Water quality — field and analytical procedures

M.J. Devlin and M.J. Lourey

Long-term Monitoring of the Great Barrier Reef

Standard Operational Procedure Number 6

Australian Institute of Marine Science
Townsville
2000

Copies available from:
Science Communication
Australian Institute of Marine Science
PMB No 3, Townsville Mail Centre
Townsville Q 4810. Australia

Long-term Monitoring of the Great Barrier Reef Standard
Operational Procedure: ISSN 1327-0184

National Library of Australia Cataloguing-in-Publication data:

Devlin, M.J.
Water quality - field and analytical procedures.

Bibliography.
ISBN 0 642 3224 2.

1. Water quality - Queensland - Great Barrier Reef -
Measurement. 2. Water quality management - Queensland -
Great Barrier Reef. I. Lourey, M.J. II. Australian Institute
of Marine Science. III. Title. (Series : Long-term monitoring
of the Great Barrier Reef standard operational procedure ; no. 6).

333.91009943

CONTENTS

LIST OF FIGURES AND TABLES

PREFACE

This document describes the field and analytical techniques used by the Water Quality personnel in the Long-term Monitoring Program at the Australian Institute of Marine Science. A description of the program is detailed in Oliver et al. (1995). Part one describes the various procedures used to collect and store water samples from sites on the Great Barrier Reef. Analytical procedures for parameters, including dissolved nutrient species, salinity, suspended solids and chlorophyll, are described in Part two. This document is intended as a guide only, and some modifications may be required to suit other brand names.

INTRODUCTION

Water Quality field sampling is conducted in conjunction with surveys of reef benthic communities, crown-of-thorns starfish and reef fishes. Water quality sampling forms part of the Long-term Monitoring Program (LTMP) which has the following objectives:

1. To monitor the status and trends in the distribution and abundance of reef biota and in water quality, on reefs of the Great Barrier Reef (GBR).

2. To provide environmental managers (and other decision makers) with information that is pertinent to managing the GBR according to the principles of ecologically sustainable use.

The majority of field trips are carried out on the *RV Sirius,* an Australian Institute of Marine Science (AIMS) vessel. Details of field procedures are specific to sampling cruises aboard the *RV Sirius,* however these techniques can be readily adapted for use on other vessels. This document sets out the Standard Operational Procedures for the completion of the field work and the analysis of water samples for dissolved nutrients, salinity, suspended solids, chlorophyll *a* and phaeophytin. Adherence to these procedures ensures that high quality data is collected.

PART 1: FIELD PROCEDURES

SAMPLING DESIGN

Water quality, reef fish, benthic communities, and crown-of-thorns starfish are surveyed annually within six sectors of the Great Barrier Reef (Cooktown/Lizard Island, Cairns, Townsville, Whitsunday, Swain and Capricorn Bunker sectors). In each of these sectors (with the exception of the Capricorn Bunker sector) three shelf positions (inner, mid and outer) have been identified. Three reefs are nested within each of these shelf position/sector combinations. In the Capricorn Bunker sector, only outer shelf reefs are represented, with six reefs being surveyed. Shelf position is determined by the position of the reef relative to the coast and continental slope, with inner shelf reefs closest to the coast. These reefs are the survey reefs for the Long-term Monitoring Program. Water sampling is conducted twice at two sites near each survey reef. There are also water sample sites that are additional to the survey reefs, where only one sample is taken. The total number of water samples collected will depend on the sampling design being used. There are two basic designs for sampling. A site may have replicate casts (drops), with duplicate subsamples taken from each bottle, or only one cast and no duplicates. Replicate casts require four Niskin bottles, over two drops, with duplicate top and bottom samples (Table 1.3). Single casts require only two Niskin bottles for only one drop, with single top and bottom samples (Table 1.4).

QUALITY CONTROL

Procedure

1. Clean all field equipment with deionized fresh water before and after each field trip.

2. Keep the laboratory clean throughout the trip. During sampling, unnecessary equipment is to be taken out of the laboratory. If the sink is to be used for other purposes (e.g. cleaning, washing) ensure that it is well rinsed with freshwater after use. Take on board a freezer dedicated to sample storage. *Do not allow the freezer to be used for any other purposes.*

3. Smoking is not allowed in the laboratory at any time or on the back deck whilst sampling is in process.

4. Use common sense in the laboratory and during the sampling process and be aware of potential sources of contamination. These include sweat, sunscreen lotions, washing detergent, clothes, food and fishing equipment.

5. Do not handle the inside of Niskin bottles, nutrient tubes and caps during the sampling and filtering process.

Blanks and standards for nutrient samples

1. Seawater blank is made up at AIMS from reagent grade (AR) NaCl and Super QTM water (36 g/litre). The seawater standard has a concentration of 4 µmol for nitrite-nitrate and ammonia, 2 µmol for phosphate and 20 µmol for silica.

2. Before each trip, fill six 10 mL polypropylene tubes with seawater blank and a further six with seawater standards. These will be stored for the duration of the voyage either on the vessel or at AIMS (Table 1). Four blanks and four standards will be stored frozen, whilst the remaining blanks and standards will be stored at room temperature. *These blanks and standards will demonstrate whether contamination of samples is occurring through the storage stage.*

3. One litre of the seawater blank is stored on the boat at room temperature for the duration of the sampling trip. Dispense seawater blanks into appropriately labelled polypropylene tubes after every fourth sample taken throughout the trip (Tables 1.1 and 1.2). *This will account for any contamination occurring during the sampling and filtering process.*

Table 1. Labelling of blanks and standards for quality control of nutrient samples.

	Sample type	Storage	Ship storage	AIMS storage
Blank	Total	frozen	CCIT*	CC4T
	Dissolved	frozen	CC2D	CC5D
	Silica	room temp.	CC3S	CC6S
Standards	Total	frozen	CC7T	CC10T
	Dissolved	frozen	CC8D	CC11D
	Silica	room temp.	CC9S	CC12S

** CC is two letter code which is unique for each sampling trip. The number and letter following, differentiate between the blanks and standards.

SAMPLING METHODS

Equipment

- 8 L lever action Niskin bottles (4)
- Niskin rack
- Reversing thermometers (2)
- Magnetic bars (2)
- Thermometer housing (4)
- Aluminium alloy single 20 cm, 8.3 cm bore, “A” section pulley
- Dump weight (1kg)
- Messengers - enquire AIMS marine store (3)
- Secchi disc (30 cm diameter and divided into black and white quadrants)
- Field data sheets

Procedure

1. At a sampling site, record acoustic depth as measured by the depth sounder. Record weather details and geographical position on the field data sheet (Appendix 2). Note reef or station name, date and time. Observe water surface for presence of trichodesmium and record observations on data sheet.

2. Take a secchi disk reading from the sampling side of the vessel and record value on data sheet.

3. The Niskin bottles are set for sampling by locking the top and bottom lids into the open position and connecting the thermometers to the front of the bottle. *The reversing thermometers are secured within a holder which is screwed to the bottle. Care should be taken when attaching the screws to the Niskin bottle.*

4. Reverse the thermometers by turning them upside down until they click into place. Set the thermometer by swiping with a magnet three times at 2-3 second intervals. On the first swipe the thermometer display will read ‘hold’. The second will read ‘continuous’ and the third swipe will set the thermometer up for sampling (the display reads ‘sample’).

5. A hydro cable consisting of a capstan and a drum containing stainless steel wire is used to lower the Niskin bottles. The wire is run along a boom which extends from the side of the vessel. It is lowered through a meter wheel which measures the length of wire released. The meter wheel is calibrated from the length of wire run out over a specified time. Attach the dump weight to the end of the wire. *The cable should be kept clean at all times to minimize contamination from oil or rust and should be replaced if rust sets in.*

6. Take the first Niskin bottle from the rack, set and attach it to the wire, approximately half a metre above the lead weight. Lower the wire until the Niskin bottle is 7 metres above the acoustic depth. Attach the second cocked Niskin bottle to the wire. Lock the messenger onto the quick release pin located just below the top Niskin bottle. Allowing one metre for the freeboard of the boat, lower the Niskin bottle 4 metres from the side of the boat. *Sampling depths will thus be three metres from the bottom and three metres from the surface.*

7. Wait approximately two minutes for the reversing thermometers to equilibrate with the surrounding water, then attach and release a second messenger down the wire. Place a hand on the wire to feel the vibrations as the Niskin bottle fires. *The messenger triggers the closing mechanism on the Niskin bottle, reverses the thermometers to take the* in situ *temperature reading and releases the attached messenger to trigger the lower Niskin.*

8. Wait 10 seconds and winch the bottles up. Release each Niskin bottle from the wire and replace in the bottle rack. Secure the bottles in the rack with elastic cord.

9. Read the *in situ* water temperature by swiping a magnet over each reversing thermometer. Immediately record the temperature and time on the field data sheet.

10. Commence laboratory water sampling procedures once the Niskin bottles have been secured in the rack.

LABORATORY PROCEDURES

Equipment

- Extech N-07061-22 vacuum pump
- Extech N-07061-32 regulator for vacuum pump
- Water trap
- Rubber hoses (2)
- Siphon hose
- Nalgene 315-0047 47 mm filter units (4)
- Nalgene 3804203 25 mm filter units (4)
- Rubber stoppers (4)
- Forceps (3)
- 100 mL measuring cylinders (4)
- 100 mL displacer
- 1L plastic bottles (8)
- 500 mL wash bottle
- Magnesium carbonate powder
- 10 mL polypropylene natural cap nutrient tubes (as required)
- Uniwire racks (as required)
- Terumo syringes s/use 50 mL (as required)
- Minisart 114024 filter devices, 0.45 µm (as required)
- Poretic filters, 47 mm * 0.4 µm (as required)
- Whatman GF/F filters, 25 mm (as required)
- 500 mL plastic beaker
- 500mL plastic bottles (as required)
- Nally bins (8)
- Milli Q™ de-ionised water
- Aluminum foil
- Pen lumocolour (3)
- Paper tape (3)
- Quartz vials (as required)
- Scissors
- Tape dispenser (2)
- Marker pens, black (4)

- Field data sheets
- Pelican case for thermometers
- 150 L freezer
- Artificial sea water blank 2.5 L
- Standard artificial seawater
- LTMP Water Quality site list

Procedure

Open the vent knob at the top of the first Niskin bottle to allow water flow. Attach a rubber hose to the outlet spout and start flow by pulling the spout out. Allow water to flow through the spout for at least three seconds to clear any debris or contamination.

A. Nutrients

1. Pre-label the acid-washed 10 mL tubes (Tables 1.1 and 1.2) and place in a clean rack (numbers depend on sampling regime). Water for nutrient samples should be taken first to minimize any risk of contamination. Remove the lids of the nutrient tubes and lay upright to avoid contamination from the bench. Rinse the tubes twice with the water from the Niskin bottle. Flick the sample tubes dry and place back in the rack.

2. Rinse a 50 mL syringe, (connected to a 0.45 µm filter device), with seawater from the first Niskin bottle. Fill the syringe completely and insert the plunger. Gently push the plunger down until a steady flow occurs, allowing at least 10 mL of the sample to flow through the filter. Without stopping, place the syringe over the top of the first rinsed nutrient tube and gently push the plunger down until the tube is filled to 80% capacity. Do not draw back on the syringe while the filter is in place as this will displace the filter paper.

3. Repeat until the labelled nutrient tubes have all been filled to 80% capacity. Close tubes and place in the rack until all sampling has been completed. Do not overfill tubes as they may burst when frozen. Replace filter device after every 8 samples.

4. If duplicate subsamples are being collected, repeat steps 1 and 2 with the same

Niskin bottle. If only one subsample is required, carry out steps 1 and 2 with the next Niskin bottle.

5. For each set of nutrient samples per site, dispense three seawater blanks in the labelled tubes (Tables 1.1 and 1.2). One blank will be used for total dissolved nutrients, one for dissolved inorganic nutrients and one for silica analyses.

6. Once nutrient subsampling has been completed, place the racks of tubes in their designated storage areas. Total and Dissolved subsamples and corresponding blanks are stored frozen. Silica subsamples and corresponding blanks should be stored at room temperature.

B. Chlorophyll *a* and Phaeophytin

1. Rinse the 100 mL measuring cylinders (labelled from 1 to 4) twice with water from the appropriate Niskin bottle. Fill each cylinder from the Niskin bottle and expel the excess water using the teflon displacing cap.

2. Add 0.1 - 0.2 mL MgCO3 (10 g/L) to the sample prior to filtration. This buffers the sample against low pH, which can cause degradation of chlorophyll into phaeophytin during storage.

3. Pour the collected sample into a filter funnel and filter through a 2.5 cm diameter GF/F filter paper (glass microfibre filter). Filter sample under low vacuum pressure (<1/3 atm). Fold the filter paper in half using forceps to avoid loss of sample.

4. Place the folded filter paper carefully onto a piece of aluminium foil. Wrap the foil around the filter paper, avoiding touching the filter paper with your fingers. Label and freeze the wrapped filter papers.

C. Suspended solids

1. Label the 1 L plastic bottles, rinse twice with water from the appropriate Niskin bottle, then fill to the one litre mark.

2. Place the preweighed polycarbonate membrane filters on the filtering apparatus. This consists of a 47 mm diameter filter funnel and base connected by a clamp.

3. Filter sample under low vacuum pressure (<1/3 atm) until dry.

4. Rinse thoroughly with approximately 10 mL of deionized water to remove particulate matter adhering to the funnel and to wash salts out of the filter paper.

5. Once dry, turn the vacuum off, remove the funnel and fold each filter paper in half using fine forceps. Place each filter paper back into its labelled scintillation vial. Store vials in a box at room temperature.

D. Salinity

1. Label the 700 mL plastic bottles and rinse the bottle and lid twice with water from the appropriate Niskin bottle.

2. Fill the bottle to the top to avoid air bubbles from forming.

3. Place Parafilm™ over the opening of the bottle and screw lid on tightly.

4. Store the bottles in a dry environment at room temperature until returned to the laboratory (refer to Part 2).

PART 2: ANALYTICAL PROCEDURES
NUTRIENTS

Ultraviolet oxidation for dissolved nutrient analysis

Phosphorus occurs in water samples as free or esterified phosphates-orthophosphates, polyphosphates and organically bound phosphates. The major forms of nitrogen present in seawater are nitrate, nitrite, ammonia, organic nitrogen and particulate nitrogen (Franson et al. (eds)1980). The inorganic species of phosphorus and nitrogen can be readily analyzed using a variety of methods, but organically bound forms must also be measured to determine the total amount of dissolved phosphorus or nitrogen in a sample. This is done by converting organically bound forms to a more readily analyzed form. There are a number of methods in use to oxidize dissolved organics in water samples. These include acid digestion methods to hydrolize esterified phosphorus in samples, and kjeldahl digestion for organic nitrogen (Wangersky & Zika 1978). The method described here utilises strong ultra-violet light to simultaneously photo-oxidize organic nitrogen and phosphorus fractions. This is a technique that is commonly used to oxidise organics in seawater samples (Manny et al. 1971). The technique uses a high intensity ultraviolet light source to irradiate samples so the organic nitrogen is oxidised to nitrate and nitrite, while organic phosphorus is converted to orthophosphates. Strickland and Parsons (1972) stated that this method gave an accurate and precise indication of organically bound nutrient fractions.

Synopsis of the technique

Filtered water samples are stored frozen in 10 mL, acid-washed nutrient tubes. Before analysis, water samples are thawed and placed under ultra-violet photo-oxidation to convert organic nutrient components to inorganic forms. Samples then undergo colourimetric analysis using a flow-through auto-analysis system. As the samples are initially filtered to remove particulates the final result gives total dissolved (or filterable) phosphorus and nitrogen. *The dissolved inorganic value is subtracted from the total to give the organic value*. These results are given to the database manager and transferred to an ORACLE database. The particulate forms of both nitrogen and phosphorus are presently not measured due to limited ship and laboratory time.

Equipment

- La Jolla Scientific Co. Ultra-Violet Photo-oxidation Unit.
- Silica sample vials (24)
- Silica stoppers with teflon sleeves (24)

Method

1. Place frozen nutrient tubes into microwave oven for two minutes on 'high' setting to thaw samples.

2. Remove cap from each nutrient tube and pour entire contents into a clean, dry quartz sample vial. *Ensure no hand contact is made with top of nutrient tube or inside of cap.*

3. Select a silica stopper fitted with a teflon sleeve. *Care should be taken to avoid touching the stopper surface*. Place stopper tightly into quartz sample vial and recap the plastic nutrient tube.

4. Place quartz sample vial in holders in the pattern illustrated in Figure 1.

5. Load first holder into the slot marked No. 1 on the photo oxidation unit and continue loading subsequent holders in a clockwise direction.

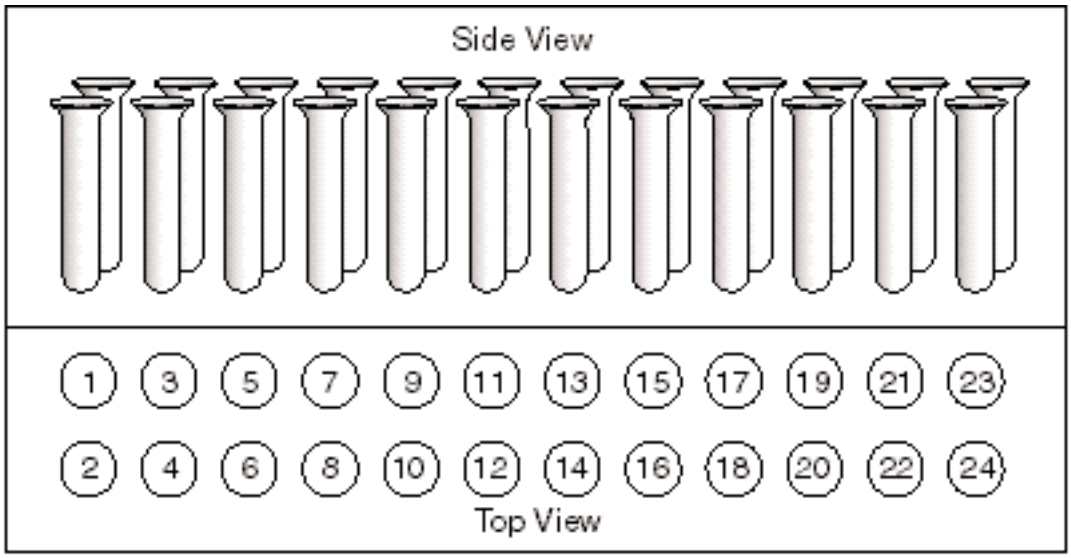
6. Set the power and lamp switches to the 'on' positions. Place the timer switch on automatic and set the timer to 7 hours.

7. Upon completion of the oxidation period, remove holders from photo-oxidation unit and remove the individual quartz sample vials. Transfer contents of vials back into original nutrient tubes.

8. Refreeze samples to await inorganic nitrogen and phosphorus analyses.

9. Wash quartz vials and stoppers thoroughly using Super Q™ water and place into 60°C oven to dry.

Figure 1. Standard method used to load holders with sample vials.



CHLOROPHYLL

Background

Estimation of chlorophyll *a*

Plant pigment concentrations in natural waters provide a semi-quantitative index of phytoplankton biomass. From a practical perspective, the pigment most useful for estimating total phytoplankton biomass is chlorophyll *a*. Concurrent concentrations of chlorophyll *b* and *c* are usually much smaller and vary in response to community floristic composition. All chlorophyll-containing materials are fluorescent. When the organisms are microscopic, such as phytoplankton, this fluorescence may be measured directly in bulk water solutions or extracts of filtered materials. In the method outlined below, the concentration of chlorophyll *a* is estimated using a sensitive photomultiplier for detection of long wavelength light (red) fluoresced from pigment extracts irradiated with short wavelengths (blue), (Yentsch & Menzel 1963).

Estimation of phaeophytin

Direct estimations of chlorophyll *a* concentration from fluorescence can be misleading due to interferences caused by the fluorescence of chlorophyll decomposition products (i.e.: phaeophytin). In some circumstances, chlorophyll degradation products can form a significant fraction of the total plant pigment in a seawater sample (Parsons et al. 1984). The concentration of chlorophyll degradation products can be determined by acidification of the original sample and measurement of the decrease in fluorescence. ***Note***. *Other water constituents can also fluoresce which may result in incorrect readings. Studies currently underway are attempting to identify these interferences using HPLC methods (pers. comm. Miles Furnas).*

Synopsis of the technique

Following collection and filtration at sea (refer to Part 1) the chlorophyll samples filtered through Whatman™ GF/F filter papers are individually wrapped in aluminum foil and stored frozen. Filter papers are ground in 90% acetone (V/V) and centrifuged to extract the chlorophyll pigments. The fluorescence emitted from the chlorophyll is measured

directly using a fluorometer. The analogue output is recorded in millivolts using a digital voltmeter. Phaeophytin levels are measured by taking fluorescence readings before and after acidification of the sample. Digital fluorescence readings (as mV) are converted to measurements of chlorophyll and phaeophytin using a spreadsheet.

Equipment

- Turner Designs™ 005R fluorometer
- 10 mL quartz cuvette
- Digital multimeter.
- High speed tissue grinder (Potter Elversham No. 23)
- 90% acetone (AR grade diluted with the deionized water)
- Hettich Rotanta/p™ centrifuge
- Centrifuge tubes (12 mL with caps)
- 6N hydrochloric acid
- 2.5 cm Whatman™ GF/F filter papers
- Chlorophyll data sheets

Method

Use of the Turner Designs™ 005R fluorometer

No internal controls require setting. Past experience has shown that the following settings on the fluorometer are successful with reef and oceanic water samples, when 100 mL water samples are filtered and extracted into 10 mL of acetone.

1. Turn the ON/OFF switch to the ON position at least one hour before reading the samples.

2. Set the AUTO/MAN switch to MAN. When this control is in this position, ranges are changed manually by using the STEP switch.

3. Change the ranges over which the meter can read fluorescence levels by depressing the STEP switch. The ranges are set over X31.6/X10/X3.16/MIN SENS. Range lights located on the front panel reveal which range the setting is on. Read the majority of reef ocean samples under the X31.6 sensitivity.

Minimize sensitivity by adjusting the range if reading is over 0.999.

4. Set the X1 - X100 knob to the X1 position. This demonstrates that the sensitivity of the instrument is as indicated by the range lights.
5. Adjust the BLANK control to minimize the residual or “blank” fluorescence as shown by the front panel meter corresponding to zero.

Use of the Fluke multimeter

Switch the multimeter knob to the V position and read all samples from the digital board. The analog output from the fluorometer (mV) is read with the digital multimeter, eliminating observer error.

Quality Control

Analyze a blank filter paper at the start of every eight single samples or every four duplicate samples.

Treat a clean microfibre filter paper according to the method below.

Place the appropriate mV readings next to the F0 and F1 blank values on the data sheet. Analysis of blanks throughout the procedure will determine machine drift and possible contamination.

Glass and labware to be used for chlorophyll analyses should be kept aside and never used with acids.

Keep all acids (except the 6N HCl) out of the fume cupboard used for pigment analyses. Initial degradation of chlorophyll to phaeophytin is caused by acidification of the chlorophyll molecule, irreversibly replacing the Mg++ with a proton.

The fluorometer is standardised spectrophotometrically (Jeffery & Humphrey 1975) against extracts of pigments from exponentially growing cultures of the diatom Chaetoceros simplex (chlorophylls a and c).

Extraction process

1. Remove filter papers from long term freezer storage and place in a lab freezer close to the work bench. Work with one sample at a time.

2. Work in a darkened room to minimize photo-degradation of the pigments. Carry out extraction of the pigment in a well ventilated fume cupboard. *Working within the fume cupboard will alleviate risk of contamination from outside sources and minimize inhalation and contact with the acetone.*

3. Record the 'sample id'* of the wrapped filter paper onto the data sheet.

4. Unwrap the frozen filter paper from the foil, and place in a glass grinding tube, avoiding hand contact with paper. Add 4 - 5 mL of 90% acetone. Homogenize the filter for 30 to 60 seconds by grinding the filter paper with the high speed tissue grinder. *Studies by Yentsch and Menzel (1963) show this to be sufficient time for extraction of the chlorophyll pigments. Prolonged grinding can cause excessive heat to be generated which can accelerate degradation of the chlorophyll pigments.*

5. Carefully pour the homogenized filter and raw extract into a 12 mL polypropylene screw-cap centrifuge tube designated for use in chlorophyll *a* determination. Rinse the glass grinding tube twice with small amounts of 90% acetone from the squeez* e bottle. Add each rinse to the centrifuge tube. Make up the volume of the extract t[1]o 10 mL, using graduation on the side of the [2]centrifuge tube. Shake the tube to ensure the extract is well mixed

6. Place centrifuge tube in the dark for 30 minutes. *This ensures complete extraction of the pigment and allows the sample to come to room temperature.*

7. Repeat steps 3 to 6 until all samples have been extracted and placed in centrifuge tubes. Place the tubes in the centrifuge in the same order as blanks and sample ids have been recorded on the data sheets.

8. Centrifuge the tubes before reading the fluorescence. The number of tubes per centrifuge run depends upon the centrifuge and heads in use. *For the Hettich*

Rotanta/p™ centrifuge, the following settings are recommended:
Braking speed (0) = 9
r/mm = 150
n/min = 3500
t/min = 10

9. After centrifuging, pour the contents of the tube into a 10 mL fluorometer quartz cuvette *(available for use with the fluorometer)*. Due care should be taken to avoid resuspension of the centrifuged pellet as it is transferred into the quartz cuvette.

10. Wipe the cuvette with a tissue to remove any fingerprints or solvent on the outside. Place the cuvette into the fluorometer. Cover with cap provided and wait 30 seconds for reading to stabilize.

11. Record the range scale on the data sheet. Record the stabilized mV reading under the F0 column on the data sheet.

12. Remove cap from fluorometer and take out the cuvette. Add 2 drops of 6N HCl and carefully invert to ensure adequate mixing of the acid within the cuvette. Rewipe the cuvette with a tissue, replace in fluorometer and cover with cap provided.

13. Wait until reading has stabilized, then record the mV reading under the F1 column on the data sheet.

14. Repeat steps 9 to 13 until all centrifuged samples have been analyzed for chlorophyll and phaeophytin fluorescence levels.

15. Conversion of the fluorometer readings into chlorophyll *a* and phaeophytin levels, and integration of the blank data is achieved using a spreadsheet. Values of the fluorescent levels with the specific settings and sample id are entered directly into the spreadsheet. The spreadsheet converts the digital readings into a chlorophyll *a* and phaeophytin reading using the blank value and the difference before and after acidification of the sample.

16. Final values are given to the database manager and transferred to the ORACLE database.

---

* ** ‘ Sample id’, is the sample identification number consisting of a two letter ‘trip code’ which is incremented for successive survey trips, followed by a unique three digit number for each sample.

SUSPENDED SOLIDS

Background

Analysis of suspended solids estimates the total amount of particulate matter in a water sample. An increase in the amount of suspended sediment, phytoplankton cells or other solids within the water column can lead to a reduction of light penetration into ocean waters. Such a reduction in ambient light can be detrimental to biota whose survival is dependent on sunlight. Sediment loading can be increased as a result of natural and human disturbances, including river input, storms, strong winds, trawling and dredging (Hatcher 1989). Extraction of the suspended material from a water sample is a necessary step in this procedure to permit easy calculation of total suspended solid. One of the most widely used and popular concentration methods is filtering of the sample onto a pre-weighed filter paper (Gibbs 1974). This is the method described here.

Synopsis of the technique

Particulate matter is extracted by filtration upon a pre-weighed filter paper of nominal pore size. The weight difference between filter papers before and after filtration and drying is used to calculate the amount of suspended solid in the sample. Final suspended solid weight is calculated using a PL/SQL™ program (Baker, in prep.).

Equipment

- Mettler AE 163 analytical balance, (reading to 5 significant places)
- Millipore polycarbonate membrane filters, (0.4 µm pore diam., 47 mm filter diam.)
- Forceps
- Glass vials with screw-top lids
- Oven (temperature set at 60°C)
- Suspended solid data sheets

Method

Preweighing of filter papers (prior to field trip)

1. Turn on the balance 15 minutes before weighing of filter papers, by depressing the bar.

2. Place the date of the analysis under “Date Initial”on the data sheet.

3. Set balance to zero by depressing the rezero bar.

4. Separate the polycarbonate membrane filter papers from the surrounding blue protective paper using the forceps. *Do not touch the filter paper with fingers at any stage during the analysis*. Place the filter paper gently on the balance tray.

5. Weigh the filter paper on the balance to five decimal places. Record the result on the data sheet under “Initial Weight”. Place filter paper in a pre-labelled scintillation vial and record this number on the data sheet under the “Vial number” that corresponds to the Initial weight value.

6. Weigh filter paper as a blank after every 14 samples and record result under ‘BLANK’. Place the blank filter paper into the corresponding vial.

7. Store vials in a box in preparation for field sampling.

Weighing of used filter papers (after field trip)

1. Release the vial cap slightly and place the vials in a clean 60°C oven and leave to dry for 48 hours.

2. After drying, take vials out of the oven and tighten lids to seal the vials. Allow sealed vials to cool to room temperature.

3. Carefully remove the dried filter paper from the vial using forceps when weighing the samples, and place on the balance tray. *It is essential that the filter paper is in a horizontal position during this transfer. Particulate matter is not*

stable on the membrane surface and can be dislodged. Record weight on data sheet under 'Final Weight' next to the corresponding initial weight. Record the sample id labelled on the scintillation vial in the 'Sample id' column.

4. Reweigh blanks and place value under "BLANK final weight" *The recording of blanks before and after a cruise will account for balance drift and possible contamination of filter papers.*

5. Enter the suspended solid data into a text file

6. The final text file is handed to the database manager and loaded into the relevant ORACLE™ table. A PL/SQL™ called Calc_ss.com then calculates the amount of suspended solid from the weight difference of the filter papers.

SALINITY

Background

Salinity is formally defined as the total amount of dissolved inorganic solids in sea water, expressed as parts per thousand (‰) by weight, when all the carbonate has been converted to oxide, the bromide and iodide to chloride, and all organic matter is completely oxidised (Fairbridge & Rhodes 1966). Salinity, in conjunction with temperature, largely determines the density of seawater and as a conservative property can be used to identify specific water masses. The salinity in a marine ecosystem may be affected by a number of factors. An increase in freshwater runoff due to high rainfall, coastal land clearing and urban development may cause a reduction in salinity, whereas evaporative concentration near shallow reefs may lead to an increase in salinity levels (Hatcher 1989). One obvious way of measuring salinity is to take a known mass of seawater, evaporate it to dryness and then weigh the remaining salt. In practice, this method tends to be highly variable and unpredictable. As a result, salinity is rarely determined directly but is routinely computed from chlorinity, electrical conductivity, refractive index, or some other property where a functional relationship to salinity is well established. 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.*

Synopsis of technique

Salinity in seawater samples is determined through the precise measurement of conductivity using a salinometer. The conductivity of individual samples is expressed as a ratio to the conductivity of a sample of standard seawater. The electrical conductivity measured by the salinometer is proportional to the salinity of the sample. Electrical conductivity values are transformed to a salinity value using a BASIC program (Baker, in prep.).

Equipment

- Hytech™ model 6220 portable laboratory salinometer
- IAPSO Standard seawater, k15 = 0.9999
- Standardized seawater (sub standard)
- Salinity data sheets

Method

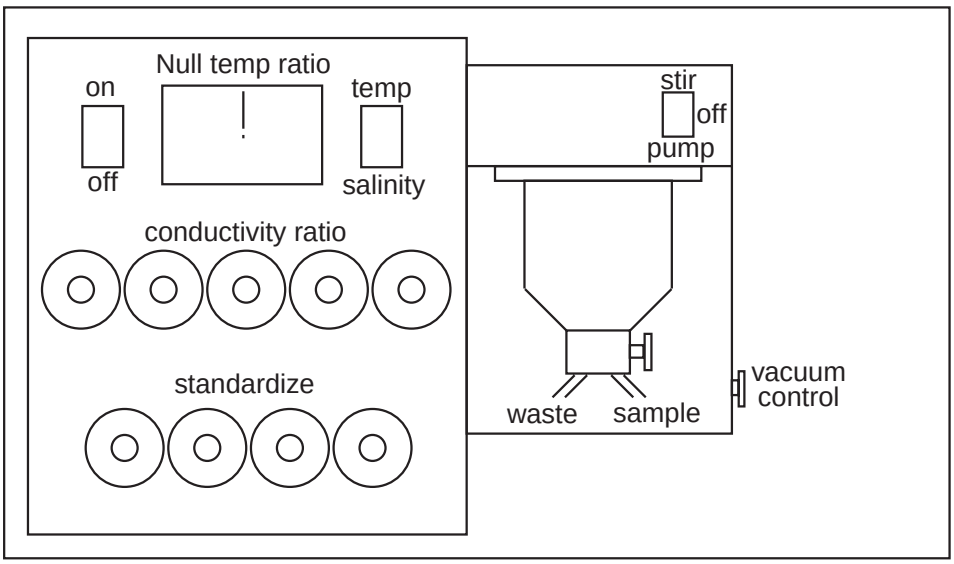
1. Collect approximately 20 litres of seawater to be used as analytical sub-standard. The sub-standard should have a nominal salinity close to that of the actual samples, so the seawater should be collected during a sampling trip.

2. Before analysis, store samples in a cold room (10°C) to prevent evaporation of sample.

3. At least 24 hours before analysis of the sample, move the samples and a seawater standard to the analysis site to allow the salinity samples, a working sub-standard and the seawater standard to reach room temperature.

4. Switch on the salinity meter, by turning power switch to the ON position, **one hour** before commencement of analysis and allow to stabilize.

Figure 2. Hytech™ model 6220, portable laboratory salinometer.



Analysis of IAPSO standard and working sub-standard

1. Connect outlet pump to a water tap. Rinse the meter and all connecting hoses out with the sub-standard seawater. Ensure all samples pass through the meter with a constant and even flow.

2. Draw the IAPSO seawater standard through the cell via the inlet pump, taking care not to draw in bubbles. Turn the three way valve (on side of cell) to BLACK to turn the pressure valve on.

3. Turn pump stir button to STIR position, and turn valve to RED position to release the pressure valve.

4. Repeat steps 2 and 3 twice to rinse salinometer cell.

5. Set conductivity ratio knobs until the numbers match the value on the IAPSO standard seawater ampule.

6. Adjust the standardization knobs (at bottom of machine) until needle on meter reaches the ‘null’ position. Record the position of these calibration dials on the data sheet under ‘standardize’. These dials then remain unchanged throughout the analysis.

7. Drain the salinometer of solution by turning the three way valve to YELLOW position.

8. Repeat steps 2 to 4 using the working sub-standard solution instead of IAPSO standard.

9. Adjust conductivity ratio knobs until needle once again reaches ‘null’ position. Place the value of the substandard under ‘new conductivity ratio’ on the data sheet.

10. Drain substandard out by turning the three way valve to YELLOW position.

11. Repeat a sub-standard analysis after every fourteen samples and record as ‘new conductivity’ ratio on each new data sheet. Record the previous sub-standard

ratio on the new data sheet as ‘old conductivity ratio’. *Running of the sub-standard prior to and during analysis of samples allows instrument drift to be accounted for*.

Analysis of samples

1. Record the date of analysis and initials of user on each new data sheet used in the analysis. *Note the sample id of each individual sample in the space provided on the data sheet. Record duplicate number if required.*

2. Draw portions of the unknown sample through the salinometer at least three times, ensuring all connecting hoses are well rinsed with the sample. After each rinse, turn off the flow to the cell and note the conductivity reading. If the reading is constant, after three rinses, record conductivity value on data sheet next to the corresponding sample id. Check the cell for air bubbles before taking the final reading. If air has entered the cell, empty and refill with sample.

3. Rinse salinometer and all connecting hoses several times with freshwater after completion of sample analysis. *Washing with freshwater will minimise corrosion.*

4. Enter the salinity data into a text file.

5. The final text file is handed to the database manager and run through the BASIC computer program ‘Saline.bas’. This program calculates the salinity value from the conductivity measurement and integrates the standard data into the results (Baker, in prep.). Final calculated values are entered into the ORACLE database.

REFERENCES

Boto, K. and Bunt, J. (1978). Selective Excitation Fluorometry for the Determination of Chlorophylls and Phaeophytin. *Analytical Chemistry* 50:392-395.

Fairbridge, R.W. (ed) (1966). *The Encyclopedia of Oceanography.* Dowden, Hutchinson and Ross, New York, 1021pp.

Franson, M. A. H., Reenberg, Connors A. E. J. J. and Jenkins, D. (eds). (1980). *Standard Methods for the Examination of Water and Waste Water*. 15th edn. American Public Health Association, Washington, 350-412.

Gibbs, R. (ed) (1974). *Suspended Solids in Water.* Plenum Press.

Hatcher, B.G. (1989). Review of research relevant to the conservation of shallow tropical marine ecosystems. *Oceanography and Marine Biology Annual Review,* 27: 337-414.

Jeffery, S.W. and Humphrey, G.W., (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phtoplankton. *Biochemie und Physiologie der Pflanzen* 167:191-194.

Manny, B.A., Miller, M.C. and Wetzel, R.G. (1971). 'Ultraviolet combustion of dissolved organic nitrogen compounds in lake waters', *Limnology and Oceanography* 16 :71-85.

Oliver, J., De'ath, G., Done, T., Williams, D., Furnas, M., and Moran, P., (eds.), (1995). *Long-term Monitoring of the Great Barrier Reef, Status report No. 1*, AIMS, Townsville.

Parsons, T.R., Maita, Y. and Lalli , C.M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, New York, 173pp.

Strickland, J.D.H. and Parsons, T.R. (1972). *A Practical Handbook of Seawater Analysis*. 2nd ed Fisheries Research Board of Canada, Ottawa, 310pp.

Wangersky, P.J. and Zika, R.G. (1978). The Analysis of Organic Compounds in Sea Water. *National Research Council of Canada: Canada*, NRCC No. 16566, 50-63.

Yentsch, C.S. and Menzel, D.W. (1963). A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence, *Deep Sea Research* 10: 221-231.

APPENDIX 1.

Labelling scheme

Table 1.1. Labelling scheme for quality control sampling with double casts and duplicate sampling. (Blanks occur every fifth sample.)

Niskin bottles sample	Sampling equipment	1	2	3	4	Blanks
		Sampling Code				
Total filtered nutrient	10 mL tube	CC701 T(a)	CC702 T(a)	CC703 T(a)	CC704 T(a)	CC705 T
		CC701 T(b)	CC702 T(b)	CC703 T(b)	CC704 T(b)	
Dissolved filtered nutrient	10 mL tube	CC701 D(a)	CC702 D(a)	CC703 D(a)	CC704 D(a)	CC705 D
		CC701 D(b)	CC702 D(b)	CC703 D(b)	CC704 D(b)	
Silica	10 mL tube	CC701 S(a)	CC702 S(a)	CC703 S(a)	CC704 S(a)	CC705 S
		CC701 S(b)	CC702 S(b)	CC703 S(b)	CC704 S(b)	
Salinity	500 mL	CC701 (a)	CC702 (a)	CC703 (a)	CC704 (a)	
Chlorophyll	bottle	CC701 (b)	CC702 (b)	CC703 (b)	CC704 (b)	
	Alfoil	CC701 (a)	CC702 (a)	CC703 (a)	CC704 (a)	
	packet	CC701 (b)	CC702 (b)	CC703 (b)	CC704 (b)	
Suspended solids	Glass vials	CC701 (a)	CC702 (a)	CC703 (a)	CC704 (a)	
		CC701 (b)	CC702 (b)	CC703 (b)	CC704 (b)	

Table 1.2. Labelling scheme for single casts and no duplicates, including the two sites per survey reef. (Blanks will be taken after every second site.)

Sites		1		2		
Niskin bottles		1	2	1	2	Blanks
Analysis	Sampling Equipment	Sampling Code				
Total filtered nutrient	10 mL tube	CC701 T	CC702 T	CC703 T	CC704 T	CC705 T
Dissolved filtered nutrients		CC701 D	CC702 D	CC703 D	CC704 D	CC705 D
Silica		CC701 S	CC702 S	CC703 S	CC704 S	CC705 S
Salinity		CC701	CC702	CC703	CC704	
Chlorophyll		CC701	CC702	CC703	CC704	
Suspended solids		CC701	CC702	CC703	CC704	

Table 1.3. Sampling regime when subsamples are taken from each Niskin bottle (4) for quality control.

Samples per Niskin bottle	Sample Containers	No. of Samples
Total Dissolved Nutrients (TDN)	10 mL acid washed tubes	2
Dissolved Inorganic Nutrients (DIN)	10 mL acid washed tubes	2
Silica (S)	10 mL acid washed tubes	2
Seawater blanks	10 mL acid washed tubes	3(TDN/DIN/S)
Salinity	700 mL plastic bottles	2
Suspended Solids	0.4 µm/47 mm polycarbonate membrane filters	2
Chlorophyll	2.5 cm GF/F filter papers	2

Table 1.4. Sampling regime when only single cast, single analyses are taken from each of two Niskin bottles (taken over two sites).

Analysis of subsamples	Sample Containers	No. of Samples
Total Dissolved Nutrients (TDN)	10 mL acid washed tubes	1
Dissolved Inorganic Nutrients (DIN)	10 mL acid washed tubes	1
Silica (S)	10 mL acid washed tubes	1
Seawater blanks	10 mL acid washed tubes	3
Salinity	700 mL plastic bottles	3 (TDN/DIN/SI)1
Suspended Solids	0.4 µm/47 mm polycarbonate membrane	1
	filters2.5 cm GF/F filter papers	1
Chlorophyll	Filter papers	1

APPENDIX II

Field data sheet

Water Quality **Long-term Monitoring Program**

Australian Institute of Marine Science

Reef name:	Tide:	H	F	L	R					
Station name:	Sea:	C	S	M	R					
Date:	Wind:	0-5	5-10	10-15		20-25	25+			
Time:	Cloud:	0	1	2	3	4	5	6	7	8
Station No.:	Trichodesmium:		Y	N						
Bio-ocean No.:										
Latitude:										
Longitude:										
Depth:										
Wind direction:										

Sample data

Secchi depth:

	Samp_id	Temp	SS1	SS2
No.1 (replicate 1):				
No. 2 (replicate 1):				
No.3 (replicate 2):				
No.4 (replicate 2):				

Blank sample:

Samplers: Filterers:

Sediment Samp_ID: Project code:

Comments: