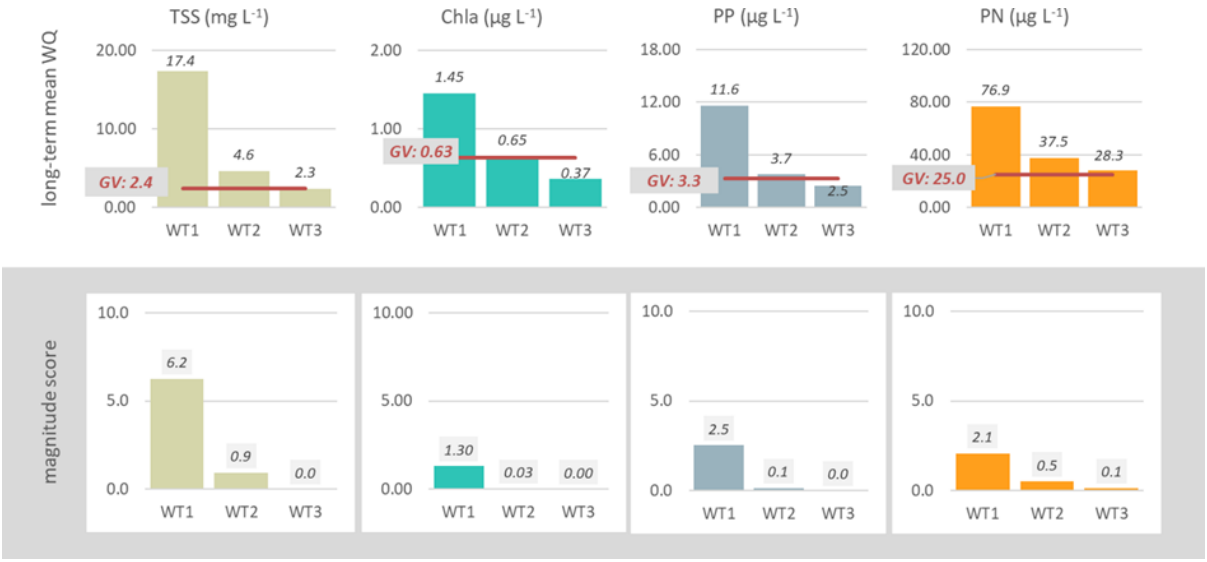

Figure 3-6: Mean long-term concentrations of water quality parameters (top) and magnitude scores across the three Reef water types (bottom). Red lines show the Reef-wide wet season GVs (Table 3-3). Magnitude scores are calculated as the proportional exceedance of the guideline: $magnitude_{water\ type} = ([Poll.]_{water\ type} - GV)/GV$ and Poll. = TSS, Chl-a, PP, or PN. Negative magnitude scores are scored as zero.

Different frameworks have been used to estimate the exposure and potential risk from exposure, (Petus *et al.* 2014b; Petus *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 'magnitude of the potential risk' corresponds to the long-term concentration of pollutants (proportional exceedance of the guideline, Table 3-3) mapped through the Reef WT1, WT2, and WT3 (Primary, Secondary, and Tertiary water types).
- The 'likelihood of the potential risk' is estimated by calculating the frequency of occurrence of each Reef water type.

The potential risk for each of the water quality parameters defined is as the proportional exceedance of the guideline multiplied by the likelihood of exposure in each of the Reef water type and calculated as below.

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

The 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 variability in the number of samples between regions and water types is primarily driven by the sampling design which was reviewed in 2014. The small number of samples in the Burnett-Mary region reflects the geographic extent of the MMP, with a majority of the samples collected by JCU in the 2011

and 2013 flood events when the design of the event monitoring was more opportunistic across the whole Reef. The relatively small number of samples in offshore waters reflects the geographic focus of the MMP design which is largely constrained to the open coastal and mid-shelf waters. The last update in the mean long-term concentrations was in the 2022–23 reporting year (Gruber *et al.* 2024), using field data collected from 2004 to 2019. Note also that the long-term and Reef-wide concentrations of water quality parameters are used rather than the seasonal and/or regional mean concentrations in water type to avoid bias due to differential regional and seasonal sampling distribution.

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 Reef WT1, WT2, or WT3 (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:

$$Poll\_\exp o = Poll\_\exp o_{WT1} + Poll\_\exp o_{WT2} + Poll\_\exp o_{WT3}$$

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 CYWP) and up to April 2023.

Region	Reef Water type	Secchi	TSS	Chl-*a*	CDOM	NO_X	DIP	PP	PN
Cape York	WT1	157	208	218	160	214	218	102	80
	WT2	225	295	301	180	301	301	188	170
	WT3	126	176	181	109	178	178	120	111
	Marine	8	13	13	4	13	13	5	4
Wet Tropics	WT1	185	406	399	388	356	356	57	58
	WT2	400	623	637	574	611	615	228	229
	WT3	203	289	296	239	273	274	143	143
	Marine	25	33	35	29	33	33	19	19
Burdekin	WT1	102	157	156	113	151	155	63	73
	WT2	202	258	260	194	258	260	99	106
	WT3	61	97	96	71	81	82	40	40
	Marine	21	33	39	23	28	29	20	19
Mackay-Whitsunday	WT1	28	45	42	43	45	45	20	20
	WT2	73	134	129	98	127	132	74	75
	WT3	20	39	39	27	33	34	27	27
	Marine	7	13	13	8	9	10	6	6
Fitzroy	WT1	22	103	104	78	105	105	17	17
	WT2	27	64	78	65	82	84	22	22
	WT3	8	20	25	11	16	17	8	8

Region	Reef Water type	Secchi	TSS	Chl-*a*	CDOM	NO_X	DIP	PP	PN
	Marine	0	6	6	1	6	6	0	0
Burnett-Mary	WT1	7	16	16	7	7	16	0	0
	WT2	5	9	9	5	5	9	0	0
	WT3	0	2	2	0	0	0	0	0
	Marine	0	8	8	1	3	3	0	0
Reef-wide	WT1	501	935	935	789	878	895	259	248
	WT2	932	1383	1414	1116	1384	1401	611	602
	WT3	418	623	639	457	581	585	338	329
	Marine	61	106	114	66	92	94	50	48

The overall exposure scores are then grouped into four potential risk 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 (20-year composite: 2003–2022), because this map presented the highest number of observations. Using the 2003–2022 mean exposure map, categories were defined as [>0–0.9] = cat. I, [0.9–3.5] = cat. II, [3.2–7.9] = cat III and >7.9 = 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 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 water quality 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 (using the Sentinel-3 FU imagery), over the long-term (2002–03 to 2021–22: 20 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) were all updated in 2023 to ensure they remain valid as a representative period and to improve their accuracy as more satellite data are available. The previous 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 Reef region, Reef waterbodies, 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 the current year and in the long-term is also calculated how current conditions deviate from the long-term average. Processed data are stored in external Medias and in Dropbox.

The marine boundaries used for the Marine Park are presented in Figure 3-7 along with NRM regions, the Reef waterbodies and the mapped 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.

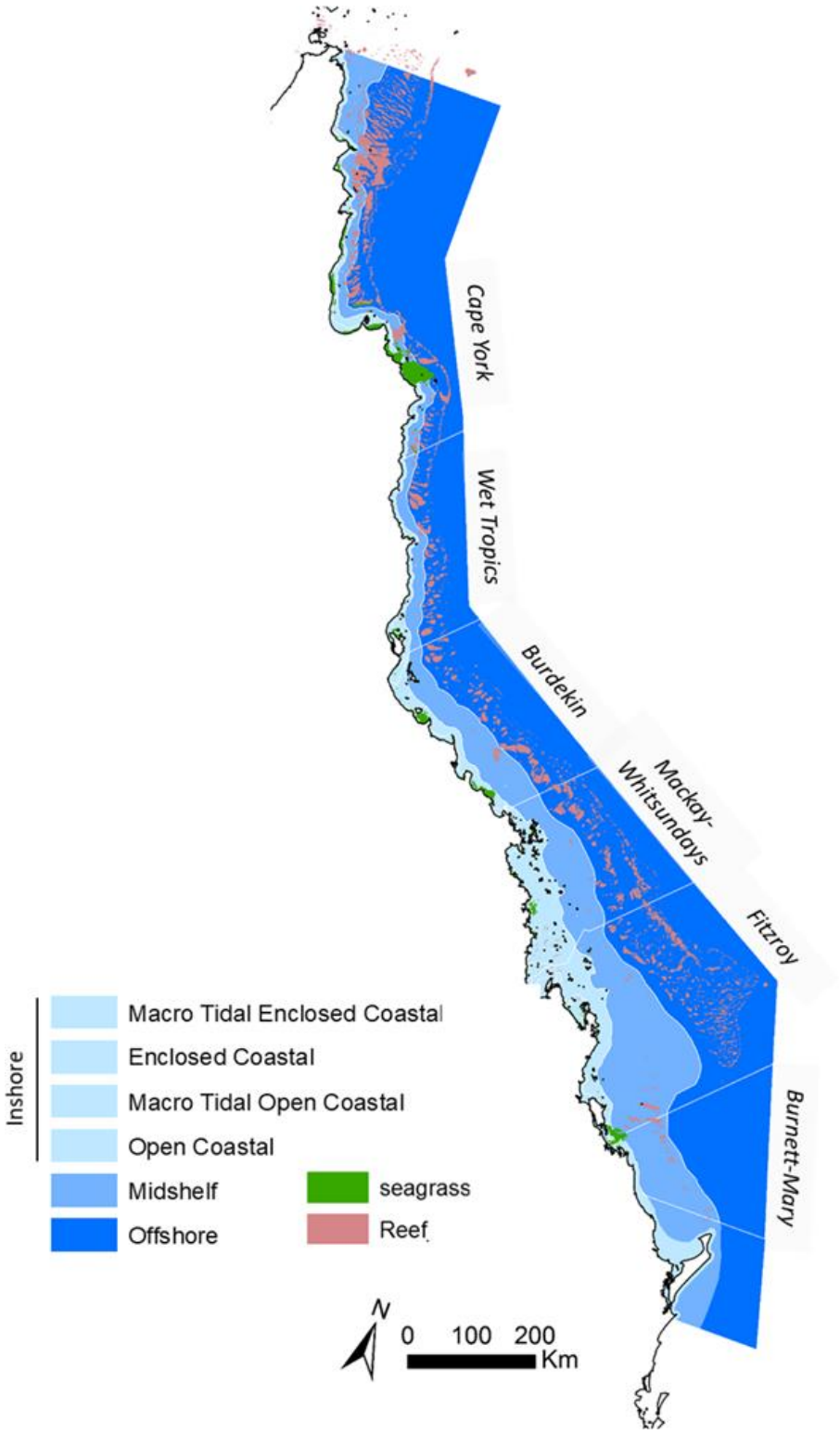

Figure 3-7: Boundaries used for the Marine Park, each NRM region, and the coral reefs and seagrass ecosystems. Coral reef and NRM layers derived from the Reef Authority, supplied 2013. Seagrass layer is a composite of surveys conducted by Department of Agriculture and Fisheries, QLD.

3.2.2 Load mapping

Surface loading maps representing the dispersion of end-of-catchment pollutant loads (DIN, TSS, and PN) into the Reef have been developed as part of the MMP since 2003 (initially

produced using the method described in Gruber *et al*. 2019). In 2018–19, a revised approach was developed using the eReefs marine models (Margvelashvili *et al.* 2018; Skerratt *et al.* 2019; Steven *et al.* 2019).

3.2.2.1 End-of-catchment loads

End-of-catchment loads are calculated using a combination of river flow and river concentration data.

River flow is reported annually and can be derived from several sources. In many cases, river flow gauges that measure discharge (and used to calculate constituent loads) are located well upstream of the river mouth and only capture a certain proportion of the catchment/basin area. Such disparities mean that river gauge data should not be directly compared across basins and NRM regions. For example, the Daintree and Barron Basins within the Wet Tropics region contain a similar area (2,100–2,200 km^2); however, the Daintree River at Bairds and the Bloomfield River at China Camp gauges collectively only measure 56% of the Daintree Basin whereas the Barron River at Myola gauge captures 89% of the Barron Basin. If gauge data are used to compare discharge between these basins, the gauge on the Barron Basin is covering a much larger proportion of the area compared to the gauges on the Daintree Basin. A scaling factor is used on these data so that discharge (and constituent loads) can be directly compared across basins and NRM regions.

To account for these differences, the relevant discharge data for each basin were compiled, where available (Table 3-5; Department of Local Government, Water and Volunteers Water Monitoring Information Portal [WMIP], 2025). The total annual discharge for each gauge was then up-scaled using the recommended scaling factors outlined in Puignou Lopez *et al*. (2025). Briefly, this work examined different upscale factors based on basin area to total gauged area, mean annual flow for the gauged basin and the mean annual basin flow from either the Bureau of Meteorology's G2G model (BoM, 2017; Wells *et al.* 2018) or the Source Catchments model (McCloskey *et al.* 2021) or the linear relationships between river gauge data and the two models; the most appropriate upscale factor was then recommended for each basin (Puignou Lopez *et al*., 2025). Where a flow gauge did not exist in a basin (e.g. Jacky Jacky Creek, Lockhart River, Jeannie River, Proserpine River, Styx River, Shoalwater Creek and Boyne River—marked with an asterisk), the gauge from the nearest neighbouring basin was used. The calculation of the long-term medians for each basin has been anchored to cover the 30-year period from the 1990–91 to 2019–20 water years.

Table 3-5: The 35 basins of the Reef catchment, the gauges for each Basin, and the correction factors used to upscale flows to provide annual discharge estimates. (AWRC = Australian Water Resources Council).

NRM Region	Basin	AWRC No.	Basin area (km^2)	Relevant gauges	% of Basin covered by key gauges	Correction factor
Cape York	Jacky Jacky Creek	101	2,963	Jardine River at Monument*	0	1.1x + 560,000
	Olive Pascoe River	102	4,180	Pascoe River at Garraway Creek	31	3.1
	Lockhart River	103	2,883	Pascoe River at Garraway Creek*	0	1.5
	Stewart River	104	2,743	Stewart River at Telegraph Road	17	5.6
	Normanby River	105	24,399	Normanby River at Kalpowar Crossing + Hann River at Sandy Creek (from 2005/06). Previous upscale period uses Normanby at Battle Camp + Hann River gauges with factor of 4.7	53	1.8

NRM Region	Basin	AWRC No.	Basin area (km^2)	Relevant gauges	% of Basin covered by key gauges	Correction factor
	Jeannie River	106	3,638	Endeavour River at Flaggy + Annan at Beesbike	0	3.2
	Endeavour River	107	2,182	Endeavour River at Flaggy + Annan at Beesbike	27	3.5x + 21,000
Wet Tropics	Daintree River	108	2,107	Daintree River at Bairds + Bloomfield River at China Camp	56	1.6
	Mossman River	109	473	Mossman River at Mossman	22	2.3
	Barron River	110	2,188	Barron River at Myola	89	1.3
	Mulgrave-Russell River	111	1,983	Mulgrave River at Peets Bridge + Russell River at Bucklands	42	2.0x + 450,000
	Johnstone River	112	2,325	South Johnstone River at Upstream Central Mill + North Johnstone at Tung Oil	57	1.6x + 540,000
	Tully River	113	1,683	Tully River at Euramo	86	1.1
	Murray River	114	1,107	Murray River at Upper Murray	14	5.0x + 600,000
	Herbert River	116	9,844	Herbert River at Ingham	87	1.2
Burdekin	Black River	117	1,057	Black River at Bruce Highway + Bluewater Creek at Bluewater	32	3.1
	Ross River	118	1,707	Ross River at Aplins Weir + Alligator Creek at Allendale (from 2001–02). Previous upscale period uses Ross River Dam HW + Bohle at Hervey Range Rd + Alligator Creek with factor of 1.6x + 75,000	52	1.9
	Haughton River	119	4,051	Haughton River at Powerline + Barratta at Northcote	62	1.6
	Burdekin River	120	130,120	Burdekin River at Clare	100	1.0
	Don River	121	3,736	Don River at Reeves + Elliot River at Guthalungra + Euri Creek at Koonandah (from 1999/00). Previous upscale period uses Don + Elliot gauges with factor of 2.9x + 170,000	46	1.5x + 210,000
Mackay-Whitsunday	Proserpine River	122	2,494	O'Connell River at Staffords Crossing + Andromache River at Jochheims + St Helens Creek at Calen	0	3.6
	O'Connell River	124	2,387	O'Connell River at Staffords Crossing + Andromache River at Jochheims + St Helens Creek at Calen	29	3.5
	Pioneer River	125	1,572	Pioneer River at Dumbleton Weir TW	95	1.1
	Plane Creek	126	2,539	Sandy Creek at Homebush + Carmila Creek at Carmila	16	5.6x + 210,000
Fitzroy	Styx River	127	3,013	Waterpark Creek at Byfield*	0	5.7x + 260,000
	Shoalwater Creek	128	3,601	Waterpark Creek at Byfield*	0	6.6x + 300,000

NRM Region	Basin	AWRC No.	Basin area (km^2)	Relevant gauges	% of Basin covered by key gauges	Correction factor
	Water Park Creek	129	1,836	Waterpark Creek at Byfield	12	5.4x + 43,000
	Fitzroy River	130	142,552	Fitzroy River at The Gap	95	1.1
	Calliope River	132	2,241	Calliope River at Castlehope	57	1.9x + 95,000
	Boyne River	133	2,496	Calliope River at Castlehope*	0	2.1
Burnett-Mary	Baffle Creek	134	4,085	Baffle Creek at Mimdale	34	2.4x + 95,000
	Kolan River	135	2,901	Kolan River at Springfield + Gin Gin Creek at Brushy Creek	37	2.4x + 19,000
	Burnett River	136	33,207	Burnett River at Figtree Ck (from 1996/97). Previous upscale period uses Burnett River at Mount Lawless with factor of 1.2x + 84,000	92	1.1
	Burrum River	137	3,362	Gregory River at Leesons + Elliott River at Dr Mays Crossing + Isis River at Bruce Highway	40	3.0x + 27,000
	Mary River	138	9,466	Mary River at Home Park	72	1.4
* Gauges used which are not in the basin area						

Current annual and pre-development TSS, DIN, and PN load estimates are calculated for all basins using a systematic approach as annual load estimates from the Paddock to Reef program are not available in time for MMP reporting. The pre-development loads represent a modelled period before 1850 when Europeans first arrived in the Reef catchment – the hydrology is kept the same, but the catchment is modelled as a pristine landscape (see McCloskey *et al.* 2021). These species of nitrogen are the most labile (readily used or converted to useable forms for uptake by phytoplankton and macroalgae) and are thus the most biologically relevant in the Reef lagoon (Great Barrier Reef Marine Park Authority, 2010). The DIN loads for the basins of the Wet Tropics and Haughton Basin are calculated using the model originally developed in Lewis *et al*. (2014) which used a combination of the annual nitrogen fertiliser applied in each basin coupled with basin discharge; a relationship is established with measured loads to calculate the average percentage of fertiliser lost as DIN (calculated as per previous description). DIN loads for the Burdekin, Pioneer and Fitzroy basins are taken from those measured in the Great Barrier Reef Catchment Loads Monitoring Program. If the measured data for the most recent years in these basins are unavailable, a mean of the long-term annual mean concentration from the previous monitoring data are coupled with the annual discharge to calculate a load. DIN loads for the remaining basins are calculated using an annual mean concentration which is multiplied by the corresponding annual basin discharge calculations. The annual mean concentration for each basin is informed using a combination of available monitoring data and Source Catchments model outputs. The pre-development DIN loads are calculated using a combination of the estimates from the Source Catchments model as well as available monitoring data from 'pristine' locations (Lewis *et al*. 2023).

The TSS and PN loads are similarly determined through a step-wise process. For the basins where the GBR Catchment Loads Monitoring Program captures >95% of the basin area (e.g. Burdekin, Pioneer, and Fitzroy) the measured/reported TSS and PN loads are used. If the measured data for the most recent years are unavailable, a mean of the long-term annual mean concentration from the previous monitoring data is coupled with the annual discharge

to calculate a load. For other basins with monitoring data, the range of annual mean concentrations are compiled and compared with the latest Source Catchment modelling values. From these data a 'best estimate' of an annual mean concentration is produced and applied with the annual discharge data to calculate loads. Finally, for the basins that have little to no monitoring data, the annual mean concentration from the Source Catchments data is examined along with nearest neighbour monitoring data to determine a 'best estimate' concentration to produce the load. The pre-development TSS and PN loads were calculated using a combination of the annual mean concentrations from the Source Catchments model and available monitoring data from 'pristine' locations. The corresponding discharge was used (as calculated previously) to produce a simulation of the pre-development load for the water year.

3.2.2.2 Loading maps

The loading maps were initially produced using the relatively simple method described in Gruber *et al.*, (2018) which had several limitations related to the influence of variable hydrodynamics, and environmental conditions including wind, tides and currents. In 2018–19 a revised approach was developed using the eReefs GBR1 model (1 km resolution) (Margvelashvili *et al.* 2018; Skerratt *et al*., 2019; Steven *et al*. 2019) to estimate river dispersion, providing a much-improved representation of hydrodynamics and environmental conditions. In keeping with the principle of continuous improvement, the method was further refined in 2024–25 to incorporate the latest eReefs marine model capability(https://marlin.csiro.au/geonetwork/srv/eng/catalog.search#/metadata/dcc8462c-8dc5-4d78-b9d8-6f1c31ae0001), using the results of a hindcast run of a newly updated version 4.0 of the GBR4 Hydrodynamic model (4 km resolution) due to the timing of several major model improvements. For the GBR4 model, recent enhancements include a better representation of shelf-break currents and upwellings, updated heat penetration, and importantly, greater representation of freshwater inputs along the GBR coast, increasing from 17 rivers to 54 rivers.

River discharge footprints are modelled using a conservative tracer in GBR4 hindcast version H4p0 (Maggiorano *et al.*, 2025) from November 2022 to April 2025. The hydrodynamic model was forced with atmospheric and ocean reanalysis: Surface Atmospheric Data from the Bureau of Atmospheric high resolution Regional Reanalysis for Australia, using Data Assimilation of Quality Control observations (BARRA R2, Su *et al*. 2019), ocean boundaries from the CSIRO Bluelink Reanalysis using Data Assimilation of Quality Control observations (BRAN2023, Chamberlain *et al*. 2021). Tides are specified using tidal harmonics from the global TPXO tide model (Egbert and Erofeeva, 2002) and stream flow for 64 rivers come from a combination of the GBR dynamic SedNet model (McCloskey *et al*. 2021) and flow gauges. River temperature is derived from the BARRA surface air temperature using a low-pass filter. The daily files, containing individual river tracer plumes available on NCI (ih54), were used to estimate the cumulative sum of each river tracer concentration per year from the beginning of the wet season (i.e. 1 November to 30 October the following year). Tracer concentrations are calculated based on the available model output data for the period.

The cumulative exposure index integrates the tracer concentration above a defined threshold (1%). It is a cumulative measurement of the exposure concentration and duration of exposure to dissolved inputs from individual river sources. It is expressed as Concentration × Days (Conc.Days). For example, if a grid cell was exposed to concentrations of 5% river water for 2 days, this gives an exposure index of 0.1 (0.05 x 2). If a grid cell was exposed to concentrations of 50% river water for 10 days, this gives an exposure index of 5 (0.5 x 10). Whenever river water concentration is greater than 1%, the exposure index is calculated and added to all other exposures in that wet season (i.e., it is cumulative). This index provides a consistent approach to assessing relative differences in exposure of Reef shelf waters to inputs from the 54 rivers.

The mathematical formulation that expresses this concept is given below:

$$\text{Conc.Days} = \sum_{t=0}^{T} \text{Conc}_{exceedance} * \text{t}$$

where,

$$\text{Conc}_{exceedance} = \begin{cases} \text{Conc(t) - Conc}_{threshold}, & \text{where Conc(t)} > \text{Conc}_{threshold} \\ 0, & \text{where Conc(t)} \leq \text{Conc}_{threshold} \end{cases}$$

and $\text{Conc}_{threshold}$ is defined here as 1% of the river concentration, Conc(t) represents the time-varying tracer concentration, and t is time in days from the beginning of the wet season (t_0 = 1 October) and $T_{\text{end of wet season}}$ = 1 May. Cumulative exposure is calculated for each grid point in the model domain.

In this step, the end-of-catchment load for fine sediment, DIN, or PN is dispersed for each river assuming a direct relationship between pollutant and tracer concentration (conservative mixing). Thus, the surface load of fine sediment, DIN, or PN per km^2 was 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 was calculated by summing the surface load outputs for the 54 rivers for which tracer data were available. This includes all 35 major drainage basins in the Reef catchment, with an additional 19 smaller rivers and streams (Table 3-6).

Table 3-6: eReefs model for the loading maps: Grouping of rivers into the Great Barrier Reef 35 major basins. Notes: [1]: eReefs phase 4 (2011 to 2016) rivers in bold, all others are eReefs phase 5 rivers (this reports version: 2011 to 2022). In eReefs phase 4 these rivers were entered as river fluxes (loads without freshwater flow). All loads for rivers/basins for the eReefs marine model are supplied by GBR Dynamic SedNet model.

NRM Region	35 GBR basins with freshwater flow[1]	Additional rivers with freshwater flow in eReefs phase 5 (2011–2022) with flows reflecting affiliation to one of the 35 basin catchment parents
Cape York	Jacky Jacky	Jacky Jacky
	Olive-Pascoe	Olive
		Pascoe
	Lockhart	Lockhart
	Stewart	Stewart
	Normanby	North Kennedy
		Normanby
	Jeannie	Jeannie
	Endeavour	Endeavour
		Annan
Wet Tropics	**Daintree**	Bloomfield
		Daintree
	Mossman	Mossman
	Barron	Barron
	Mulgrave Russell	Mulgrave Russell
	Johnstone	Liverpool
		Johnstone
	Tully	Tully
	Murray	Murray
		Meunga

	Herbert	Herbert
Burdekin	Black	Bluewater
		Black
	Ross	Bohle
		Ross
		Alligator
	Haughton	Haughton
		Barratta
	Burdekin	Burdekin
	Don	Elliot
		Euri
		Don
Mackay-Whitsunday	Proserpine	Gregory
		Proserpine
	OConnell	O'Connell
		St Helens
		Constant
	Pioneer	Pioneer
	Plane	Sandy
		Plane
		Carmila
Fitzroy	Styx	Styx
	Shoalwater	Shoalwater
	Waterpark	Waterpark
	Fitzroy	Fitzroy
	Calliope	Calliope
	Boyne	Boyne
Burnett-Mary	Baffle	Baffle
	Kolan	Kolan
	Burnett	Burnett
	Burrum	Eliott
		Burrum
	Mary	Mary

The difference between the estimated wet season fine sediment, DIN, and PN loadings (tonnes km^2) in the Reef lagoon for the current water year was calculated and compared to the pre-development loads derived from the Source Catchments model (which have a degree of uncertainty; refer to McCloskey *et al.* 2021). This can be interpreted as 'anthropogenic' fine sediment, DIN, or PN loadings, highlighting the areas of greatest change with current land use characteristics.

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 $sampler^{-1}$). 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 systems utilised include:

- **Styrene divinyl benzene reverse phase sulfonate (SDB-RPS) Empore™ 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; and
- **Polydimethylsiloxane (PDMS) passive samplers**, used to detect more hydrophobic pesticides such as propiconazole, pendimethalin, chlorpyriphos and trifluralin) have been used during previous monitoring years, but were not deployed in the 2024–25 wet season.

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). Following a review of the program in 2019, 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 2020–21, a total of 3 high risk sites in the Mackay-Whitsundays (Repulse Bay, Flat Top Island and Sandy Creek) were selected for wet season monitoring. For the 2023–24 wet season, ten locations were routinely monitored. This was reduced to 8 sites during the 2024–25 wet season due to site access issues. Passive samplers were deployed monthly during the wet season. Grab samples were taken during each passive sampler deployment/retrieval as a complementary sampling technique. Additional grab samples were also taken during flood events to capture pesticide loads being transported to inshore Reef locations via floodwaters.

In addition to the scientific requirements of the project, the selection of passive sampling deployment sites is governed by practicalities, which include safety, security, availability of sites and site access. Site establishment is ultimately determined by the Reef Authority.

4.2.2 Target pesticides

The chemicals targeted for analysis and the limits of quantitation (LOQ) for ED and grab samples are indicated in Table 4-1. This list of target chemicals was derived through consultation with the Reef 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
- likelihood of accumulation in one of the passive samplers (exist as neutral species in the environment)
- are included in complementary Reef 2050 Water Quality Improvement Plan programs such as end-of-catchment loads monitoring.

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.

Table 4-1: List of Targeted Pesticides with corresponding limit of quantitation (LOQ) for both Empore disk (ED) and grab water samples analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Chemical	Description	Priority or of interest	LC-MS/MS	
			ED LOQ ng sampler^{-1}	Grab LOQ ng sample^{-1}
2, 4-D	Phenoxy-carboxylic-acid herbicide	Priority	5.0	0.84
Ametryn	PSII herbicide – methylthiotriazine	Priority	5.0	0.36
Atrazine	PSII herbicide – chlorotriazine	Priority	1.0	0.76
Atrazine – desethyl	PSII herbicide breakdown product (also active)	Priority	1.0	1.0
Atrazine – desisopropyl	PSII herbicide breakdown product (also active)	Priority	1.0	1.1
Bromacil	PSII herbicide – uracil	Of interest	1.0	1.2
Diuron	PSII herbicide – phenylurea	Priority	0.5	0.74
Fluazifop	Herbicide: inhibition of acetyl CoA carboxylase	Of interest	0.1	0.9
Fluometuron	PSII herbicide – urea	Of interest	1.0	0.6
Fluroxypyr	Pyridine carboxylic acid herbicide	Priority	1.0	2.0
Haloxyfop	Aryloxyphenoxy-propionate herbicide	Priority	1.0	1.0
Hexazinone	PSII herbicide – triazinone	Priority	1.0	0.96
Imazapic	Imidazolinone herbicide	Priority	1.0	10
Imidacloprid	Neonicotinoid insecticide	Priority	1.0	1.1
MCPA	Phenoxy-carboxylic-acid herbicide	Priority	5.0	1.1
Metolachlor	Chloracetanilide herbicide	Priority	1.0	0.92
Metribuzin	PSII herbicide – triazinone	Priority	1.0	1.2
Metsulfuron methyl	Sulfonylurea herbicide	Priority	1.0	1.7
Prometryn	PSII herbicide – methylthiotriazine	Priority	1.0	1.2

Chemical	Description	Priority or of interest	LC-MS/MS	
			ED LOQ ng sampler^{-1}	Grab LOQ ng sample^{-1}
Propazine	PSII herbicide – chlorotriazine		1.0	1.1
Simazine	PSII herbicide – chlorotriazine	Priority	1.0	1.1
Tebuconazole	Conazole fungicide	Priority	1.0	0.64
Tebuthiuron	PSII herbicide – thiadazolurea	Priority	1.0	0.22
Terbuthylazine	PSII herbicide – triazine	Priority	1.0	1.0
Terbutryn	PSII herbicide – triazine	Of interest	5.0	0.92

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.

4.2.3 Chemical analysis

From the 2014–2015 monitoring year onwards, the analysis of ED extracts and grab sample extracts at QAEHS was transferred to the AB Sciex QTRAP 6500, a new liquid chromatography-mass spectrometry (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). LOQs of the target analytes were not negatively influenced by the change in instrumentation.

The limits of detection (LOD) and LOQs for the LC-MS/MS 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 seven 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 ten is applied to get the limit of quantitation (LOQ). The LOQ is equivalent to the limit of reporting (LOR), unless analytes are detected in blank samplers, which raises the LOR. Grab LOQs presented in Table 4-1 are based on a sample volume of 500 mL. ED LOQs are shown as ng sampler^{-1}. The reporting limits for water concentrations determined using the passive samplers 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 disks:

- 3M™ Empore™ Extraction Disks (SDB-RPS) – Phenomenex.
- Deployed in a custom-made 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 approximately one month. From January 2012 onwards, Phenomenex membranes of the same specifications have been used.

Preparation:

- Disks are washed in acetone and isopropanol.
- Disks are conditioned first with methanol, then Milli-Q water, using a vacuum manifold.
- Disks are loaded into acetone rinsed Teflon Chemcatcher housing.
- Disks are covered with membrane and solvent rinsed wire mesh.
- Chemcatcher housing is filled with Milli-Q water.
- Chemcatcher samplers are sealed packaged for transport and stored at ~4°C
- Chemcatcher samplers are transported via insulated bags filled 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.

- Concentrate to 1 mL using evaporation under purified N_2 then centrifuge to separate any particles in extract.
- Supernatant is transferred to a clean tube and water added.
- Sample is then extracted with solid phase extraction (SPE; Strata-X polymeric reversed phase cartridges, Phenomenex)
- Cartridges are dried and eluted with methanol and acetone.
- Extracts are concentrated to 0.1 mL, then 0.4 mL 20% methanol in Milli-Q water is added.
- Extracts are filtered (0.22 µm PFTE) into HPLC vials.
- 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 (left) and an assembled housing ready for deployment (right)

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.* 2011a). 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.* 2011b).

Figure 4-2: Passive flow monitors pre-deployment (left) and post-deployment (right)

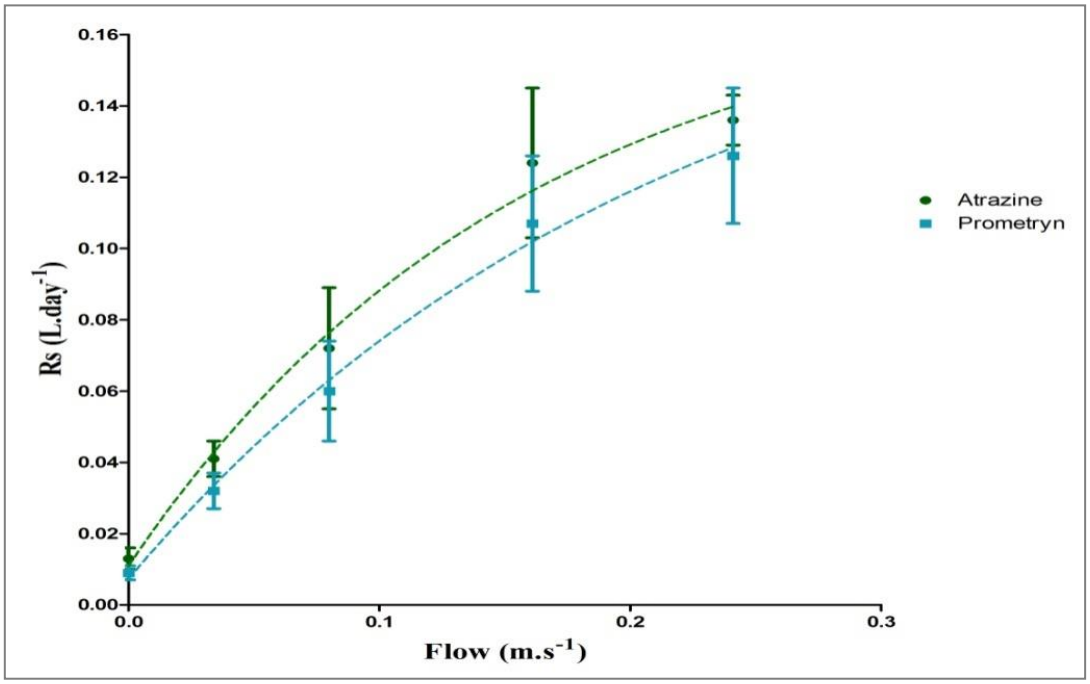

Figure 4-3: Relationship between flow and sampling rates of specific herbicides indicating a shift from aqueous boundary layer control to diffusion limiting membrane control under higher flow conditions

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.* 2011b; Vermeirssen *et al.* 2009; Stephens *et al.* 2009; Shaw and Mueller 2005). 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 mass (in ng) accumulated per sampler per day. Chemicals for which the R_s of atrazine are assumed are highlighted red in Table 4-1. An in-field calibration would be beneficial in determining R_s for the newly included chemicals.

Water concentration is estimated from passive sampler data using Equation 1, and incorporating the site-specific sampling rates derived from PFMs.

Equation 1: Estimation of water concentration based on linear phase sampling.

$$C_W = \frac{C_S \, x \, M_S}{R_S \, x \, t} = \frac{N_S}{R_S x \, t}$$

Where:

C_W = the concentration of the compound in water (ng L^{-1})
C_S = the concentration of the compound in the sampler (ng g^{-1})
M_S = the mass of the sampler (g)
N_S = the amount of compound accumulated by the sampler (ng)
R_S = the sampling rate (L day^{-1})
t = the time deployed (days)

4.2.5 Grab sample extraction

Grab samples were prepared and extracted according to established SOPs and previously published procedures and methods described in Kaserzon *et al.* (2014).

- Hydrophilic-lipophilic balanced solid phase extraction (SPE) cartridges (Strata X, Phenomenex) were placed on a vacuum manifold and conditioned with methanol, then Milli-Q water.
- Water samples were spiked with labelled internal standard mix then loaded on to SPE cartridges
- Sample bottles and cartridges were rinsed with MQ water after sample loading
- SPE cartridges were dried under vacuum, then eluted with methanol
- Extracts were evaporated under a gentle stream of nitrogen, then MQ water added to reach a final methanol:water ratio of 20:80
- Samples were passed through a 0.2 µm PTFE syringe filter into HPLC vials
- Analyse using LC-MS/MS

4.2.6 Risk assessment metrics

For the 2024–25 monitoring period, the pesticide risk metric (PRM) was calculated and applied to the estimated pesticide water concentrations (Warne *et al*., 2023). The PRM uses 22 pesticides (nine PSII herbicides, ten ‘other’ herbicides and three insecticides) to calculate the combined risk to aquatic species, expressed as the average percentage of species affected. These pesticides (2,4-D, ametryn, atrazine, chlorpyrifos, diuron, fipronil, fluroxypyr, haloxyfop, hexazinone, imazapic, imidacloprid, isoxaflutole, MCPA, metribuzin, metolachlor, metsulfuron-methyl, pendimethalin, prometryn, simazine, tebuthiuron, terbuthylazine, triclopyr) were chosen due to their previous detection in GBR waterways, and because they had species sensitivity distributions (SSDs) available. SSDs were combined with the multisubstance-potentially affected fraction **(**msPAF) method, Independent Action model of joint toxicity and Multiple imputation method to calculate the PRM.

The multisubstance-potentially affected fraction msPAF 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 2050 Water Quality Improvement Plan.

The msPAF 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 modelled percentages of species that will be affected (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 concentrations and half maximal effective concentrations.

4.2.7 QA/QC procedures in the pesticide monitoring program

The methods described above have been developed as a result of this work in collaboration with analytical method development. These methods are formalized as Standard Operating Procedures (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:

- Internally approved and reviewed SOPs for the preparation, deployment, extraction, and analysis of passive samplers.
- Staff training in relevant SOPs (laboratory) and maintaining staff training records.
- 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.
- Participation in interlaboratory studies, cross-checking a selection of samples with Queensland Health Forensic and Scientific Services (QHFSS) and routine participation in the international passive sampling proficiency studies run via the Dutch QUASIMEME organisation.

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, chemical analysis, analytical results, data processing, storage and access. This protocol may be summarised as:

- The unique alphanumeric identifier code attached to each sample 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.
- LC-MS/MS results files with a unique identifier code are transferred from the instrumentation computer to the UQ QAEHS server.
- Excel spreadsheets used for data processing and a summary results file (concentration in water estimates) are stored on the UQ QAEHS secured 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.

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, exposure to freshwater flood plumes, high water temperatures causing coral bleaching or elevated populations of 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 acute 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 includes 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.

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).

A broad survey 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 replicated at 2 m and 5 m depths below the zero tide datum. Within each site and depth, fine scale spatial variability is accounted for by the use of five replicate 20 m long transects.

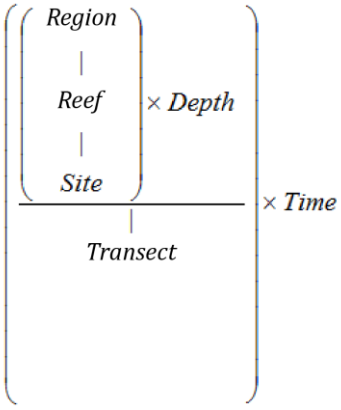

Figure 5-1: Sampling design for coral reef benthic community monitoring. Terms within brackets are nested within the term appearing above.

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, Middle Reef removed from the program as this was also a site monitored by the AIMS Long-term Monitoring Program, and all reefs scheduled for sampling biennially, half surveyed in ‘odd-years’ and half surveyed in ‘even-years’. Importantly, when an acute disturbance was suspected of having impacted survey reefs during the preceding summer, the biennial design allowed 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 2021 the program returned to an annual frequency of monitoring for all reefs and Peak Island was removed from the program on the basis of not having substantive development of a carbonate substrate. 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 since 2021 is presented in Figure 5-2.

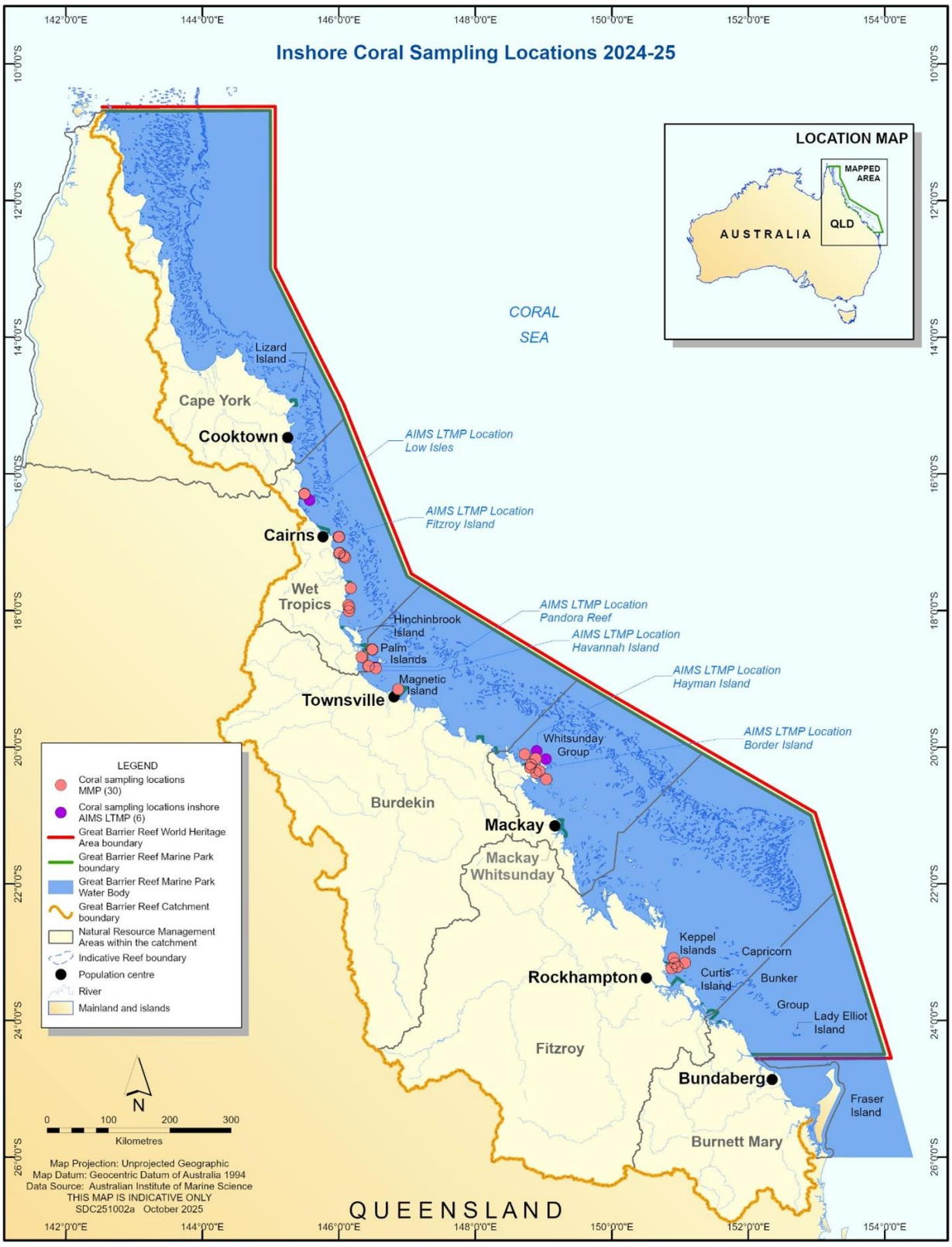

Figure 5-2: Coral monitoring sites as of 2021.

Table 5-1: Scheduled coral monitoring sampling design for the MMP. 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 B1.

NRM Region	Catchment	Monitoring location	2005 to 2006	2007to 2014	2015 to 2020	Since 2021
Wet Tropics	Barron Daintree	*Low Isles*	O	O	O	A
		Snapper North	A	A	O	A
		Snapper South	A	A	E	A
	Russell / Mulgrave Johnstone	*Green Island*	O	O	O	
		Fitzroy East	A	E	E	A
		Fitzroy West	A	A	O	A
		Fitzroy West	O	O	O	A
		Frankland East	A	O	O	A
		Frankland West	A	A	E	A
		High East	A	O	O	A
		High West	A	A	E	A
	Herbert Tully	Barnards	A	O	O	A
		Bedarra			A	A
		Dunk North	A	A	E	A
		Dunk South	A	E	E	A
		King	A	O		
Burdekin	Burdekin	Havannah Island	A	O	O	A
		Havannah Island (North)	O	O	O	A
		Lady Elliot	A	E	E	A
		Magnetic	A	A	O	A
		Middle Reef	O	O	O	
		Palms East	A	E	E	A
		Palms West	A	A	O	A
		Pandora	A	A	E	A
		Pandora (North)	O	O	O	A
Mackay Whitsunday	Proserpine	*Border*	O	O	O	A
		Daydream	A	A	E	A
		Dent	A	E	E	A
		Double Cone	A	A	E	A
		Hayman	O	O	O	A
		Hook	A	E	E	A

NRM Region	Catchment	Monitoring location	2005 to 2006	2007to 2014	2015 to 2020	Since 2021
		Langford	**O**	**O**	**O**	
		Pine	**A**	**A**	**O**	**A**
		Seaforth	**A**	**O**	**O**	**A**
		Shute	**A**	**O**	**O**	**A**
Fitzroy	Fitzroy	Barren	**A**	**A**	**O**	**A**
		Keppels South	**A**	**A**	**E**	**A**
		Middle	**A**	**E**	**E**	**A**
		North Keppel	**A**	**O**	**O**	**A**
		Peak	**A**	**A**	**O**	
		Pelican	**A**	**E**	**E**	**A**

A, Reefs designated as 'core reefs' and sampled annually, prior to 2015 or from 2021
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

5.2.3 Depth selection

From a broad-scale survey 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 zero tide datum because coral communities rapidly diminish below this depth at many reefs. Two metres below zero tide 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 5–7 metres below zero tide datum and reported along with the 5 m depths of the MMP.

5.2.4 Field survey methods

Site marking

Each 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 metre or two metre depth contours. Digital depth gauges were 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) and waypoints maintained in the AIMS database.

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 Table 5-1.

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 35 cm wide images at 50 cm intervals along upslope side of transect from which 160 points are sampled	Full sampling design
Juvenile counts *	2005–06 Size structure of coral communities, recorded into: 0–2 cm, >2–5 cm, >5–10 cm, >10–20 cm, >20–50 cm, >50–100 cm and >100 cm categories	Colonies intersecting a 34 cm belt along the upslope side of each transect	First 10 metres of each transect
	2007–2018, density of juvenile corals in size classes: 0–2 cm, >2–5 cm, >5–10 cm	Colonies intersecting a 34 cm belt along the upslope side of each transect	Full sampling design
	2019 on, density of juvenile hard corals in size classes: 0–2 cm, >2–5 cm	Colonies intersecting 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

Photo point intercept method

This method is used to gain estimates of the proportional 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 most hard and soft corals, 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. In general, the MMP avoids the use of species level categorisations of the benthos as most species cannot be consistently identified for the transect photos.

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 metre 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 metre described by Jonker *et al.* 2008).

Juvenile coral surveys

These surveys aim to provide an estimate of the number of hard coral colonies that have successfully recruited and survived 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 is estimated to have been physically damaged since the previous year's observation, 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 and any consistent bias in identification discussed among observers. 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 and inconsistencies discussed among observers to resolve.
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 >40 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., prior to 2020 every 12–24 months and every 12 months since) or every four months at sites where FLNTU loggers are co-located (Table 2-1). Four 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. From 2019, Sea-Bird Scientific SBE 56 high-accuracy temperature loggers (https://www.seabird.com/sbe-56-temperature-sensor/product?id=54627897760) were introduced to gradually replace the Vemco loggers. In 2020, these loggers were joined by the RBR high-accuracy temperature loggers (https://rbr-global.com/products/compact-loggers/rbrsolo-t-2/), a logger similar to the SBE 56. By 2024, both loggers had replaced the Vemco 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. Those loggers were calibrated against a certified reference thermometer after each deployment and generally accurate to ± 0.2 °C. Both SBE 56 and RBR loggers have increased data storage capacity and are set to take readings every five minutes. The loggers are calibrated every two years by the CSIRO (Hobart), a NATA accredited organisation. Both SBE 56 and RBR loggers have an accuracy of ± 0.002 °C. Detailed data download, quality checks and data management methods are described in Appendix A11.

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 MMP data. Data from the AIMS MMP inshore coral reef monitoring are stored in a custom-designed data management system in Oracle databases to allow cross-referencing and access to related data (see Appendix A11 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 are available here:

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

All coral monitoring field data are 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 include lookup fields to make sure that data entered are 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 are uploaded to an Oracle Database using a custom written synchronisation process. All keyed data are 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 Estimation of indicator scores

A key component of the coral program reporting are annual estimates of indicator scores that when aggregated for the Coral Index scores reported by Reef and Regional report cards. These scores are estimated as detailed in Thompson et al. 2020. The calculation of scores is performed by R scripts maintained by AIMS and annually archived in GitHub to ensure the derivation of scores is entirely transparent and repeatable. These include the entire process of coral community scoring from extraction of data from the AIMS database through to the tabulation of scores that are provided to the Reef Authority and organisations responsible for provision of regional report cards.

The GitHub repository for scores reported for 2024–25 can be found at: https://github.com/open-AIMS/MMP_Coral_Report/commit/eb5833b5eeb24eeec318849b80594ba4d853e21e

6 Inshore seagrass monitoring

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6.1 Introduction

Approximately 3,464 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; and
- 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, intertidal, subtidal). 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 all 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 three 50 metre tape measures, 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 for drop-camera assessments. Drop-camera sampling is conducted from a surface vessel using a real-time underwater action camera (DJI Osmo) mounted to a frame with a 0.25 m^2 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. At sites which were originally assessed on SCUBA, the drop-camera is deployed along 50-metre transects two-to–three metres apart (aligned along the depth contour). 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-wet (April) and late-dry (October) periods each year; extra sampling is conducted at more accessible locations in the dry (July) and wet (January) periods.

6.2.0 Field survey methods

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 intertidal 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). The centre of each subtidal site is marked with a waypoint (accuracy ±3 m). This guarantees that the same site is monitored at each event.

Seagrass cover and species composition

Survey methodology follows standardised protocols (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-wet (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 6.2.1).

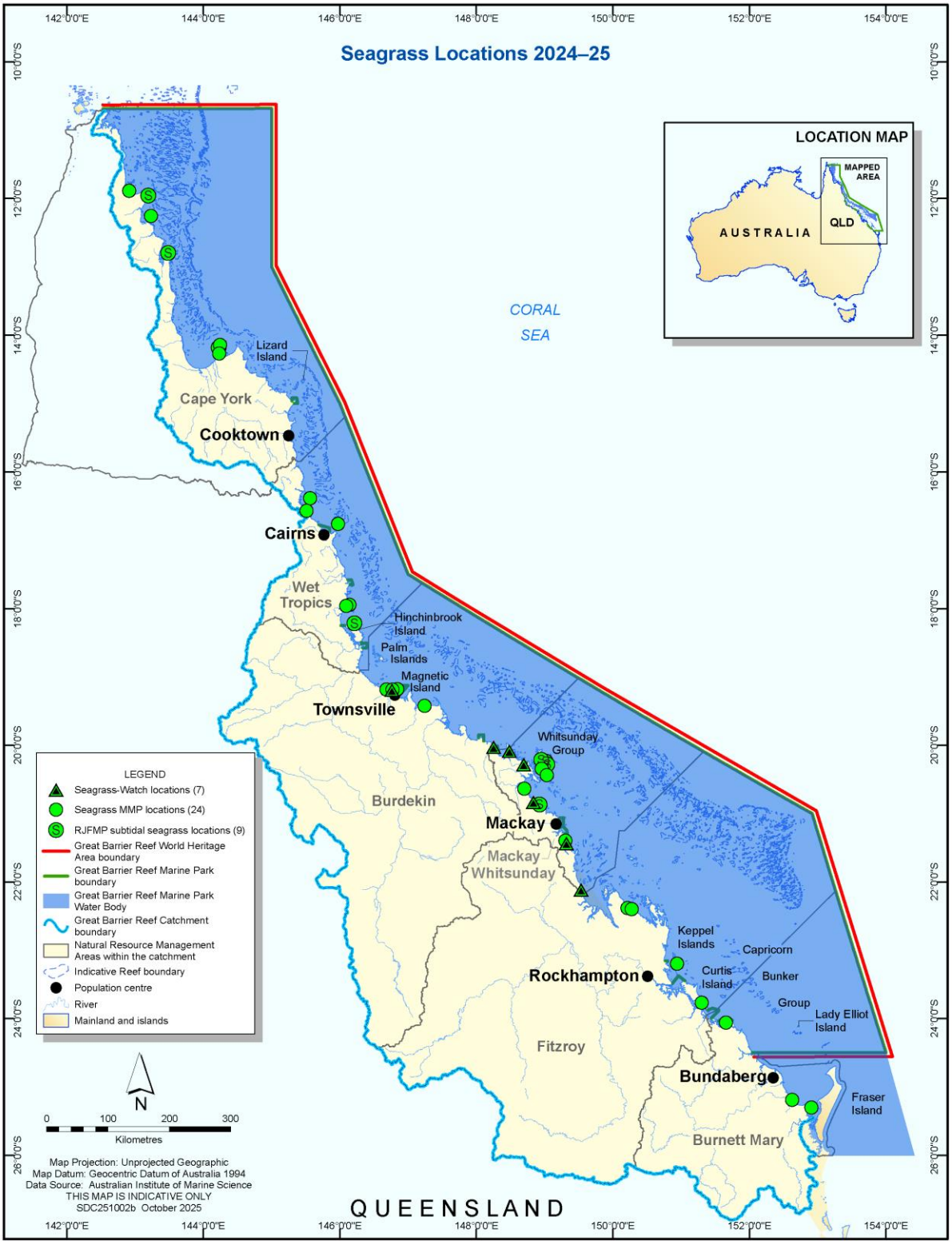

Figure 6-1: Inshore seagrass locations that exist as of 2024–25. However, not all locations were assessed or sampled in 2024–25.

Table 6-1: Inshore seagrass long-term monitoring sites surveyed in 2024–25. 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. serrulata*
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*
				LN3*	Lindeman Is.	149.033	-20.438	*H. uninervis* with *H. ovalis*
			Repulse Bay *coastal*	MP2*	Midge Point	148.702	-20.635	*Zostera muelleri* with *H. uninervis/H. ovalis*
				MP3*	Midge Point	148.705	-20.635	*Z. muelleri* with *H. uninervis/H. ovalis*
			Hamilton Island *reef*	HM1*	Hamilton Island	148.957	-20.344	*H. uninervis* with *H. ovalis*
				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	*Z. muelleri* with *H. ovalis*
				WH1*	Wheelans Hut	150.275	-22.397	*Z. muelleri* with *H. ovalis*

	NRM region	Basin	Monitoring location	Site		Longitude	Latitude	Seagrass community type
			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	*Z. muelleri* with *H. ovalis*
				GH2*	Gladstone Hbr	151.304	-23.765	*Z. muelleri* with *H. ovalis*
	Burnett-Mary	Baffle	Rodds Bay *estuarine*	RD1*	Rodds Bay	151.655	-24.058	*Z. muelleri* with *H. ovalis*
				RD3*	Rodds Bay	151.589	-24.038	*Z. muelleri* with *H. ovalis*
		Burrum	Burrum Heads *coastal*	BH1*	Burrum Heads	152.626	-25.188	*H. uninervis / Z. muelleri with H. ovalis*
				BH3*	Burrum Heads	152.639	-25.210	
		Mary	Hervey Bay *estuarine*	UG1*	Urangan	152.907	-25.301	*Z. muelleri* with *H. ovalis*
				UG2*	Urangan	152.906	-25.303	*Z. muelleri* with *H. ovalis*

Table 6-2: Extra inshore seagrass long-term monitoring sites from the Seagrass-Watch and QPWS drop-camera programs surveyed in 2024–25. 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	Jacky	Margaret Bay *coastal*	MA1^	Margaret Bay	143.194	-11.957	*H. uninervis / H. ovalis with H. spinulosa*
				MA2^	Margaret Bay	143.203	-11.956	
		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*	BW2*	Port Dennison	148.252	-20.017	*Z. muelleri / H. uninervis* with *H. ovalis*
				BW3*	Port Dennison	148.251	-20.018	
	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	*Z. muelleri / 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	
			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 / Z. muelleri* with *H. ovalis*
				CV2*	Clairview	149.535	-22.108	

At each intertidal 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 intertidal 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). For sites assessed using drop-cameras, the 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.

Monitoring at an extra 33 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 6.2.1; 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.

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 at each site each sampling event.

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*, 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.

Meadow extent

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-wet (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 (https://www.seagrasswatch.org/seagrass-monitoring/) (McKenzie *et al.* 2001). Meadow, patch, or scar (meadow-scape) edges are recorded as tracks (one second polling) or a series of waypoints in the field using a portable GNSS receiver (i.e. Garmin GPSmap® 65s). Accuracy in the field is dependent on the portable GNSS receiver (Garmin GPSmap® 65s is <2 metres and how well the edge of the meadow is defined. Generally, accuracy is within that of the GNSS (i.e. 2–3 metres) and datum used is WGS84. Tracks and

waypoints are downloaded from the GPS to a portable computer using 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 of the 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.

6.2.1 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 Seagrass-Watch HQ.

6.2.2 Laboratory analysis

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*, 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.

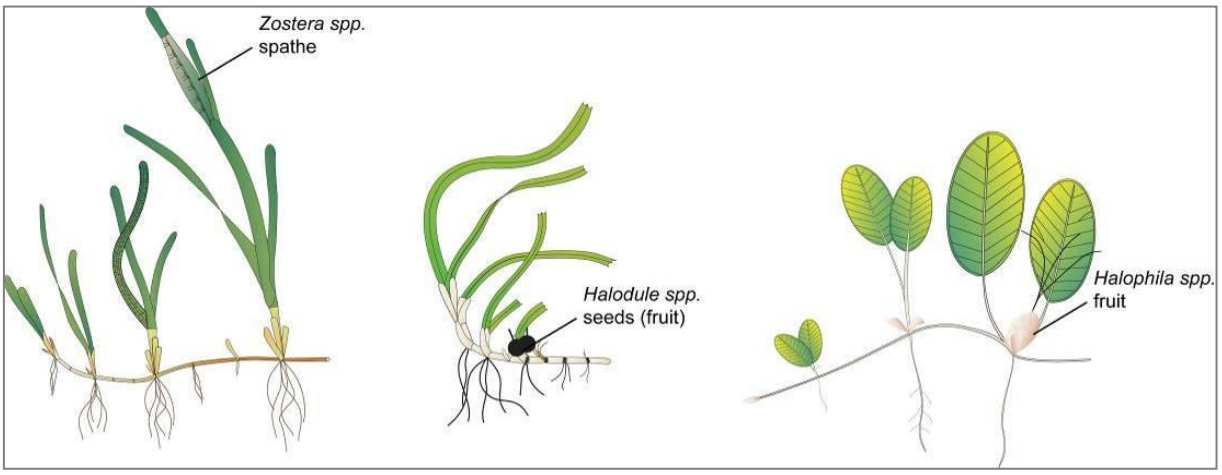

Figure 6-2: Form and size of reproductive structure of the seagrasses collected: *Halophila ovalis*, *Halodule uninervis* and *Zostera muelleri.*

Meadow extent

Mapping is conducted by trained and experienced scientists using ESRI® ArcGIS Pro (Environmental Systems Research Institute). 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).

6.2.3 Temperature monitoring within seagrass canopy

Autonomous HOBO ® submersible temperature loggers (HOBO® MX2201 Pendant and HOBO® MX2204 TidbiT) are deployed at all sites. The HOBO® MX2201 and MX2204 loggers record temperature (resolution 0.01–0.04 °C, accuracy ±0.2–0.5 °C) every 15 minutes, which is downloaded every three to twelve months, depending on the site.

The HOBO Pendant and TidbiT loggers are used as they are designed for durability, compact, and waterproof in salt water to a depth of 1500 meters. The loggers are also Bluetooth® Low Energy-enabled for wireless communication with a phone, tablet, or computer.

The main features of the HOBO MX2201 Pendant include:

- operating temperature range: -20 to 50 °C
- resolution of readings: 0.04 °C
- accuracy: ±0.5 °C from -20 to 70 °C
- sampling Rate: 1 second to 18 hours
- number of readings: 96,000 measurements
- password protection, with separate passwords for read only and full access.

The main features of the HOBO MX2204 TidbiT include:

- operating temperature range: -20 to 70 °C
- resolution of readings: 0.01 °C
- accuracy: ±0.2 °C from 0 to 70 °C
- sampling Rate: 1 second to 18 hours
- number of readings: 96,000 measurements
- password protection, with separate passwords for read only and full access.

The HOBO® 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 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.

6.2.4 Light monitoring at seagrass meadow canopy

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-	Midshelf intertidal & subtidal	Green Island	-16°	45.789	145°	58.31

Region	Basin	Water body	Site	Latitude		Longitude	
	Russell / Johnstone	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
		Macro tidal Enclosed coastal intertidal	Shoalwater Bay	-22°	23.926	150°	16.366
	Calliope / Boyne	Enclosed coastal intertidal	Gladstone Harbour	-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 15 minutes (this is a cumulative 15 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 include only 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).

6.2.5 Light logger calibration procedure

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 Meadow abundance, community structure, reproductive health and extent

TropWATER has systems in place to manage the way MMP data are collected, organised, documented, evaluated and secured. All data are 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 secure relational database. 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.

After meadow extent field mapping, data points are downloaded from the GPS into computer memory and the data exported to ESRI® ArcGIS™. An administration file (*.gpx) is generated by the 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.

6.3.2 Temperature monitoring within seagrass canopy

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 database.

6.3.3 Light monitoring at seagrass meadow canopy

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 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.

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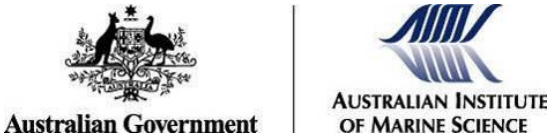
Appendix A Detailed Manuals and Standard Operating Procedures

Prepared by

Renee Gruber, Susan de Boer, and Jack Massuger



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Appendix 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), photosynthetically active radiation, chlorophyll fluorescence (WET Labs), turbidity (beam transmissometer, Sea Tech, 25 cm, 660 nm) 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 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 two meters below the sea surface. *Some bubbles will be expelled from the tubing at the top of the CTD.*
8. Whilst keeping the CTD two meters below 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, raise the CTD so the ring at the top of the cage is just at the sea surface and the PAR sensor is just under the surface, then lower the CTD to within 2-3 m of the bottom at no more than 1 m per sec. Slightly slower is preferred under calm conditions however the profiling speed should not be too slow such that heaving of the ship will cause the instrument profile to move up and down throughout the cast. *[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 backup 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.

Appendix A2: Measuring salinity using the Portasal Salinometer 8410A

AIMS-SOP-(EFWQ)-v1.0

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.

PERSONNEL PREREQUISITES & COMPETENCIES

Laboratory safety induction

Read and understood procedure

Training by experienced technician

TOOLS & EQUIPMENT

- 250 mL screw cap polyethylene bottles – RO water rinsed and air-dried
- Primary standard seawater IAPSO P-series from OSIL (IAPSOSW) at room temperature – 1 x 250 mL bottle
- Secondary standard seawater (SSW) from Coral Sea around 34 to 36 PSU at room temperature as a secondary standard – 1 L to 2 L
- Milli-Q water at room temperature, approx. 500 mL
- Kimwipes

- Drain bucket and salinometer drain tubing.
- Laptop with OSIL salinity data logger installed.
- RS232 cable and USB to RS232 adapter
- 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 Instruments (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.
- Sub-standard 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 1L to 5 L aliquot bottle/carboy in the salinity measurement room. 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

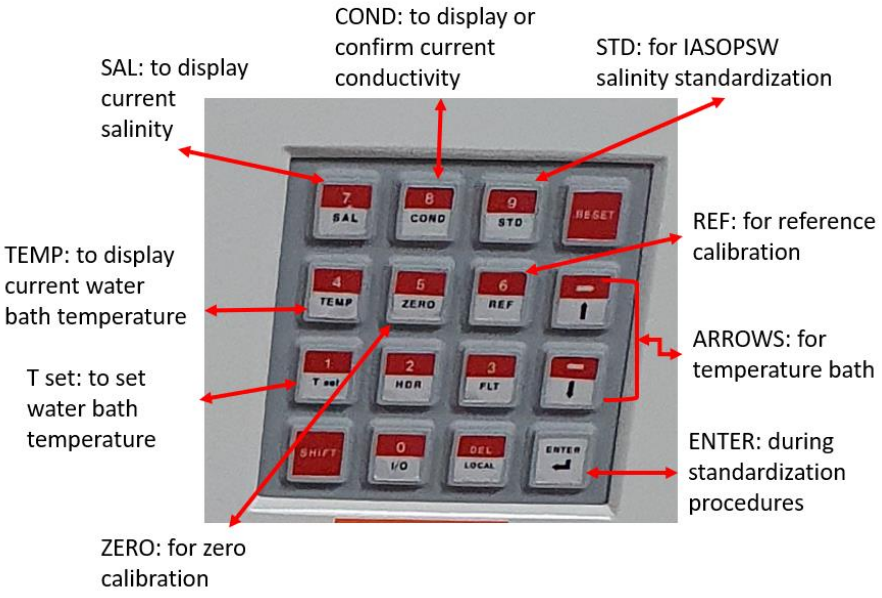
a. Preliminary to using salinometer

- Bring the salinity samples out of the cold room 48 hours before analysis and put them in the laboratory where the salinometer to fully equilibrate to room temperature. Ideally the room temperature should be approximatively 23ºC.
- The temperature of the bath water should be set to about 2ºC above ambient room temperature. See section .b to change bath temperature. Get a new sealed 250ml bottle of IAPSO standard seawater (IAPSOSW) and a 1L to 2L bottle of Sub-standard sea water (SSW) let both equilibrate next to the salinometer as well.
- Make sure you have enough (>1 L) secondary standard seawater (SSW) in a full dedicated plastic bottle to allow for several (5 to 10) drift correction measurement during your salinity measurements. 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 sample water. As the bucket fill make sure the drain tubing does get submersed under the wastewater to avoid back pressure issue in the sample water fill/drain system and consequently in salinity measurement cell.
- Before turning the salinometer power make sure the FUNCTION switch is in the STDBY 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 four speed setting (0, 1 2 and 3). Normally the lowest speed (1) will be sufficient for regular measurements and the higher speed settings (2 and 3) are used for flushing the conductivity cell at the end of salinity measurement session. Always leave the FLOW RATE switch on the salinometer in the “ON” position during use although its only function since the installation of the peristaltic pump is to keep the venting line open.
- Ideally samples to be measured should be 2ºC below the bath temperature. In extreme cases, 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.
- Ensure the salinometer is communicating with the associated laptop and salinity logger by opening the software and starting a new run that will be discarded (see below). This will ensure that you can both standardize the salinometer and record the value of the IAPSOSW using only one primary standard sea water.

b. Salinometer set up

- Power the salinometer and attached peristaltic pump by turning ON the 2 power outlet next to the salinometer the salinometer unit as well as the main power switch located at the back of the salinometer instrument
- Set the salinometer water bath to the required temperature (approx. 2°C above room temperature) by:

- Pressing the “T-Set” key on the salinometer keypad
- Increment or decrement the displayed valued on the salinometer screen using the ⬆ or ⬇ on the keypad until the required temperature is displayed (each arrow button press increment or decrement by 1°C)
- Press “ENTER”
- Water bath should be left approx. 1.5 hours to reach a stable and desired set temperature ± 0.02°C at which time a salinity standardization can be undertaken
- Water bath temperature can be check at any time by pressing the TEMP key on the salinometer keypad.

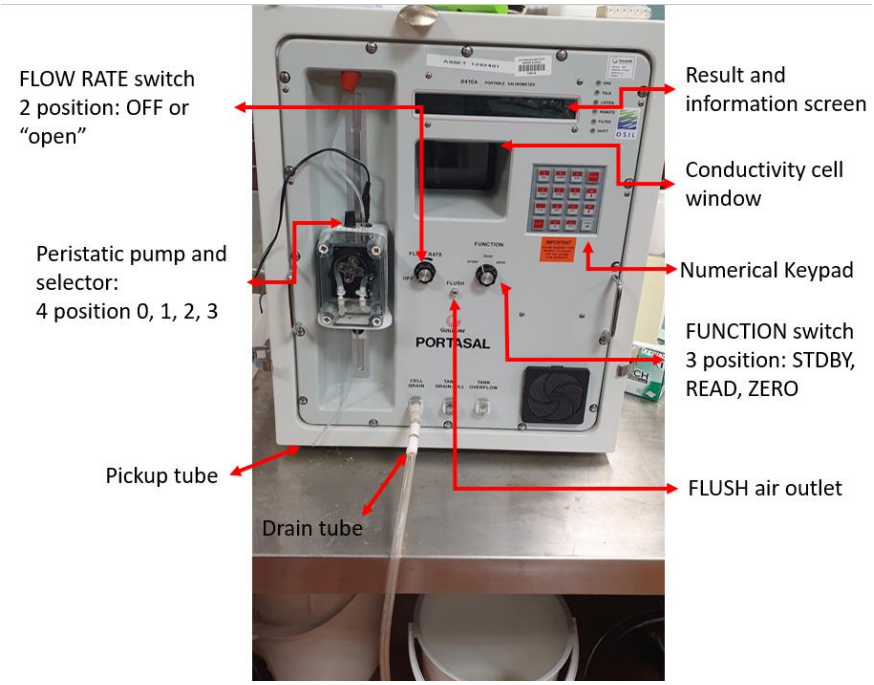


- Perform a flow rate check by:

- Placing the intake pipe in the SSW water bottle
- Turning the FLOW RATE switch on “OPEN” on salinometer
- Ensuring the FUNCTION switch is set to STDBY
- Turning the peristaltic pump speed to 1
- 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 3 times
- Turn peristatic pump off and FLOW rate to OFF to keep the salinity cell filled with SSW while the salinometer water bath reaches the required temperature.

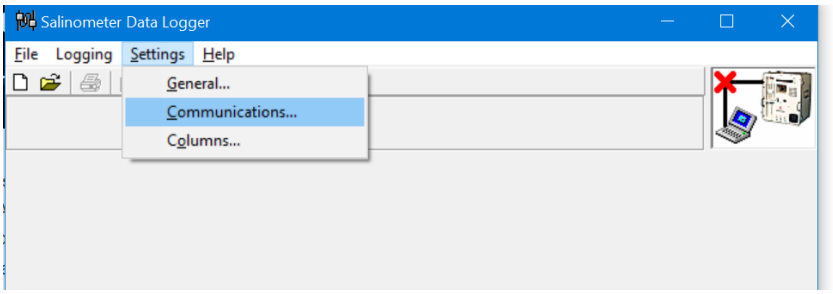
- This step ensures a reduction of observed salinity drift when the salinometer has not been used for a couple of days and the conductivity cell has been left dry, in Milli-Q, or has gone through a cleaning procedure.
- If air bubbles are adhering to the cell and are hard to dislodge by flushing, it may be necessary to clean the cell.
- Each time the Portasal has been powered up or the bath temperature has been changed, allow 1.5 hours with the bath temperature regulating at 2°C 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.



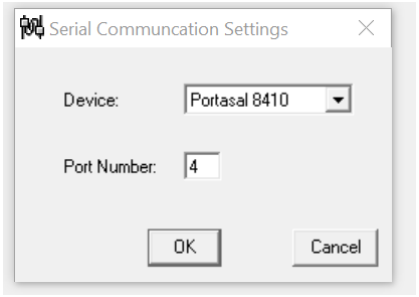
c. Salinometer to salinity logger software check

- Perform a software to salinometer communication check and salinity data acquisition set up:

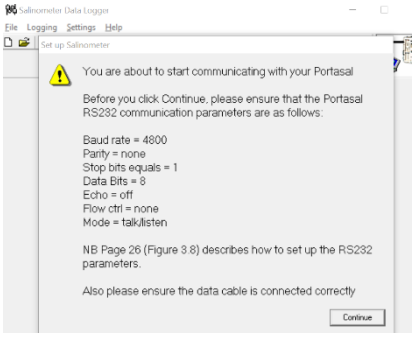
- Ensure salinometer RS232 is plugged at the back of the instrument and is connected to the laptop USB to RS232 adapter.
- On the laptop go to "device manager" and in "ports" sub menu check which COM port is connected (eg:COM4)
- Start the OSIL salinometer data logger on the computer and in the "settings" sub menu select "communications"
-



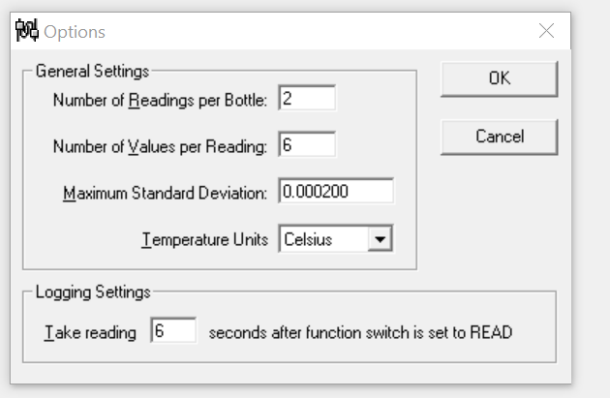
- In the communication windows select Portasal 8410 as a device and enter the COM port number identified previously un the “port number” window (eg:4).



- Press “OK”
- Go to “File” then “New”, a window called “Set up salinometer” will appear, press “Continue”



- The salinometer and logger software should start communicating and a new logger sheet menu should be created.
- Cancel the creation of the new logger sheet before starting the salinity standardization process (If the communication is not established, re-check the COM port number in the “device manager” and repeat communication protocol.)
- Prior to start any salinity measurement, in the OSIL logger software, go to “settings” then “general”.



- In the “option” windows set the followings
- Number of readings per bottle: 2
- Number of values per readings: 6
- Maximum standard deviation: 0.0002
- Temperature unit: Celsius
- Take reading “6” seconds after function switch is set to read.
- Press “OK” to set data acquisition values for the subsequent salinity measurements.

d. Standardization

The Portasal is usually quite stable but to ensure quality control, a salinity standardization routine should be performed using a new sealed IAPSOSW bottle every time the instrument is powered up, the bath temperature is changed, or a set of salinity sample measurements are taken.

To perform the Standardization and drift Correction Routines the instrument needs to be powered up for a minimum of 1.5 hours and the displayed temperature be stable ± 0.02°C to the previously set up temperature.

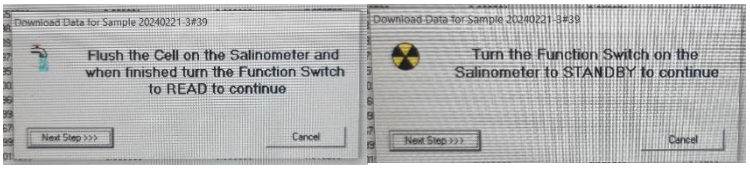
The salinometer standardization is independent of any data acquisition by the OSIL salinometer software and should be carried out without the software logging any values (except in the last step of the standardization process when the IAPSOSW standard value is checked and logged).

Using a new sealed bottle of Standard Sea Water (IAPSOSW) as the primary standard, fill and flush 3 times and fill the cell similarly to what us described in the Sample Measurement procedure (below) steps 1 through 15.

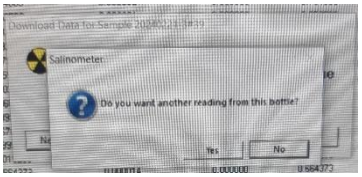
- Rocking the IAPSOSW bottle gently to eliminate gradients without creating air bubbles
- Ensure the FUNCTION switch is set to STDBY
- Turn the FLOW RATE switch on open on the salinometer
- Turn the peristaltic pump speed to 1
- Wipe the intake tube using a Kimwipe
- Place the intake tube in the IAPSOSW standard bottle (use Kimwipe to handle the intake tube)

- Filling and flushing the conductivity cell 3 times ensure 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 the FUNCTION switch to READ
- 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 (approx. for 10s), press ENTER key. This will terminate the standardisation operation and display the conductivity ratio using the new calibration values.
- Turn the FUNCTION switch to STDBY
- Start a new salinity log sheet on the OSIL salinity data logger as described in salinometer to logger software check section (ensure acquisition conditions are properly set) and fill the relevant information on the log sheet (mostly file name, technician and room temperature)
- When a new salinity log sheet is created on the OSIL software, computer will prompt the user to turn the FUNCTION switch to ZERO (approx. 5s). Please make note of this value (usually 0.00000±0.00005) as zero reading will be required at the end of a salinity log sheet session.
- Turn the FUNCTION switch to STDBY as prompted by the software.
- Flush and refill the cell one time

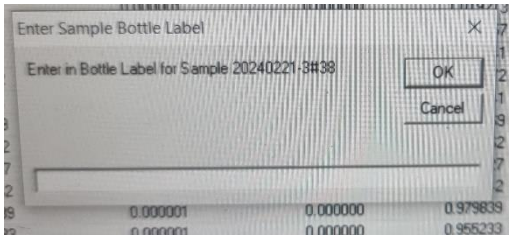
- Turn the FUNCTION switch to read
- Following onscreen direction operate the FUNCTION switch from STDBY to READ two times for acquisition of 2 averaged salinity values from 6 readings each.



- Once the measurement is taken and CV value of repeated reading and average values is satisfied the software will prompt you to re-take a measurement from this bottle.
- Press “NO”



- Once the salinity acquisition is completed, confirm that the measured average salinity value acquired on the computer correspond to the IAPSOSW salinity value written on the bottle ±0.001PSU.
- On the computer log sheet, right click on the bottle#1 on the log sheet list and press “enter bottle label”, then enter the name ”IAPSOSW” for this sample



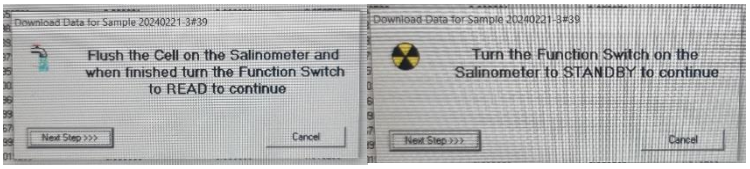
- Remove the IAPSOSW bottle from holder, flush the salinometer cell and wipe the intake tube with a Kimwipe
- If the 1st salinity measurement correspond to the IAPSOSW bottle value, the salinity standardization is successful and you can continue with the 1st drift correction routine measurement and subsequent samples measurements.
- If the standardization is unsuccessful, please repeat the standardization protocol using a new IAPSOSW bottle until successful.

e. Drift Correction Routine

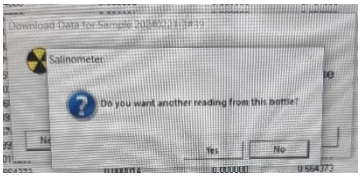
- To minimise the use of expensive commercial primary standard (IAPSOSW), a secondary standard (SSW) is used. This is just clean seawater (preferably collected off the continental shelf to ensure maximum clarity). This samples are run similarly to a regular salinity sample as described in the Sample Measurement section. The SSW value is acquired directly after the IAPSOSW salinity confirmation, every 20 samples thereafter and as the last sample measured in a given session preferably at samples position #2, 23, 44, 65, 86, 107,128.etc on the salinity log sheets for the automated excel drift analysis. A drift calculation is then applied to the sample readings assuming linear drift between the two substandard readings.

3. SAMPLE MEASUREMENTS

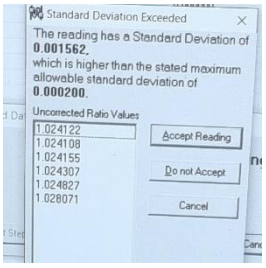
1) Rock sample bottle gently to eliminate gradients. Do not introduce air bubbles by shaking it.
2) Wipe salinometer pickup tube using a Kimwipe
3) Insert the intake tube in a sample bottle using a Kimwipe. Ensure that the pick-up tube reaches almost to bottom of bottle such that flow will not be restricted. 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 and ensure no air bubbles are present in the conductivity cell, especially along the four electrode along the length of the conductivity cell.
7) Turn the FUNCTION switch to READ
8) Following the onscreen direction operate the FUNCTION switch from STDBY to READ two times for acquisition of 2 averaged salinity values from 6 readings each.



9) Once the measurement is taken and CV value of repeated reading and average values is satisfied the software will prompt you to re-take a measurement from this bottle.
10) Press “NO”



If the CV value conditions are not satisfied the following window will appear



In such case press “Do not Accept” and then press “YES” when prompted to take another reading from the bottle and repeat step 4) to 10)

11) On the computer log sheet select the last sample acquired by the software, right click on the bottle number and press “enter bottle label”, then enter the name

written on the bottle label for this sample (If the sample measured is the secondary standard enter “SSW)

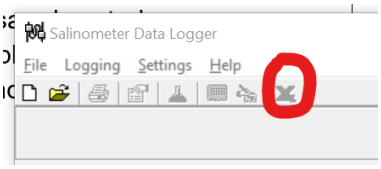
12) Remove the intake tube from the sample bottle

13) Flush the cell

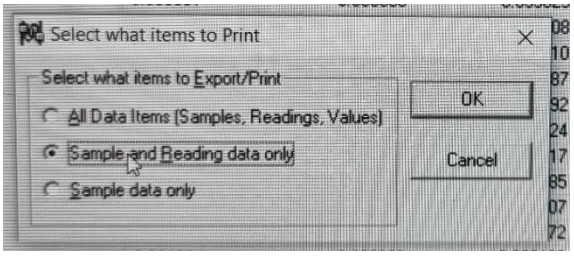
14) Wipe the intake tube using a Kimwipe

15) Repeat the protocol for the following sample bottle to be measured from step .1)

16) At the end of a salinity measurement session, export the acquired results into an excel spreadsheet by pressing “X” symbol in the salinometer logger software.



On the following export windows select to export data as “sample and Readings data only. Save and name the exported excel file “SAL_YYYYMMDD” and place it in the relevant folder for drift correction analysis



17) When closing the salinity log sheet, the computer will prompt you to acquire a final zero value by turning the FUNCTION switch to “ZERO” similarly to when the log sheet was created. This value should be close to 0.00000±0.00005 and similar to the value measured initially. If this value differs, please inform laboratory manager or trained technician for re-calibration.

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, turn the peristaltic pump on the “0” position 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, turn the peristaltic pump to the “3” position, fill and flush the conductivity cell at least 5 times using Milli-Q water 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 readings are logged by the salinity software during measurements and then exported as a Microsoft Excel spreadsheet. Average salinity values for each samples are then exported into a template called "SAL_dritcorr.xls" which will automatically perform all salinity drift measurement calculation according to the first and subsequent SSW values measured in a given salinity measurement session.

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 standardization the salinity from the IAPSOSW returns a value drifting by ±0.001PSU, the standardization is repeated until the standardization reaches the expected IAPSOSW standard.
- If during the analysis the conductivity from the SSW returns a value drifting by ±0.0005, the samples measurements are stopped and repeated after instrument re-calibration.

a. 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.

- Repeated analysis of IAPSO (IAPSOSW) and SSW
- Using the SSW the standard deviation of all samples taken within a daily session is usually ±0.003 PSU.

Appendix A3: Automated analysis of Dissolved Inorganic Nutrients in Seawater (DIN5 / ATSOP002)

1. Objective

Analyses of concentrations of ammonia (NH_4^+/NH_3), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate ($Si(OH)_4$), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) in water samples, with a focus on the high precision trace nutrients analysis of seawaters.

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 and AA500 segmented flow analysers. See method references (7.1) for detailed methodological information and additional references for each analyte.

Dissolved inorganic nutrients (DIN5) are measured using colorimetric and fluorescence spectroscopy methods. Ammonia (in the forms of both NH_3 and NH_4^+) is analysed by fluorescence spectroscopy. Phosphate (PO_4), Nitrite (NO_2), Nitrate + Nitrite (NO_x) and Silicic acid or Silicate (Si) are analysed by colourimetry. The raw data is corrected for baseline drift, carryover effects, sensitivity drift, and salinity effects before final results are calculated. The units for reporting are µmol/L @ 24°C unless otherwise specified.

3. Sample analysis

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.

Nutrient analysis is performed using segmented flow autoanalyzers (SEAL AA3 and AA500 Analysers) and their accompanying software (SEAL AACE Software). Segmented flow methods offer the greatest precision and lowest detection limits for low-level nutrient analysis.

3.2. Laboratory facilities and labware

- Lab air intakes in location protected from atmospheric contaminants, with a scrubber used for air injection on the ammonia channel.
- Sample storage is in dedicated, clean, locked and alarmed freezers. The freezers are on power circuits with UPS/generator backup and are checked regularly by security personnel.
- Sample management is through a Laboratory Information Management System (LIMS). Samples are logged into the LIMS and assigned a unique LIMS identification number.
- Weighing balances (serviced/calibrated annually).
- Volumetric flasks and positive displacement pipettes for preparation of standard solutions.
- All glass and plastic ware used for the analysis is washed with 10% v/v HCl and rinsed with 18.2MΩ water
- Nutrient standards are prepared from pure chemicals. Wherever possible, they are made from certified reference materials with a corresponding certificate of analysis. [$(NH_4)_2SO_4$, KNO_3, $NaNO_2$, KH_2PO_4, Na_2SiF_6].
- Wherever possible, analytical reagents are prepared with AR grade chemicals.
- Water is 18MΩ quality or better is used for the analysis

4. Method

4.1. Samples

• Ammonia, nitrite/nitrate, and phosphate (NH_3, NO_x, NO_2, PO_4) samples are received and stored frozen in dedicated freezers at -20°C. Before analysis, samples are thawed in air at room temperature, then homogenised, uncapped, placed in sample racks on the covered autosampler.

• Silica samples (Si) are stored in a 4°C refrigerator. Before analysis, they are warmed to room temperature, homogenised, placed in sample racks on the covered autosampler.

• Samples for analyses of total dissolved nitrogen and phosphorus (TDN and TDP) are oxidised with persulfate reagent. Bound forms of N and P require different conditions for oxidation and decomposition into inorganic forms of N+P that can be quantified colorimetrically. In AIMS' method, N+P are decomposed simultaneously with a boric acid/NaOH system. The pH of the reaction starts ca. pH 9.7 and ends at ca. pH 4-5 (Appendix A4).

4.2. Standards

• Primary stock standards are prepared in 18.2 MΩ water and stored for between 3-6 months at room temperature. New calibration stock standards undergo a validation procedure prior to use in calibrations.

• Between six and eight working standards (plus a calibration blank) are prepared fresh from stock standards or intermediate standards prior to each analysis run.

• Working standards are prepared over the following ranges: NH_3: 0-6 µmol-N L^{-1}; NO_3: LR: 0-10 µmol-N L^{-1} / HR: 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 from the stock solutions in an aged and filtered low-nutrient seawater (LNSW) matrix, which is made to match the salinity of the samples (e.g. fresh, estuarine or marine waters). By matching the matrix of the seawater samples with the calibrants, AIMS ensures the greatest level of accuracy in the analytical measurements. Residual nutrients (if present) in the LNSW are accounted for through an appropriate zero-point correction.

• Reference materials include In-house QCs, CRMs (i.e. RMNS standards from KANSO Technos, Osaka), and periodic proficiency samples (i.e. Wepal-Quasimeme Laboratory Performance studies, Wageningen Research, Netherlands).

• In-house sea water storage reference samples:

  Aged LNSW is filtered through a 0.4 µM polycarbonate filter. 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 12 months and are prepared as required. Inhouse reference samples are typically prepared in three concentration ranges – high, medium, and low, to span across the calibration range so that each part of the calibration can be monitored.

4.3. Calibration

• Pipettes are gravimetrically calibrated before use. Conformance checks are performed to ensure that volumetric ware meets ISO/Australian Standards.

• The auto-analyser is calibrated daily for each analyte (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.

Each tray protocol as part of every analysis session includes:

- Between six and eight working standards plus calibration blank.
- QA/QC standards: Replicate in-house seawater reference samples and certified reference materials (CRMS).
- Drift and blank samples for drift and baseline corrections, included at regular intervals within the sample set, with typically 30 samples per interval.

4.4. Calculations

- The AACE software automatically assigns peak markers to the peaks in the instrument charts during post-run processing; however, each peak is also checked manually by the analyst and the marker adjusted where necessary to ensure signals are correctly detected. Editing is performed within the standard auto-analyser software by moving the peak markers to the correct position on the sample peak.
- The raw instrument data is exported in a CSV file and processed in Excel, to correct for baseline drift, carryover effects, sensitivity drift, and salinity effects including refractive index, before final results are calculated.
- The calculation steps are semi-automated through the use of a controlled Excel processing template with macros. This ensures consistency between different analysts.
- Interferences are minimal for filtered natural oligotrophic seawaters with normal pH, salinity, and concentration range.

4.5. Instrument performance

- The Method Detection Limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. The MDL and Limit of Quantitation (LOQ) values are an estimate and may change from run to run. Some recent (FY25/26) MDL values for nutrients in seawater were 0.02 µmol L^{-1} NH_3, 0.01 µmol L^{-1} PO_4, 0.03 µmol L^{-1} NO_x, 0.01 µmol L^{-1} NO_2, 0.05 µmol L^{-1} Si. with the MDL calculation method from the EPA (2016).
- Calibration quality, baseline, sensitivity, and drift: Strict QAQC protocols ensure that performance is consistent and meets the requirements of the method.

5. Data management

Suitable sample identifiers are supplied by staff requesting analyses and submitted to the analytical laboratory via the Laboratory Information Management System (LIMS). The LIMS creates a unique identifier for the samples as well as job number so that the nutrient submissions can be tracked. All nutrient data including the excel calculation sheets and QAQC data is traceable and maintained through LIMS.

6. Quality control

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

6.1. Assessment of the method limit of detection (MDL)

Replicate LNSW readings are taken during each analytical run, and these results are tracked through LIMS. The blank readings may be used to monitor for changes in precision that may mark a deviation from the established MDL.

6.2. Assessment of accuracy of the analysis

Reference materials are used to assess recovery of a known amount of analyte. AIMS performs both intralab and interlab testing.

- External certified reference standards are included in each run and analysed daily (e.g. Kanso RMNS CRMS). These CRMs have been especially developed for trace-level oceanographic nutrients testing.
- Regular validation of the analyses by continued participation in inter-laboratory trials, e.g. biannually with Wepal-Quasimeme.
- Spikes of known concentration are added to natural seawater samples and analysed as part of the sample batch, unbeknown to the analyst.
- The laboratory conducts method validation activities and periodically updates and reviews its methods in accordance with current analytical needs and best practices, and with a continuous improvement philosophy.

6.3. Assessment of precision of the analysis

Precision is ascertained through repeated analyses of the same concentration of analyte to check for repeatability and reproducibility.

- Repeatability and reproducibility of in-house seawater storage reference samples for each nutrient species.
- Repeatability and reproducibility of other materials such as blanks, CRMS, standards etc. are also tracked and monitored through LIMS.

6.4. Procedural blanks:

Blank samples for drift and baseline corrections (part of the autoanalyzer software) are included at regular intervals within the sample set with typically 30 samples per interval.

6.5. 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 analyses. Before data analysis, results are generally averaged over duplicates.

Coefficients of variance are calculated from analytical results of sample duplicates. Duplicates with CV>20% are flagged during QC checking by the water quality group, and the analysis repeated using extra duplicates that are generally collected as a backup.

7. References

7.1. Methods

Ammonia/Ammonium:

- SEAL Analytical Autoanalyser Applications, Ammonia in Water and Seawater Method No. G-327-05 Rev. 7 (OPA Fluorescence Method)
- ‘Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis’, Kerouel, R, Aminot, A. Marine Chemistry Vol. 57, no. 3-4, pp. 265-275., Jul 1997.
- Method Summary: The sample is reacted with o-phthalaldehyde (OPA) at 75°C in the presence of borate buffer and sodium sulfite to form a fluorescent species proportional to the ammonia concentration. The fluorescence is measured at 460 nm following excitation at 370 nm.

Free Reactive Phosphate:

- SEAL Analytical Autoanalyser Applications, Phosphate in Water and Seawater Method No. G-297-03 Rev. 4 (Multitest MT19)
- EPA 365.1 DETERMINATION OF PHOSPHORUS BY SEMI-AUTOMATED COLORIMETRY, 1993
- Method Summary from EPA365.1. “Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.”

Nitrate and Nitrite:

- SEAL Analytical Autoanalyser Applications, Nitrate and Nitrite in Water and Seawater and Total Nitrogen in persulfate digests, Method No. G-172-96 Rev. 19 (Multitest MT19)
- USEPA Method 353.4, Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis, 1997, accessed via EPA website https://cfpub.epa.gov/
- Method Summary from EPA353.4: “Samples are passed through a copper-coated cadmium reduction column. Nitrate in the sample is reduced to nitrite in a buffer solution. The nitrite is then determined by diazotizing with sulfanilamide and coupling with N-1 naphthylethylenediamine dihydrochloride to form a color azo dye. The absorbance measured at 540 nm is linearly proportional to the concentration of nitrite + nitrate in the sample. Nitrate concentrations are obtained by subtracting nitrite values, which have been separately determined without the cadmium reduction procedure, from the nitrite + nitrate values.”

Silica:

- SEAL Analytical Autoanalyser Applications, Silica in Water and Seawater Method No. G-177-96 Rev. 11 (Multitest MT19)
- Standard Methods, 22nd Ed. (2009), 4500-SiO2 Silica C.
- Method Summary: The method can be compared to Standard Methods, 22nd Ed. 2009, 4500-SiO2 Silica C. From SEAL G-177-96: “This automated procedure for the determination of soluble silicates is based on the reduction of silico-molybdate in acidic solution to molybdenum blue by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to minimize interference from phosphates.”

7.2. In-text 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.

EPA, Definition and Procedure for the Determination of the Method Detection Limit - Appendix B to Part 136, Title 40, Revision 2, (2016) EPA 821-R-16-006, available from https://www.epa.gov

Wepal-Quasimeme Proficiency Testing: AQ1 and AQ2 programs, Wageningen university and Research, https://www.wur.nl/en/research/products-services/proficiency-testing-wepal-quasimeme/seawater

KANSO RMNS Certified Reference Materials (CRM) KANSO TECHNOS CO., LTD., Available from https://www.kanso.co.jp/eng/services/production/

Attachment 3.1 Analysis of Total Persulfate Oxidisable N and P in Water (ATSOP-010)

Introduction:

The AIMS Analytical technology laboratory analyses marine, estuarine and freshwater samples for Total Dissolved (or Filterable) Nitrogen (TDN) and Total Dissolved (or Filterable) Phosphorus (TDP). Persulfate digestion is used to decompose nutrients that are bound organically or in other non-reactive forms. The resulting nutrients, in the form of Phosphate (PO_4^{3-}) and Nitrate (NO_x) can then be quantified by colourimetry on the AA3 or AA500 segmented flow analyser (See AIMS SOP: ATSOP002 Analysis of Dissolved Inorganic Nutrients in Seawater (5DIN)).

Measurement principle:

Bound forms of nitrogen and phosphorus require different conditions for oxidation and decomposition into inorganic forms of N + P that can be quantified colorimetrically. The oxidation of nitrogenous compounds for determining TDN must occur in an alkaline medium. The oxidation of phosphorus compounds for determining TDP must occur under acidic conditions. In this method, N and P are decomposed simultaneously with a boric acid/NaOH system. The pH of the reaction starts ca. pH 9.7 and ends at ca. pH 4-5.

The digest converts organic and other bound forms of N and P into phosphate (PO_4^{3-}), and nitrate (NO_x), which are subsequently quantified by colorimetric analysis. The raw data is corrected for baseline drift, carryover effects and sensitivity drift before the final results are calculated. The units for reporting are TDN and TDP µmol/L @ 24°C unless otherwise specified.

Scope:

The method is for analysis of oligotrophic seawater and estuarine waters with salinities from 0-36 and a pH range of 6-8.5.

This procedure related to the analysis of dissolved inorganic nutrients by Seal Analytical AA500 and AA3 Segmented Flow Analysers in the AIMS Analytical technology laboratory. The digested samples are compatible with the ATSOP002 (5-DIN) NO_x and PO_4 methods for the colorimetric analysis step.

Calibration and standards:

Working standards are prepared over the following ranges: NO_3: LR: 0-10 µmol-N L^{-1} / HR: 0-30 µmol-N L^{-1}; PO_4: 0-3 µmol-P L^{-1}

Working standards are prepared from the stock solutions in an artificial seawater (ASW-OX) matrix, which is made to match the salinity of the samples (e.g. fresh, estuarine or marine waters). By matching the matrix of the seawater samples with the calibrants, AIMS ensures the greatest level of accuracy in the analytical measurements. Residual nutrients (if present) in the ASW-OX are accounted for through an appropriate zero-point correction.

Each digest as part of every analysis session includes:

- Between six and eight working standards plus calibration blank.

- Blank samples for zero-point and baseline corrections.
- Oxidisable QCs including spike recovery standards and in-house QCs.

Limit of detection, limit of quantification:

The Limit of Detection (LOD or MDL) and Limit of Quantification (LOQ) are estimated mathematically and can vary from run-to-run. Oxidised MilliQ Blank (MQ-OX) standards are used for baseline drift and zero-point correction and can be used to estimate the LOD. The QC target is for TDN LOD <0.5 uM and TDP LOD <0.05 uM.

Accuracy and interference:

Interferences are minimal for natural oligotrophic seawaters with normal pH, salinity, and concentration range. The TDN/TDP digestion method is expected to be selective for natural seawater and estuarine samples of pH 6-8.5.

Matrix-matching of the calibrants ensures the greatest accuracy for seawater analysis. A zero-point correction with MQ-OX corrects for the residual nutrients present in the artificial seawater (ASW-OX) standards. Refer to method validation documentation for further information.

Quality control / quality assurance:

- The laboratory autoclave and reaction vials are reserved for low-level nutrient samples only and undergo a rigorous cleaning procedure between each digest batch.
- Reaction vials are sealed during the digestion to protect from N contamination.
- Calibrants undergo the same digestion procedure as the samples and are matrix-matched for salinity.
- QA/QC standards: Replicate in-house seawater reference samples and spikes of known concentration containing oxidisable nutrients are included in each digest batch.
- Matrix spikes/QCs containing organic oxidisable N+P are included in each run along with blank replicates for baseline and zero-point correction. The expected recovery for TDN and TDP digest recovery check standards is 100% +/- 10%.
- Each digest batch is monitored for precision and LOD.
- Reference materials include In-house QCs, SRMs (e.g. oxidisable nutrients spikes) and periodic proficiency samples (i.e. Wepal-Quasimeme Laboratory Performance studies, Wageningen Research, Netherlands).
- The laboratory conducts method validation activities and periodically updates and reviews its methods in accordance with current analytical needs and best practices, and with a continuous improvement philosophy.

Calculations and records:

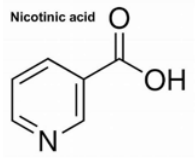
- Autoclave records are kept in the Autoclave logbook next to the instrument. Run data, QCs and results are recorded in Matrix LIMS. All calculations are attached to the Job File in the LIMS, along with the accompanying Runsheet file.
- The digested samples are analysed using the DIN5/ATSOP002 procedure.
- The raw instrument data is exported in a CSV file and processed in Excel, to correct for baseline drift, carryover effects, sensitivity drift, and salinity effects including refractive index, before final results are calculated.
- The calculation steps are semi-automated using a controlled Excel processing template with macros. This ensures consistency between different analysts.

Application note:

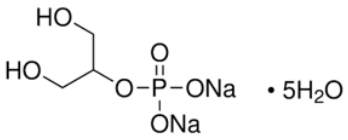
Persulfate digestion is a milder form of digestion and is often favoured over other means due to its safety and ease of use, as well as offering lower detection limits for many forms of N + P. The digestion recovery (%) will vary with the form of the bound N + P in the sample (Dayton E., 2017).

Pure organic N + P compounds (see molecular structures and table below) have been selected for use in the recovery check/spike solution for reasons of purity, safety, and stability, as well as for their organic nature. They are used as a traceable reference material to allow calculation of the digest recovery (%) and for process control.

It is recommended they be used in conjunction with a suitable CRM containing oxidisable N + P, and with a matrix as similar as possible to the sample.



B-glycerol phosphate disodium salt pentahydrate (CAS# 13408-09-8)



Organic nutrients spike solution - Final Spike Value

(100uL of Working Stock Solution is added to 10mL of sample to give 10.1mL final volume)

Standard Name	Chemical Name	Aliquot working stock solution (uL)	Matrix	Final concentration in solution (uM)
Final Spike Value Org N+P	B-Glycerol phosphate disodium salt pentahydrate	100	Sample	2.00
	Nicotinic Acid			20.00

References:

- ATSOP002 AIMS Method for Analysis of Dissolved Inorganic Nutrients in Seawater (5-DIN)
- Grasshoff, K., Kremling, K, Ehrhardt, M. ‘Methods of Seawater Analysis’, 3rd Ed., Wiley-VCH, 1999 (Section 10.2.17, pp. 205-206)
- Dayton, E., Whitacre, S., Holloman, C., ‘Comparison of three persulfate digestion methods for total phosphorus analysis and estimation of suspended sediments’ Applied Geochemistry, 78, 2017, pp.357-362

Appendix A4: Dissolved Organic Carbon and Total Nitrogen Assay in Water using the Shimadzu TOC-L

AIMS ATSOP-006

INTRODUCTION

Marine, estuarine and freshwater 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.

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.

0.05 M 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.

1000 ppm 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.

1000 ppm 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 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 A5: Measuring CDOM in water samples using the Shimadzu 1900UV-Vis spectrophotometer

AIMS-SOP-EFWQ-v2.0

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).

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, PVC, 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, PVC, 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. 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:
  - Remove and store safely the 1 cm cuvette inserts
  - 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.

2. 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(λ) 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.

3. 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/”).
    - 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.

4. 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.

Appendix A6: Analysis of particulate phosphorus in marine waters

AIMS-SOP-EFWQ-v2.0

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).

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.

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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 duplicated pair of deionised water blank filters and four 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 1 hour and repeat spectrophotometer reading

- All measurements should be recorded on the sample analysis logsheet.

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 allows for variation of these numbers for individual samples.

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

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. 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" 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. If for a given analytical batch the phosphate recovery after persulfate digestion is calculated to be less than 90%, all samples within this analytical batch are rerun.

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.
- A pair of wet filter blanks (WFB) are collected during each sample collection to measure contamination of GF/F filters or through handling. The WFB phosphorous value is subtracted from all the filters collected on a similar trip to normalize the results and account for any contamination.

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.

Others

- 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.

Appendix A7: 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

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 day's 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

Appendix A8: Analysis of Chlorophyll-*a* in marine waters

AIMS-SOP-EFWQ-v1.0

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).

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). 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. 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.

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
    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.
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
14) An unused blank GF/F filter should also be processed in the same manner to give a blank value.

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 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
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:

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 (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.

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%.

Appendix A9: Measuring total suspended solids in seawater

AIMS-SOP-EFWQ-v2.0

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.

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. Handle the filters only with the forceps.
- Make a record of the filter weight against the vial number.
- Repeat for required number of filters.

3. SAMPLE COLLECTION AND HANDLING

- 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.
- 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 for 72 hours 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. 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 all filter blanks for a given trip or subsequently a sampling year is then removed from the entire dataset to account for weight blank correction. Negative weights are considered to be 0 mg if the negative weight is less than 0.0001 mg.

6. CALCULATION

$$\text{Suspended Solids} = \frac{(\text{final weight} - \text{initial weight}) - \text{wfb}}{\text{Sample volume}}$$

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.

Appendix A10: AIMS In-house procedures for time-series loggers

Wet Labs ECO FLNTUSB

The Wetlabs ECO (Environmental Characterization Optics) line is a range of optical instruments with a multiple sensor and configuration options. The AIMS MMP team uses a fleet of ECO-FLNTUSB units, which measure chlorophyll and turbidity, and are configured with a bio-wiper and internal batteries.

From the WetLabs Manual - Theory of Operation: The fluorometer allows the user to monitor chlorophyll concentration by directly measuring the amount of chlorophyll-a fluorescence emission from a given sample volume of water. Chlorophyll, when excited by the presence of an external light source, absorbs light in certain regions of the visible spectrum and re-emits a small portion of this light as fluorescence at longer wavelengths.

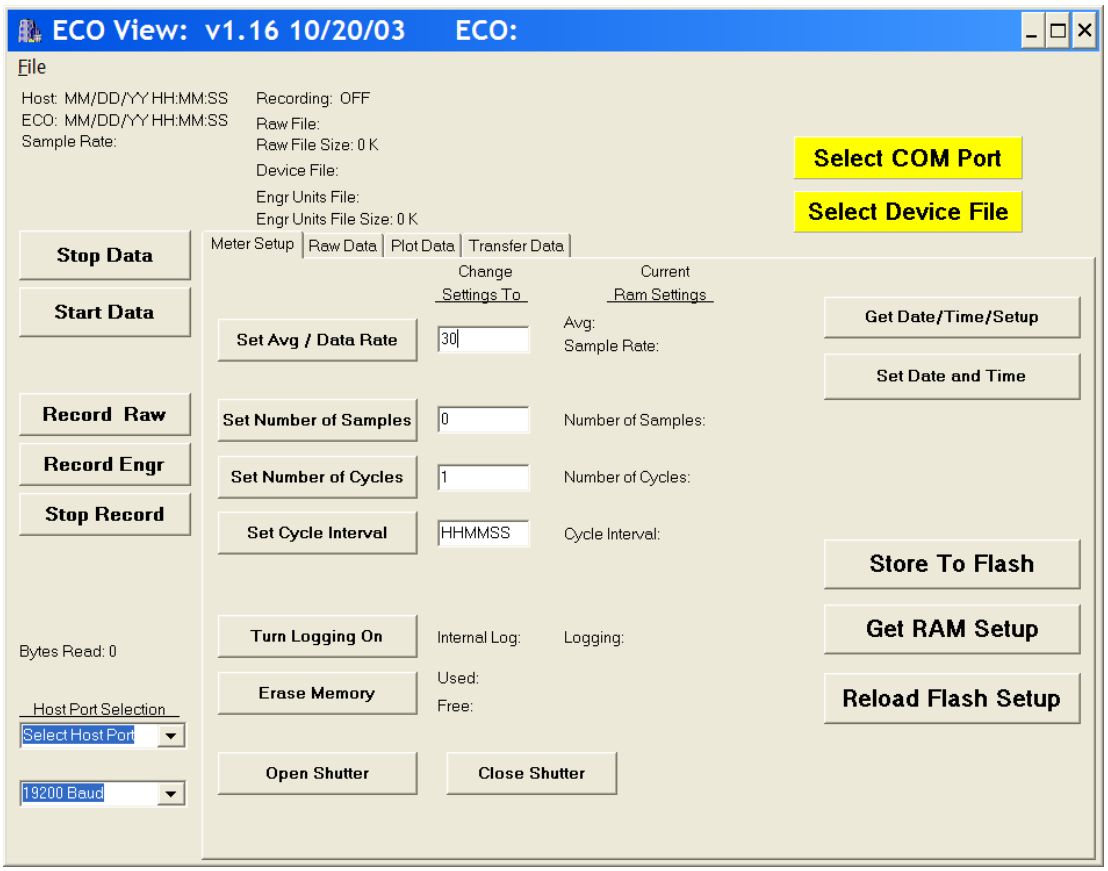
Two bright blue LEDs (centred at 455 nm and modulated at 1 kHz) provide the excitation source. A blue interference filter is used to reject the small amount of red light emitted by the LEDs. The blue light from the sources enters the water volume at an angle of approximately 55–60 degrees with respect to the end face of the unit. Fluoresced light is received by a detector positioned where the acceptance angle forms a 140-degree intersection with the source beam. A red interference filter is used to discriminate against the scattered blue excitation light. The red fluorescence emitted is synchronously detected by a silicon photodiode.

Turbidity is measured simultaneously by detecting the scattered light from a 700 nm LED at 140 degrees to the same detector used for fluorescence. The turbidity measurement is performed at the same 140 degree angle as the chlorophyll fluorescence.

FLNTU units are sent to IMBROS (Australian based service agent) after 12 months of in-water time for servicing and calibration.

ECO View Software

ECO View software has a manual available on the SeaBird website. The below instructions outline use of this software for the MMP project.



ECO View is a program created by WetLabs to interface with the FLNTU Units. Begin by plugging a 6-pin F Subconn to serial or USB-Serial cable into your PC and finding the COM port number of the cable in the systems device manager. Prior to connecting to the instrument, open ECO View, and select the correct COM port. A device file does also need to be selected, though MMP processing does not utilise this software to calculate engineering units hence it is not important for this to be correct to the instrument.

With a 9 V battery connected to the serial cable, plug into the instrument with the 6-pin subconn connection. The wiper should rotate 180 degrees, and the sampling window should flash LED lights. Click the “Stop Data” button multiple times during this sample to open comms with the instrument. The Host and ECO time should appear in top corner, as well as the current instrument RAM settings.

Performing Dark Counts

Each instrument will capture data in raw “counts”, which will need coefficients applied for conversion to engineering units. These instrument coefficients can shift over time and between services, and thus need to be “Field Characterized” prior to each deployment. Dark Counts are a “zero” measurement, taken when the unit should not receive a value, which is used to set the baseline offset on the engineered results.

To perform a Dark Count, set up instruments as follows:

- Wiper removed
- Collar on
- 2 layers of black electrical tape over the optical window
    - Set Avg / Data Rate = 50

  - Set Number of Samples = 1
  - Set Number of Cycles = 0
  - Set Cycle Interval = 000007 (up to 000057)
- Turn Logging On

Place all instruments receiving dark counts into an instrument rack and turn on all units with a red “hot” plug. Immerse instruments in conditions similar to intended deployment location for approx. 3 hours. Note: Be sure to remove the electrical tape after dark count is complete.

Download and plot all raw count data in Excel – the Dark Count value will be the lowest consistent measurement (i.e. non outlier spike) during the submerged period.

All factory and in-house calibration values are stored in a master calibration document. Field dark count values are used in conjunction with factory scale factors to convert raw count data into engineering units of µg L^{-1} for chlorophyll and NTU for turbidity.

Device files (.dev)

Device (.dev) files are text-based files which store calibration coefficients (scale factors and dark counts) for each individual FLNTU. These files are unique to each calibration event for a FLNTU (e.g., each time it is factory or in-house calibrated, a new .dev file will be required to process data files). Below is an example of a .dev file for a particular FLNTU.

WHI4_REC_20240214_FLNTU-827.dev - Notepad

File Edit Format View Help

```
ECO     FLNTUSB-827
Created on:     24/01/2023

COLUMNS=7
N/U=1
N/U=2
N/U=3
Chl=4   0.0132  34
N/U=5
NTU=6   0.0216  50
xtemp=7 71.443205       -0.005595
```

Prepare for Deployment

To prepare FLNTUs for MMP deployment, run through the following procedure:

- Pre-setup
  - Consult deployment plan to establish locations for each instrument.
  - Perform dark count (see Performing Dark Counts)
- Drop Sheet

MMP FLNTUSB Dropsheet

FLNTU Hardware Information	
Logger ID	
Firmware	

Trip Information		*Mark R above for recovery*
Trip Number		
Trip start date		
Site ID		

FLNTU Setup Information		
Setup date		
Sync time		*Programming checklist*
New batteries?	☐	*Average: 50*
Time sync?	☐	*Number of sample: 1*
Memory clear?	☐	*Number of cycles: 0*
Set date & time?	☐	*Cycle interval: 00095X*
Connect test lab?	☐	
Time Interval		*Logging: On*
Voltage (Vin | Vpins)		*Used:0*
Time zone EST?		*Free:1055K*

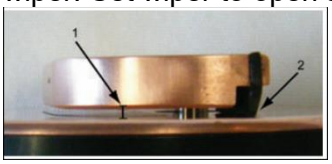
FLNTU Deployment Information		
Deployment technician		**If on mooring, use discretion to decide last good and first good data points*
Deploy Voltage (Vpins)		
Logger Date on		
Logger Time on		
WQ Station		
Time wet *(dive start)*		*.=recovered last good data*
Time stable *(dive end)*		*.=recovered time on deck*
Deployment comments		

FLNTU Recovery Information		
Recovery Trip Number		**Logger status**
Recovery Technician		
Recovery Date		
Recover WQ number		
Last good data *(dive start)*		
Time on deck *(dive end)*		
Time off		
Shutter status		

FLNTU Data Download Information		
Host date/time (atomic)		
Logger Date/time		
Wiper function		
File Size		
Memory used / free		
Downloaded filename		*SITE_REC_yyyymmdd_FLNTU-serial*
Voltage out (Vpins)		
Data comments		

-
- Physical set up
  - Place collar on if unit will be deployed by divers, remove collar for mooring.
  - Start and fill out dropsheet
  - Install new set of batteries, measuring within .05 V of each other
  - Install new (dry) silica packet
  - Inspect harness and optical face
  - Install a clean and functional copper faceplate
- Programming

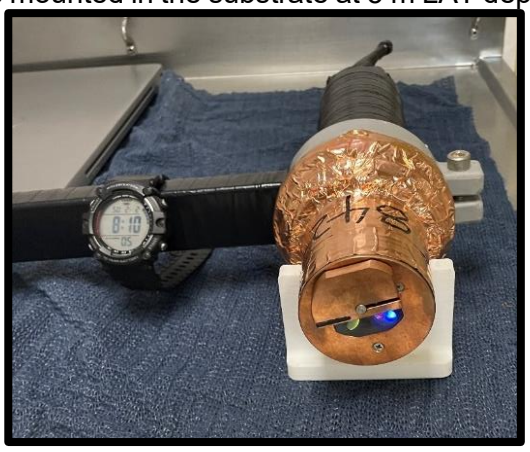
- Erase Memory
- Confirm wiper functionality with Open / Shut Wiper
  - Set Avg / Data Rate (MMP Precedent) = 50
  - Set Number of Samples (single reported sample) = 1
  - Set Number of Cycles (indefinite) = 0
  - Set Cycle Interval (10 minutes) = 000957
- Confirm Logging On
- Set Date and Time

- Physical field prep
  - Wrap whole instrument with plastic wrap and electrical tape, then place copper tape around the sensor end of the body to protect from excess biofouling.
  - Attach wiper
    - The wiper should be placed such that the blade edge is just touching the sensor face, with minimal bend in the rubber.
    - It is important not to rotate the wiper shaft by hand. The wiper motor contains fine toothed gears which can be damaged by force.
    - Reconnect to unit via ECO View. Set wiper to closed and carefully attach wiper. Set wiper to open and confirm positioning, adjusting if necessary.
    - 

  - Place a white protective cap on the instrument and put a dummy plug in both subconn connections. The instrument is now ready for field deployment.

FLNTU deployment

FLNTUs are attached using custom clamps to pickets mounted in the substrate at 5 m LAT depth. Each clamp will have a wrapped FLNTU mounted in the circular opening, and a temperature logger (if applicable for site) zip-tied along the clamp arm. The clamp should also have a permit tag attached to it such that it will not interfere with the instrument.



In the hour prior to conducting the logger dive, the instrument should be turned on with a “hot” red plug (ideally at a neat 10 minute interval) and the dropsheet should be filled out. The wiper functionality should be confirmed on the surface. The 10 minute interval should be noted so that divers know when to check for subsequent wipes.

During the dive, the FLNTU should be laid down on its side in a clear area, so the wiper is not impeded. The existing logger clamp should be removed, and the picket cleaned and assessed for degradation. The new logger should then be attached in the same orientation, and wiper functionality should be confirmed again once in place. Care should be taken with the recovered FLNTU for the remainder of the dive until the wiper can be turned off.

As soon as practicably possible, the instrument should be turned off by removing the “hot” red plug and replacing with a green dummy plug. Users should then be able to find the end of the electrical tape and proceed to unwrap the instrument (alternatively making a small cut by scraping the blade on the wrap near the plug end and unwrapping from here). A white safety cap should be attached to the sensor head of the unit to protect the wiper and optics. The FLNTU should be rinsed well with fresh water, and placed somewhere safely to dry. The clamp components should be initially scrubbed, then left to soak in a 50% vinegar solution to enable further cleaning.

Downloading .raw file

Prior to downloading the files from a FLNTU, locate the instrument dropsheet and ensure recovery metadata is up to date. Assess the instrument state, including its wiper position, battery voltage and optical face condition. Remove the wiper prior to connecting to the download computer to avoid any scratches from wiper debris which may still remain.

Plug in the instrument and take note of the presence/absence of flashing LEDs on the optical face, as well as the wiper shaft rotation. Click STOP 5 times during the LED pulses to stop the instrument from sampling and open a connection to the instrument.

Fill in the required metadata on the dropsheet, including the instrument time, sample size, and voltage. Also take the opportunity to reconfirm that the instrument was correctly setup:

- Avg / Data Rate: 50
- Number of Samples: 1
- Number of Cycles: 0
- Cycle Interval: 000957
- Logging On

Navigate to the “Transfer Data” tab, and select “Receive Data”, following the below file naming structure. Downloads may take multiple minutes, the “Receive File Status” will change to “Complete” when the transfer in finished.

Note: .raw file and .dev file should be named the same, and should always be located together.

- Structure: SHORTNAME_REC_YYYYMMDD_FLNTU-SERIAL.raw
- Example: WHI4_REC_20240214_FLNTU-827.raw
- REC in this instance denotes recovery date

Sea Bird SBE37

The SeaBird SBE 37 MicroCAT is a high-accuracy conductivity and temperature (pressure optional) recorder with RS-232 interface, internal batteries, data storage, and optional pump. The MMP has 9 sites with SBE 37s deployed, utilising a fleet of instruments maintained and managed by the Oceanographic team. This section will provide an overview of the procedures and considerations involved with the SBE 37 fleet. SBE 37 units are sent to SeaBird after 12 months of in-water time for factory servicing and calibration.

Prepare for deployment

Prior to deployments, SBE 37s are loaded with a set of new SAFT LS14500 batteries as per manufacturer specifications. Instruments are wrapped with plastic wrap, then duct tape to protect against biofouling. Copper tape is also placed around the sensor inlet and outlets to further reduce biofouling risk to these areas.

Pre-run checks / Programming for Deployment

The MMP has a specific SBE 37 drop sheet, which can be stepped through to conduct pre-run checks and program for deployment. The AIMS SBE 37 fleet is made up of instruments running different firmware. Those running firmware versions below 3.0 will need to use the software Seaterm V1, where as all later models will use Seaterm V2 for programming.

MMP SBE 37 Dropsheet

Trip Information	
Trip Number	
Trip Dates	
Site ID	

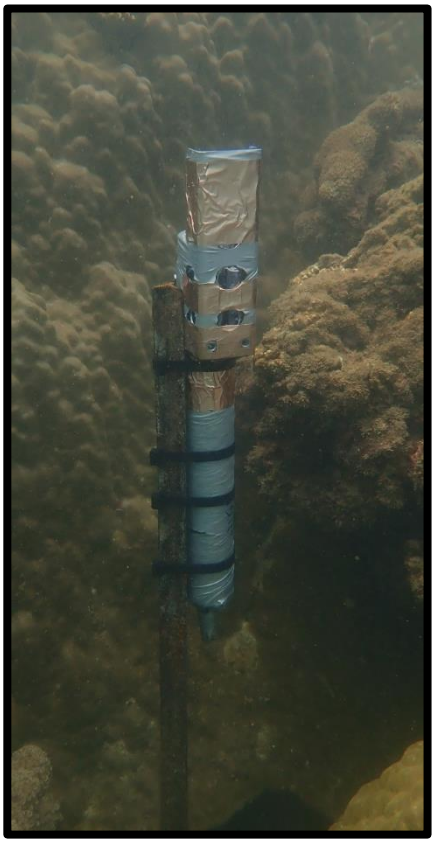
AUSTRALIAN INSTITUTE OF MARINE SCIENCE

SBE Hardware Information	Minimum Sample Interval (6 months)
Intrument Model	
Serial Number	
Firmware	

SBE Setup Information		Commands @ S>
Baud Rate (select which)	9600 14400 19200 38400 115200	
Status		ds
Calibration Date		
battery voltage	V Main	V Lith
Set instrument Date & Time	DateTime=mmddyyyyhhmmss (**EST**) or SetLocalTime from Menu	
Initialize memory		SampleNumber=0
Set interval		SampleInterval= **600**
Set Salinity Output		OutputSal=1(yes)
Set Real Time TX		TxRealTime=0(no)
Check MinCondFreq	(should be around 3000)	(zero conductivity frequency + 500 Hz)
Test Pump	****immerse conductivity cell****	
		PumpOn
		PumpOff
Check/clean bleed port		
Test deployment/download		File format: Serial#YYMMtest
Set start Date & Time	StartDateTime=mmddyyyyhhmmss (EST)	
Start later		StartLater
Set QS State		qs
Setup Technician & PC		

Instrument can now be prepared for deployment

SBE 37 deployment



SBE 37s can run for an extended period out of water, and should not need specific pre-dive checks completed. The optional pump is the only moving part of the instrument, and will not run unless the conductivity sensor has an appropriate reading.

Prior to deployment, divers should familiarise themselves with the required orientation of the specific instrument. They will also need to ensure they have snips and at least 4 large zip-ties. A catch bag is available for transporting the SBE 37 to the 2 m picket location, enabling the diver to keep one hand free for BC control.

SBE 37s are attached to pickets mounted in the substrate at 2 m LAT depth. Similar to the FLNTU, lay the new instrument in a safe location nearby, and begin by removing the existing unit by cutting the zip-ties. Attach the new unit in its intended orientation, and return to the surface.

Once returned to the vessel and time available, note the condition of the inlet and outlet ports for the pump. Remove the outer wrapping from the instrument and discard, and scrub down the main body of the unit in fresh water with a soft bristle brush. Remove the cage and hardware from the unit and soak / scrub in vinegar. Wear appropriate PPE and remove the spent TBT cartridge, storing in the appropriate waste container. Use a scouring pad to carefully scrub around the conductivity tube and remove as much of the buildup / excess Sudocrem as possible. Leave to dry, reassemble and store safely.

Downloading .hex file

The MMP has a specific SBE 37 drop sheet, which can be stepped through to complete instrument download. Instruments running firmware versions below 3.0 will need to use the software Seaterm V1, whereas all later models will use Seaterm V2 for download.

SBE Data Download Information		*Commands @ S>*
Recovery Trip Number		
Recovery Trip Dates		
Recovery Technician		
SBE status		*ds*
Instrument stop date/time		*"stop" YYYY/MM/DD HH:MM:SS (EST)*
Clock Check GPS		
Clock Check SBE		
Clock drift		*+/- HH:MM:SS*
Number of samples		
Download default hex file. Use SBE datcnv to convert to cnv file. Default vars are "timeK: Time, Instrument [seconds]", "tv290C: Temperature [ITS-90, deg C]", "cond0S/m: Conductivity [S/m]", "Salinity, Practical [PSU]". If instrument has a pressure add "prdM: Pressure, Strain Gauge [db]".		
Downloaded filename		*SITE_REC_YYYYMMDD_SBE37_SERIAL*
Comments		
		Updated Aug24

Connect the appropriate cable to the users PC, and identify the relevant COM port using the device manager. Open Seaterm and configure the connection parameters, including instrument type (SBE 37 RS232), COM port and BAUD rate. Connect to the instrument, and type “ds” to display status. Note the concurrent time of an atomic clock (www.time.is) against the internal clock with another “ds” to log the time drift. Instruct the instrument to stop logging with a “stop” command. Upload the data file with the naming convention below.

- Structure: SHORTNAME_REC_YYYYMMDD_SBE37_SERIAL
- Example: WHI4_REC_20240214_SBE37_9283
- REC in this instance denotes recovery date

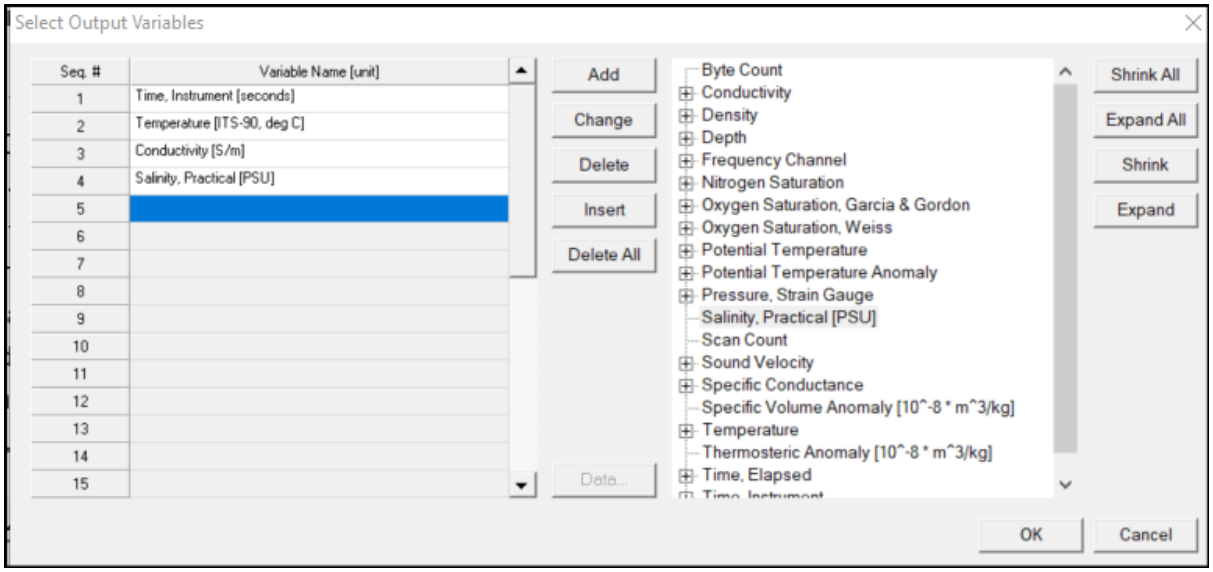
Conversion

The hex file downloaded directly from the SBE 37 needs its contents converted from strings and calibration coefficients applied to the resultant values. The software SBEDataProcessing uses the instruments configuration file (.xmlcon) for this process. Open the software, and select “Run” – “1. Data Conversion”.

Select the Instrument configuration file, the input directory and the input file in their respective boxes. Output directory can be made to match the input directory when prompted.

To confirm the output variables, navigate to the Data Setup tab – Select output variables and ensure they are as below (include depth if applicable)

- # name 0 = timeK: Time, Instrument [seconds]
- # name 1 = tv290C: Temperature [ITS-90, deg C]
- # name 2 = cond0S/m: Conductivity [S/m]
- # name 3 = sal00: Salinity, Practical [PSU]
- # name 4 prdM: Pressure, Strain Gauge [db] (if applicable)



Confirm choices and click “Start Process” – The .cnv file should be located in the same directory as the input .hex file.

Appendix A11: 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.

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.

12.2 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.

12.3 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.

12.4 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.

12.5 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.

Each evening photo transect images are copied from the cameras to laptop computers and renamed with the reef, site and depth from which they belong and sorted into folders for each individual transect. The raw and renamed and sorted versions of the day's images are backed up to an external hard drive. Upon return to the office, the data is uploaded to an Oracle Database using the Oracle Lite synchronization process. All keyed data are 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 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.

12.6 FLNTU (Fluorescence and Turbidity) and SBE37 (Temperature and Conductivity) loggers

For protocols pertaining to MMP logger data prior to 2023, see previous MMP QA/QC reports. Metadata from all field trips with associated instrument deployments are stored using OceanDB, a custom Microsoft Access database developed by the AIMS Physical Oceanography team. Information is entered into OceanDB post-trip, and cross checked for accuracy. OceanDB deployment data is subsequently used to populate NetCDF metadata, as well as informing deployment time shifts / trimming required to automated QC routines. The IMOS Matlab Toolbox provides a semi-automated, easy to use interface for converting raw instrument data into IMOS compatible Quality Controlled NetCDF files. The MMP has begun QCing data using IMOS Toolbox due to its ability to provide robust metadata, and more nuanced flagging to our data.

The following pre-processing routines are run on each dataset:

- timeOffsetPP – Used to check and apply UTC correction requirement from database
- timeMetaOffsetPP – Used to check and apply UTC correction to metadata
- timeDriftPP – calculates and applies time drift from DB to dataset

The following automated QC routines are run on each dataset:

- ImosImpossibleDateQC
- ImosImpossibleLocationSetQC
- ImonInOutWaterQC
- ImosGlobalRangeQC
- ImosImpossibleDepthQC
- imosHistoricalManualSetQC

After running Auto QC, users can manually investigate the data channels, and flag any data points required with a range of flag levels.

- 0 :No QC performed
- 1: Good data
- 2: Probably good data
- 3: Bad data that are potentially correctable
- 4: Bad data
- 5: Value changed
- 6, 7, 8: Not used
- 9: Missing value

Data are initially observed for erroneous data in the form of step changes, or single point outlier spikes, as well as compared to historic data to give site specific comparison to readings. Data are then manually verified against a range of publicly available data sources to inform and contextualise data trends and features. Below is a non-exhaustive list of comparison data sources for each focus region.

Fitzroy

- Daily satellite imagery
- AIMS research vessel underway thermosalinographs
- AIMS temperature logger program
- 3 x FLNTU sites
- 2 x SBE 37 sites
- Wave buoy (Emu Park)
- River discharge (Fitzroy at The Gap)

O'Connell

- Daily satellite imagery
- AIMS research vessel underway thermosalinographs
- AIMS temperature logger program
- 4 x FLNTU sites
- 2 x SBE 37 sites
- Wave buoy (Mackay and Abbot Point)
- River discharge (OConnell at Staffords crossing)

Burdekin

- Daily satellite imagery
- AIMS research vessel underway thermosalinographs
- AIMS temperature logger program
- 4 x FLNTU sites
- 1 x SBE 37 site
- Wave buoy (Townsville and Abbot Point)
- Port of Townsville Marine buoy array
- River discharge (Burdekin at Clare, Alligator creek at Allendale, Herbert at Ingham)

Tully

- Daily satellite imagery
- AIMS research vessel underway thermosalinographs
- AIMS temperature logger program
- 2 x FLNTU sites
- 2 x SBE 37 sites
- River discharge (Tully at Euramo)

Russell-Mulgrave

- Daily satellite imagery
- AIMS research vessel underway thermosalinographs
- AIMS temperature logger program
- 4 x FLNTU sites
- 2 x SBE 37 sites
- Wave buoy (Cairns)
- River discharge (Russell at Bucklands, Barron at Myola)

Instrument data are also validated against concurrently collected water samples. The relationship between optically measured turbidity and TSS analysed on filters is relatively good 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.

After QC is complete, IMOS Toolbox is used to export a NetCDF file, with SBE 37 files containing variables TEMP, CNDC, PSAL (Pressure if available)) and FLNTU files containing variables CPHL and TURB. IMOS Toolbox will automatically name the files to meet the IMOS naming convention.

- Data code: CSTZ and KUZ identify the types of data in the file.
- Date: Start date of data
- FV01: File version, indicates Level 1 Quality Controlled Data

Data are then uploaded in NetCDF format on the AODN Thredds server.

12.7 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. CTDs record data in a similar format to SBE 37s. For CTD profiles, 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's calibration and configuration 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 QC 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 are averaged into 1 m vertical bins for each parameter.

Once the data have been Bin Averaged, the data are then ingested into an Oracle 9i database.

12.8 Temperature loggers

Temperature loggers are deployed at, or in close proximity to, all locations at both 2 m and 5 m depths and routinely exchanged at the time of coral surveys.

The two types of temperature logger used for the sea surface temperature logger program are similar in structural design and accuracy. The Sea-Bird Scientific SBE 56 high-accuracy temperature logger (https://www.seabird.com/sbe-56-temperature-sensor/product?id=54627897760) has a storage capacity for 15.9 million readings and is set to take readings every five minutes. The RBR high-accuracy temperature logger (https://rbr-global.com/products/compact-loggers/rbrsolo-t-2/) has a storage capacity for 130 million readings and is set to take readings every five minutes. Both the SBE 56 and RBR temperature loggers have an accuracy of ± 0.002 °C. They are calibrated every two years at the CSIRO (Hobart), a NATA-accredited organisation.

Prior to deployment, the temperature loggers are calibrated to within 0.02 °C 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 are downloaded and imported into an Oracle database as raw or level 0 data. To accomplish this task the SBE 56 loggers have proprietary software to download data into a Microsoft Excel xml file via a micro-USB port. This is then exported as an Excel .csv file to a designated repository folder on a server onsite at AIMS. RBR loggers download data using proprietary software via a USB-C port as a proprietary *.rsk file. This is then exported as a Excel .xlsx file to the same designated repository folder on the AIMS server.

The data files in the repository folder are uploaded into the Oracle database using a Java application written by the AIMS Data Centre (ADC). Once in the Oracle environment the data is processed through a QAQC application program written in Java by the ADC. Data from the same location (the flat and the slope) and nearby locations are plotted together for comparison. Any bad points are removed, and any drift in recorded data points is corrected. After this process the corrected data are considered quality controlled or Level1 data. The Level1 data is uploaded to the Amazon Web Services (AWS) cloud environment, into the PostgresSQL database. Data are made available by AWS through Time Series Explorer (TSE), or, when accessing data through R scripts, via the AIMS Data Platform API using an API key.

12.9 Data validation

As the previous section describes, the resulting data from sampling and instrumentation are ingested into several Oracle database schemas. 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 three 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 – are the data within acceptable limits;
- Logical checking – are the data logical (is the value possible);
- Comparative checking – are the data similar to other comparable data (such as previously collected data or better previous equivalent data); and
- 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.

Appendix B Waypoints for Coral Monitoring Transects

Appendix B1 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
		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
			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

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			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
	Herbert Tully	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
		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
			2	2	146.540683	-18.846367
Burdekin	Burdekin		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

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			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
		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
			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

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
		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
		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
			1	5	151.070467	-23.157483
Fitzroy	Fitzroy		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
		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

NRM Region	Catchment	Monitoring location	Site #	Depth (m)	Longitude	Latitude
			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