Field and analytical techniques for the collection of marine sediments

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Long-term Monitoring of
the Great Barrier Reef

Standard
Operational Procedure
Number 4

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CONTENTS

LIST OF FIGURES AND TABLES

PREFACE

The Long-term Monitoring Program at the Australian Institute of Marine Science (AIMS) monitors benthic communities, reef fish communities, crown-of-thorns starfish and water quality on the Great Barrier Reef on an annual basis. This document describes the field and analytical techniques used for the collection, analysis and storage of sediments and associated water samples by personnel of the Long-term Monitoring Program. The procedures described here detail equipment and techniques that are specific to those held and used at the Australian Institute of Marine Science. However, the techniques described in this document could be modified to suit conditions at other institutions.

INTRODUCTION

There has been much concern regarding the role river systems play in transporting dissolved and particulate material from anthropogenic sources into the Great Barrier Reef (GBR) lagoon. The catchments of river systems are often highly cultivated, using large amounts of nitrogen and phosphorus based fertilisers that are leached into rivers during periods of rain. Riverine outflows have been identified as a major source of nutrients to the Great Barrier Reef lagoon. It has been suggested (Bell and Gabric 1991; Bell 1991; 1992) that river run-off has been responsible for causing some regions of the GBR lagoon to become eutrophic. There have also been arguments to the contrary and that there is insufficient evidence available to support either conclusion (Walker 1991; Kinsey 1991).

A large proportion of the terrestrially derived nutrients, sediment and organic matter is deposited and becomes bound in near shore sediments. This material may then be broken down and the constituent nutrients released into the interstitial pore water of the sediment. These nutrients may potentially be released into the water column across the sediment-water interface.

This project strives to investigate the importance of river output in transporting nutrients and sediment to the GBR lagoon and to determine the fate of this material once it is discharged. The study will also provide information on nutrient concentrations and processes over time and space. Specifically the two main objectives of this project are:

- To assess the role of river run-off in delivering nutrient and sediment loads in the GBR by monitoring changes in the quantities of nutrients (and related variables) in the interstitial porewaters and bulk sediments.

- To measure the rates of nutrient flux across the sediment-water interface in order to determine the flux nutrients between the sediments and the overlying water column.

PART 1: FIELD PROCEDURES

Sampling Design

A series of sampling stations are arranged in cross shelf transects off the mouths of the Barron, Johnstone and Pascoe Rivers (Figure 1). The Barron and Johnstone Rivers both have catchments that are located in areas that are heavily farmed and cultivated. They also have very high mean annual flows. The Pascoe River is located in an area that is not heavily cultivated or populated, thereby acting as a control for the effects of cultivation.

Six stations are sampled in a grid off each of the Johnstone and Barron Rivers. Only four of the six stations located in a grid off the Pascoe River, have been sampled to date. Half of the stations are situated within one km of the shore. The other half of the stations sampled off each river, are located 20 km seaward, adjacent to the reef complex, along the outer edge of the GBR lagoon. At each site, one cross shelf transect is situated directly adjacent to the river mouth; the other two transects are located to the north and south of this transect. Trips have been carried out after the dry season (September) and the wet season (March).

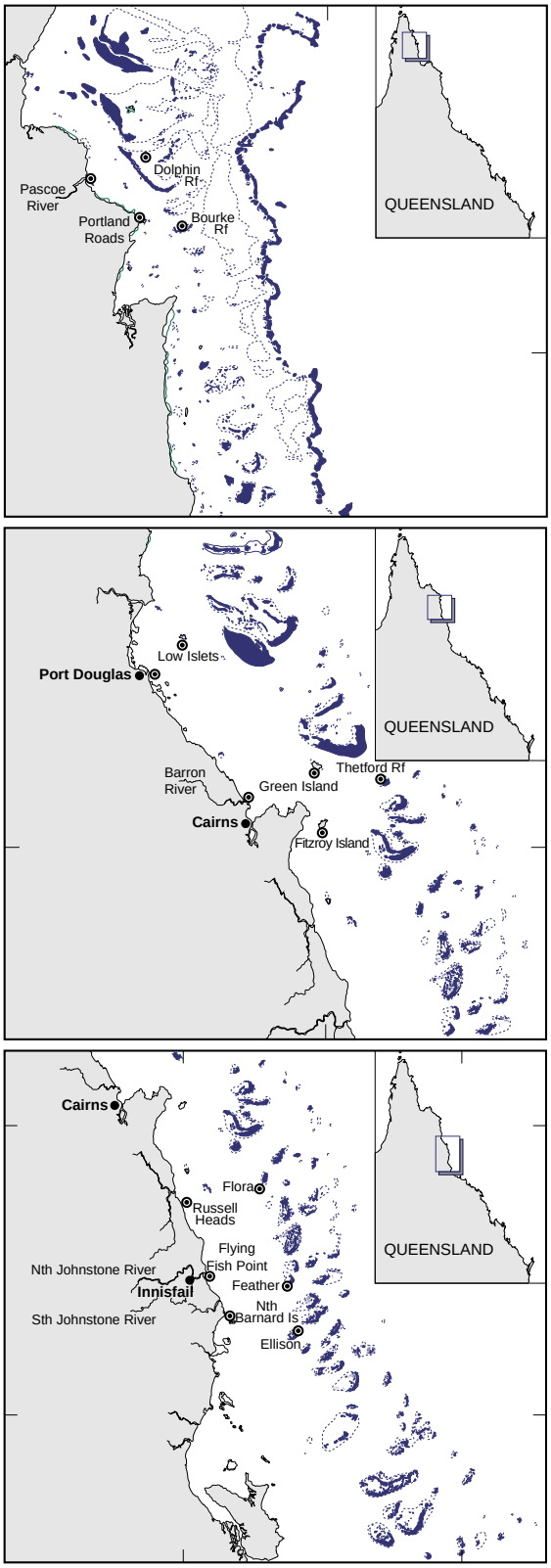

Figure 1. Map of sampling sites.

Quality Control

Procedure

1. Clean all field equipment thoroughly before and after each field trip using freshwater.

2. Keep laboratory and work areas clean throughout the trip. Unnecessary equipment should be removed from work areas. Use a freezer that is dedicated to the storage of samples only. Do not allow this freezer to be used for any other purpose.

3. No smoking in the lab area at any time or on the back deck work area while sampling.

4. Ensure waste (from galley, bathroom etc.) is not discharged during sampling.

5. Be aware of potential sources of sample contamination. These include sweat, sunscreen lotions, detergents, clothing, food and fishing equipment.

6. Do not touch sampling and filtering equipment that comes in direct contact with samples.

Blanks and standards

Before each sampling trip, collect six artificial sea water blanks and six artificial sea water standards from AIMS Laboratory Services. Three will be stored on board the vessel for the duration of the voyage; the remaining three will be stored at AIMS. Four blanks and four standards are stored frozen, two blanks and standards are to be analysed as total nutrients and two blanks and standards are to be analysed as dissolved nutrients. Two blanks and two standards remain at room temperature for silica analysis. Seawater blanks and standards are made up at AIMS from reagent grade (AR) NaCl and Milli-Q™ water.

Safety

Occupational health and safety principles must be adhered to at all times. Two areas where particular care must be taken are; when using heavy equipment on the back deck and when using acids and other chemicals.

Equipment List

The following is a list of necessary equipment that is specific to sediment sampling. Items marked with a * must be ordered on the AIMS logistics requisition . Items marked ** must be ordered in from external suppliers well in advance of trip.

Equipment Piece	Location	No required
Box Corer	Field Operations	1
Boxes	WQ store	12
Box Base Plates	WQ store	12
Large box pins	WQ store	2
Small Base pins	WQ store	>24
Lead weights	WQ store	6
Geologists pick*	Field Operations	1
Large Nelly™ bins	WQ store	3
Sediment subsampler	WQ store	2
Stainless steel slicers	WQ store	2
Plastic siphon hose	WQ store	2
Petri dishes	WQ store	12
25 ml wide mouthed polythene jars	AIMS store	200
10 ml plastic nutrient tubes	Lab Services	10 bags
25 ml teflon capped glass vials	WQ store	400
Unwire racks	Lab Services	10
Shade cloth	WQ store	3
Swivel	Field Operations	1
Messel deck table	Field Operations	1
Pore water squeezers	D. Alongi's store	12
Squeezer racks	D. Alongi's store	4
Dental Dam**		2 packs
Plastic outlet hoses	D. Alongi's store	16
Nitrogen cylinder	AIMS store	1
Regulator (with attachments)	D. Alongi's store	1
Cylinder clamp	D. Alongi's store	1
Wooden spacers	D. Alongi's store	2

Equipment Piece	Location	No required
90 mm 0.4 µm polycarbonate membrane filters**	8 packs	
Plastic beakers	WQ store	4
Plastic vial holders	D. Alongi's store	12
Plumbing tape	AIMS store	1
Conc HCL	AIMS store	1
Auto pipette	D. Alongi's store	1
Flux chambers (dark and light)	D. Alongi's store	12
Battery powered stirrers	D. Alongi's store	6
Magnetic stirrer bars	D. Alongi's store	12
Batteries AA	AIMS store	12
Tubing	D. Alongi's store	6
Main Tap	D. Alongi's store	1
50 ml syringes	AIMS store	1 box
Minisart™ filters	AIMS store	1
25 mm filter holders	WQ store	1 box
25 mm Nuclepore™ filters	AIMS store	2 boxes
Garden hose	WQ store	1
Shifting spanner	WQ store	1
Screwdrivers	WQ store	1
Clean freezer	WQ store	1
Wettex™	AIMS store	5
Bucket	AIMS store	2
Label tape	AIMS store	2
Tape	AIMS store	1
Plastic jerry can of Milli-Q™ water	WQ store	2

Sampling

Background

Sediment samples are collected from the sea floor using a box corer. The apparatus uses "boxes" which sample rectangular cores of sediment representative of the substrate at the sampling location. In this case the box core is used to collect a column of sediment from the sea floor which is sub-sampled for analysis at different depths into the sediment. Subsamples are taken from each core using subsampling tubes. Analysis of these subsamples cores provides data on chemical composition of sediment deposited over time. Collection of a box of sediment in this way also allows factors such as nutrient fluxes to be measured on samples under conditions that replicate the natural sediment water interface.

Equipment

- Box Corer
- Stainless steel boxes
- Stainless steel base plates
- Large box pins
- Small base plate pins
- Lead weights
- Geologists pick
- Nally™ bins
- Shade cloth
- Swivel

Procedure

1. A stainless steel cable is used to lower the box corer. The cable is run off the drum through the large pulley wheel on an "A" frame on the stern of the vessel. Attach the cable to a swivel and then to the box corer using large "D" shackles. Add lead weights to the corer depending on penetration required. *Start with three weights. The depth of penetration can also be altered using the pin on the main shaft of the corer.*

2. Run out sufficient slack cable to allow the closing mechanism to swing open. Fit a stainless steel box to the bottom of the corer using two large stainless steel box pins. Fit a base plate lined with rubber to the inside of the closing mechanism.

3. Place the stainless steel ring into the slot on the top of the corer and secure by lifting and holding the counter weight. Take up the slack cable slowly until the weight of the corer is supported by the cable.

4. Lift the corer smoothly, removing the pin on the main shaft that restricts full movement of the corer. Then swing the corer out clear of the transom of the vessel. Lower the corer to the sea floor. On impact, the triggering mechanism is released capturing the sediment core. Winch the corer back to the surface. Replace the pin in the main shaft and lower the corer onto the deck of the vessel. *Care should be taken when lowering and retrieving the corer so as not to disturb the core that has been collected. Care should also be taken to avoid injury. This will require the wearing of protective footwear.*

5. Wash off any mud adhering to the outside of the box using the sea water hose. Insert small pins into each side of the box and lock them down to secure the base plate. Let out enough slack cable to allow the closing mechanism to swing away. While supporting the weight of the box, remove the large securing pins and carefully remove the box. Then lower the box into a Nelly™ bin full of sea water. Cover the bin with shade cloth. *Care should be taken not to disturb the sediment core during the transfer.*

6. Rinse off the sampling equipment with sea water before resetting for the next sample. Repeat the procedure a further five times at each sampling station.

Field Laboratory Procedures

Sediment slicing

Background

Sediment cores may be subsampled at discrete depths using a sediment subsampling tube to provide samples that are representative of the depth profile of the core. The subsampler used in this case is an aluminium tube (7 cm diameter) containing a recessed inner tube consisting of series of plastic rings, each 2 cm high. Six subsamples are collected from three boxed sediment cores (ie two subsamples per box core). The subsample of the core is then divided into 2 cm sections by slicing between the plastic rings. Two replicates are stored in plastic vials for bulk sediment analysis. The other four replicates are placed into petri dishes ready for pore water squeezing, two sets are destined for organic carbon analysis the other two are used for nutrient analysis.

Labelling of plastic vials

Sample tubes and vials are always labelled with a two letter cruise code (ie AA) and a three diget site number (ie 801). Vials destined for bulk sediment analysis for total organic carbon, total carbon, total nitrogen and total phosphorus are labelled as shown in Table 1.

Table 1. Labelling system for samples destined for bulk sediment and pore water analysis**.**

Sample ID	Replicate	Depth
AA801	1	A
AA801	1	B
AA801	1	C
AA801	1	D
AA801	1	E
AA801	2	A
AA801	2	B
AA801	2	C
AA801	2	D
AA801	2	E
AA802	1	A
AA802	1	B
AA802	1	C

Equipment

- Sediment subsampling tube
- Stainless steel slicers
- Plastic siphon hose
- Petri dishes
- 25 ml wide mouthed polythene jars

Procedure

1. Lift the box containing the sediment core out of the Nally™ bin of water taking care not to disturb the core. *If the core is disturbed allow sufficient time for sediment to settle before continuing.* Any water lying on top of the sediment core may be siphoned off carefully using plastic tubing.

2. *The aluminium outer tube is divided length ways into two halves.* Take half the outer tube and position the inner rings inside the half cylinder to form a tube. Place the other half on top and secure the two halves with cable ties. *This holds the plastic rings in a continuous tube inside the outer tube.*

3. Push the assembled subsamplers into the sediment core down to the bottom of the box. *Two subsamples may be taken from each box*. Carefully remove the box and surrounding sediment. Cut away the cable ties and open the cylinder exposing the series of rings containing sediment.

4. Slice the sediment by pushing stainless steel slicers between each ring. Then place the slices into petri dishes for pore water squeezing or transfer a section of the slice to a clean 25 ml polystyrene vial. Collect the first five sections of each core and label samples with sample id, replicate and depth (A to E, with A being the top section). Take care not to over fill the vial. Freeze immediately after sampling.

5. Continue slicing cores until duplicate samples from each depth are stored in vials and the subsamples from a further four cores are stored in petri dishes ready for pore water squeezing.

Pore Water Squeezing

Background

Terrestrially derived sediments and organic material generally settles and becomes bound up in marine sediments, mainly in river plume areas. Once deposited this material may be broken down and its constituent nutrients released into interstitial pore waters (Ullman and Sandstrom 1987). Typically concentrations of nutrients in porewaters is greater than in overlying water (Alongi *et al*. 1989). These nutrients may eventually be released into the water column across the sediment - water interface.

Concentrations of nutrients are normally lower in tropical interstitial waters than in pore waters of temperate systems (Alongi 1990a). It is thought that high rates of bacterial production in tropical sediment deposits leads to a tight cycling of nutrients (ie rapid uptake, retention and utilisation) (Alongi *et al*. 1993).

Pore waters or interstitial waters are acquired from sediment samples using apparatus modified from that described by Robbins and Gustinis (1976). The procedure is outlined in Alongi (1990b). The system uses gas to expand a rubber diaphragm which compresses the sediment contained in a teflon pore water squeezer. Pore water is forced out through a 0.4 µm polycarbonate filter. The system is adequate to obtain approximately 10 ml of pore water from a 2 cm x 7 cm diameter sediment section. Pore waters are analysed for a suite of nutrients and organic carbon.

Labelling of glass and plastic vials

Sample tubes and vials are always labelled with a two letter cruise code (ie AA) and a three digit site number (ie 801). Nutrient tubes (dissolved nutrient analysis) and glass vials (non purgable organic matter analysis) destined for pore water analysis are labelled as set out in Table 1.

Equipment

- Pore water squeezers (base, centre ring, lid and mesh)
- Squeezer racks
- Plastic outlet hoses

- Nitrogen cylinder
- Regulator
- Cylinder clamp
- 90 mm 0.4 μm polycarbonate membrane filters
- Dental Dam
- 10 ml plastic nutrient tubes
- 25 ml teflon-capped glass vials
- Concentrated Hydrochloric acid (HCl)
- Wooden spacers
- Plastic beakers
- Plastic vial holders
- Plumbing tape

Procedure

1. The teflon pore water squeezers are set up on a stainless steel base with a plastic closing handle above each one. A nitrogen cylinder is connected to each squeezer in series through a number of switches, the pressure is set on the regulator to 100 kPa. Each squeezer is divided into three sections: a base, a central ring and a lid (Figure 2).

2. Screw a small plastic outlet hose into the front of each base section. Plumbing tape may be required to ensure that the outlet does not leak. Place a mesh screen in the bottom of the base to support the polycarbonate filter. Place a 90 mm, 0.4 μm polycarbonate membrane filter onto the mesh screen. *Forceps should be used to transfer the mesh and filter papers to ensure there is no contamination of the filter surface.*

3. Place the central ring section into the base section over the filter membrane. Ensure that the large "O" rings on the top and bottom of the central ring section are inserted correctly and provide an adequate seal.

4. Place a sediment section from a petri dish into the ring.

5. Cover the section with a latex dental dam and put on the lid. The nitrogen source tube enters the rear of the lid section. Place the assembled pore water squeezer in the rack and engage the handle, securing the system. Turn on the switch to begin dispensing pore water (Figure 3).

6. Collect approximately 10 ml of pore water in either acid washed 10 ml plastic nutrient tubes for nutrient analysis or 25 ml glass vials with teflon caps for organic carbon analysis. Acidify the samples destined for organic carbon analysis by adding 100 µl of conc HCl. Cap tubes and vials securely and store plastic nutrient tubes in a freezer, store glass vials in a 4°C refrigerator.

7. Use the polycarbonate membranes for each replicate. *They should be discarded at the completion of sampling at each site*. Rinse squeezer base and mesh screen with Milli-Q™ water between each site. Wash dental dam and central ring in sea water and rinse with Milli-Q™ between each sampling site.

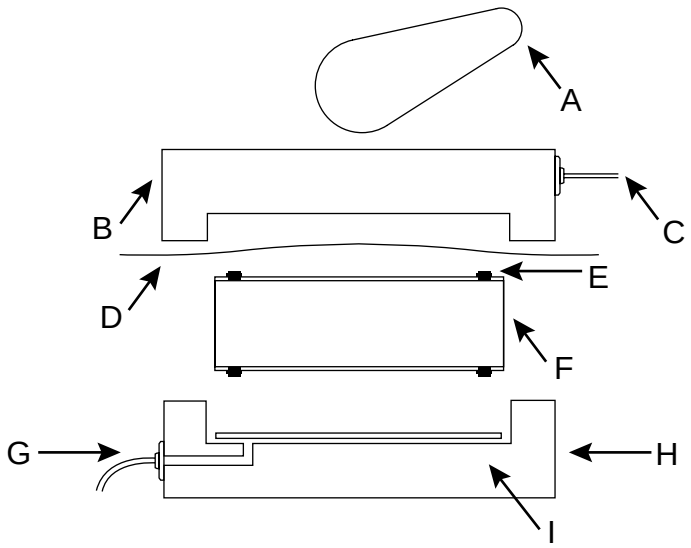

Figure 2. Cross section diagram of teflon pore water squeezer. A - Securing handle; B - Lid section; C- Nitrogen inlet; D - Rubber diaphragm, E - O-ring, F - Central ring section; G - Pore water outlet; I - Mesh and filter; H- base section.

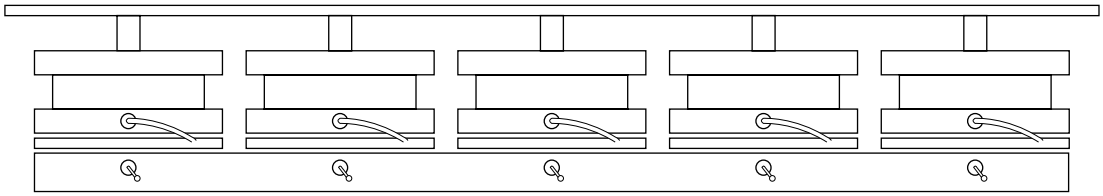

Figure 3. Assembled pore water squeezers connected in series.

Flux Chamber

Background

Water column-sediment exchanges are an important pathway for nutrient cycling in shallow aquatic habitats. In estuarine and coastal environments, nutrient regeneration from benthic sediments can supply a significant fraction of the nutrient requirements of primary producers in overlying waters (Alongi 1989; Callender 1982).

A number of physical, biological and chemical factors affect the rate of nutrient flux. These include biogeochemical diagenetic processes, molecular diffusion across a concentration gradient, mixing processes such as currents, bioturbation, adsorption, chemical precipitation, temperature, rates of organic deposition and composition of deposited organic matter (Callendar 1982; Furnas 1992). It is generally accepted that nutrient fluxes across the sediment water interface are slower in tropical than temperate systems (Ullman and Sandstrom 1987).

The method used to measure nutrient fluxes is outlined in Alongi *et al*. (1989). Nutrient fluxes are measured using bell jars inserted into undisturbed sediment cores. The cores are situated in a continuously flowing seawater bath to mimic sea floor conditions. Samples destined for nutrient and organic carbon analysis are then taken at regular time intervals. In this way a rate of flux can be calculated.

Labelling of glass and plastic vials

Sample tubes and vials are always labelled with a two letter cruise code (ie AA) and a three letter site number (ie 801). Nutrient tubes (dissolved nutrient analysis) and glass vials (non purgable organic matter analysis) for nutrient flux analysis are shown in Table 2.

Table 2. Labelling system for samples destined for dissolved inorganic nutrient analysis of flux chambers (D = Dissolved nutrient analysis, T = Total nutrient analysis and Si = Silica analysis).

Sample ID	Replicate	Time interval	Type of analysis
AA801	1	0	D, T or Si
AA801	1	45	D, T or Si
AA801	1	90	D, T or Si
AA801	1	135	D, T or Si
AA801	1	180	D, T or Si
AA801	2	0	D, T or Si
AA801	2	45	D, T or Si
AA801	2	90	D, T or Si
AA801	2	135	D, T or Si
AA801	2	180	D, T or Si
AA802	1	0	D, T or Si
AA802	1	45	D, T or Si
AA802	1	90	D, T or Si

Equipment

- Flux chambers (light and dark)
- Battery powered stirrers
- Magnetic stirrer bars
- Batteries AA
- Tubing
- Main tap
- 50 ml syringes
- Minisart™ filters
- 25 mm filter holders
- 25 mm nuclepore filters
- Garden hose

Procedure

1. Lower two box cores carefully into a Nally™ bin filled with sea water. Place a hose into the bin to allow a slow exchange of fresh sea water. Let any disturbed sediment settle out before setting up the apparatus.

2. Once the sediment has settled push two bell jars (one clear glass and one dark) side by side into each core (Figure 4). *Push each bell jar into the sediment carefully so as not to disturb the sediment. Each jar should be pushed into the sediment to a depth of around 2 cm.*

3. Push a stirrer into the top of each bell jar and screw on the battery powered stirring apparatus. To one arm on each bell jar attach a length of teflon tubing (2 mm diameter), connect the other end of the tubing to a tap. Connect the tubing from the first jar in the first box to the Position 1 inlet on Tap 1, connect the second jar to the Position 2 inlet on Tap 1 (Figure 5). Repeat procedure for the next box, attaching the jars to the Position 1 and 2 inlets on Tap 2 this time. Use a large rubber band to attach a magnet to the side of the stirring apparatus to act as a switch. Turn the magnet to vertical to initiate stirring.

4. Connect a 50 ml teflon syringe to Outlet Valve 1 and turn Tap 1 to Position 1 to start the flow from the first bell jar (Figure 5). Draw back on the syringe slowly until approximately 5 ml of sample has entered the syringe. Turn Tap 1 to the off position. Disconnect the syringe from Outlet Valve 1, attach a 0.45 µm Minisart™ filter and empty contents through the filter. Repeat rinsing procedure three more times. *Ensure syringe barrel is thoroughly rinsed with sample*.

5. Reattach the syringe to Outlet Valve 1. Turn Tap 1 back to Position 1 and pull approximately 40 ml of flux chamber sample into the syringe. Turn Tap 1 back to the off position. Remove the syringe, attach the 0.45 µm filter and rinse three 10 ml acid washed polypropylene nutrient tubes and caps with around 10 ml of the sample. Dispense the remaining 30 ml into the three tubes. Label each tube according to

6. Connect the syringe to Outlet Valve 1 and turn Tap 1 to Position 2 (for the next bell jar). Repeat steps 4 and 5. However, this time dispense into the next three labelled and rinsed tubes. *These correspond to time 0 and replicate 2*.

7. Connect the syringe to the Outlet Valve 2 and turn the Tap 2 to Position 1. Rinse the syringe as described in step 4. This time use a 25 mm Nuclepore™ filter in a Millipore™ filter holder. Collect 30 ml of sample in the syringe and rinse a 30 ml acid washed glass vial with around 10 ml of sample. Dispense about 10 ml of the remaining sample through the 25 mm Nuclepore™ filter paper into the vial. Add 100 µl of conc HCl and cap with a teflon cap. Label the vial as described in Table 3. *Since all glass vials are destined for the same (organic carbon) analysis, the fourth column is neglected. This sample represents time 0 and replicate 1*.

8. Connect the syringe to the Outlet Valve 2 and turn Tap 2 to Position 2. Repeat the procedure outlined in step 7. Dispense into another labelled and acidified vial. *This sample represents time 0 and replicate 2.*

9. Freeze samples destined for total and dissolved nutrient analysis. Store silica samples at room temperature. Place organic carbon samples in storage at 4°C.

10. At 45 minute intervals repeat steps 4 - 9. Samples will continue to be collected for three hours and are labelled as displayed in Table 3 for T, D, Si and organic carbon analysis. *These samples will represent replicates 1 and 2 and times 45, 90, 135 or 180 minutes*.

11. After three hours disconnect tubing from the bell jars and rinse with Milli-Q™ water. Loop and tie the tubing in preparation for the next site. Remove and store the magnetic switch bars. Remove the stirrers, rinse with Milli-Q™ water and place in protective casings. Remove the bell jars from the sediment and record the volume of displaced mud within the bell jars on the data sheet. Rinse the bell jars with Milli-Q™ water and place into plastic coverings. Dump the core overboard and wash the box with salt water.

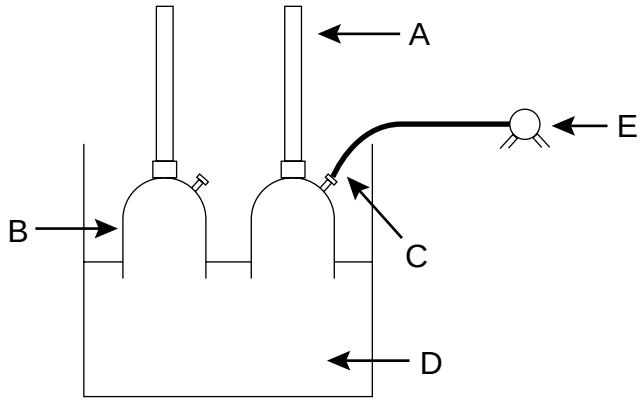

Figure 4. Diagram of flux chamber equipment. A - Battery operated stirrer; B - Bell jar; C - Water outlet; D - Sediment core; E - Tap and syringe attachment.

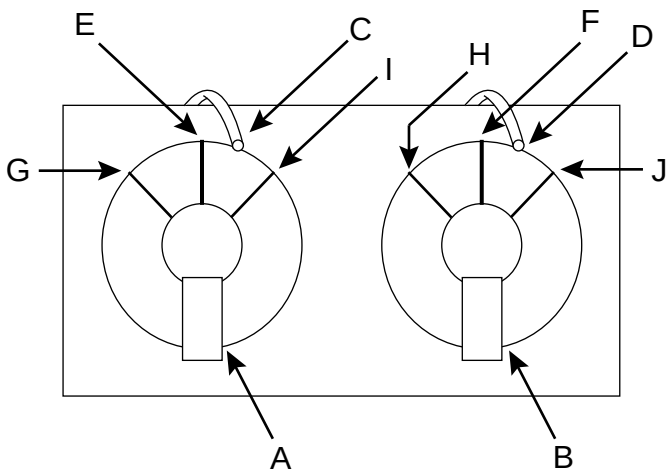

Figure 5. Diagram of the tap assembly on flux chamber equipment.
A - Tap 1; B - Tap 2; C - Outlet Valve 1; D - Outlet Valve 2; E & F - Off position; G & H - Position 1; I & J - Position 2.

Part 2: ANALYTICAL PROCEDURES

Sample Storage and Preparation

Drying sediment samples

Sediment samples must be dried before any of the chemical analyses are performed. The drying process removes any interstitial water from the sample. Oven drying is adequate for the analyses carried out on these samples. Analytical measurements made on these samples are then based on dry weight.

1. Freeze sediment samples immediately after sampling.

2. Upon returning from each field trip loosen the lids on the vials containing sediment samples. Place into an 80°C oven for 48 hours to dry.

3. When dry, remove the samples from the oven and tighten the lids. Store the samples at room temperature in air tight plastic containers until ground.

Grinding sediment samples

Grinding sediment samples serves two purposes. It provides a homogenous sample so any determinations that are made are representative of that sample. It also reduces the sample to particles with a low surface area. This ensures that all constituents are available for determination and that larger particles do not clog and foul equipment.

1. Grind the dry samples for 4 minutes in an agate grinding mill. Scrape the dry sediment from the vial into the grinding mill using a spatula. Place the central disc into the mill and put on the lid.

2. Place the mill into the grinding machine. Turn the mill until the pin in the machine locks into one of the groves on the base of the mill. Pull the locking mechanism down securing the mill. Ensure the locking mechanism is correctly engaged and the mill secure. Close the lid and press the green button to start. *The time required is set using the small dial on the left of the grinding machine.*

3. Once grinding is complete lift the lid and remove the grinding mill. Check the contents have been ground to a fine powder. *If powder consistency has not been achieved then continue grinding the sample.*

4. Transfer contents of grinding mill into a clean labelled vial. Vacuum the residue from the mill using a clean brush to dislodge particles.

Submission of samples

Sediment samples are analysed for total phosphorus using plasma emission spectroscopy. Pore water and flux chamber nutrient samples are analysed for total dissolved and inorganic nutrient species using colourimetric analysis on a flow through auto analysis system adapted for low level determinations (Ryle *et al*. 1981). These analysis are performed by staff at AIMS Analytical Services.

A 1 ml portion of each pore water nutrient sample is taken and diluted to 10 ml for total dissolved nutrient analysis. Samples destined for analysis for total dissolved nutrients require ultraviolet photo-oxidation after dilution, refer to Devlin and Lourey (1996) for description. The remaining sample is analysed for dissolved inorganic nutrient species.

Total Carbon and Nitrogen

Background

Sediments of the inner shelf are dominated by terrigenous mud and quartz sand with a low (< 20%) carbonate content. Reef sediments are predominantly calcareous in their nature (Alongi 1989). By measuring the organic carbon and the total carbon component of sediments and calculating the inorganic carbon fraction it is possible to determine whether the sediments are calcareous or terrigenous in their nature. The nature of the sediment in any area can be affected by an intense wet season and cyclonic activity.

Nitrogen is an essential plant nutrient, it is present in amino acids. Autotrouphs need to obtain nitrogen and other essential minerals from the environment in which they live. Nitrate (NO_3^-) and to a less extent ammonia (NH_4^+) are the species of nitrogen immediately available to primary producers. However, it is also useful to measure other types of nitrogen as they may be broken down and converted to available forms by micro-organisms as part of the nitrogen cycle.

The ratio of organic carbon to total nitrogen in sediments can be used to characterise the type of organic matter in the sediment. A ratio of approximately six indicates the organic matter is derived from marine zooplankton and phytoplankton. A higher ratio indicates input of organic matter from higher land plants. However, carbon to nitrogen ratios are only useful for organic carbon rich sediments (Stein 1991).

This procedure uses a CHN analyser to determine the amount of carbon and nitrogen in a dry sediment sample. Combustion of the sample at 950°C in a pure oxygen environment converts the elements under study to simple gasses. Carbon dioxide, water vapour, oxides of nitrogen, elemental nitrogen and oxides of sulphur are among the products of combustion. Elements such as halogens and sulphur are removed by scrubbing reagents, a copper reduction tube reduces NO_x compounds to N_2 gas. Gases

are separated in a column in a stepwise manner and detected as a function of their thermal conductivities. Output is corrected for calibration factors and blank values. Correction for sample weight is calculated and the results displayed as weight percentages.

Equipment

- Perkin Elmer™ Model 2400 Series II CHN S/O analyser
- Perkin Elmer™ AD-4 auto balance
- 6 mm x 4 mm tin sample capsules
- Acetanalide
- Standard reference material (eg estuarine sediment)
- 2 x flat end forceps
- Offset pointed forceps
- Spatula
- Black tile
- Brush

Procedure

Starting up the analyser

1. Set the machine to "wake up" the **day before** analysis to bring the analyser to working temperature. Press the "parameters" button and select option 11. Enter the date and time required for wake up (30 minutes before analysis is due to start). For an 8.00 am start set wake up for 7.30 am. Select "No" for helium and oxygen purge. Select "Yes" for raise temps. Combustion temperature should be set at 950°C, reduction temperature at 640°C. Press "parameters" button to return to standby mode.

2. Set the pressure on the helium valve to 20 psi before beginning the analysis.

3. Press the "monitor" button and answer "yes" to get a print out. Ensure combustion temperature is 950°C, reduction temperature is 640°C and the detector is set at 82.9°C. Press "monitor" button to return to standby mode.

4. Press the "Purge Gas" button. Press the "yes" button to purge helium and enter 100 seconds as a time. Answer "no" to purge oxygen.

5. Check instrument is in CHN mode. Select "parameters" enter option 6 and press 1 for CHN mode.

6. Check theoretical weight percentage values against acetanilide standard values. Select “parameters” enter option 3. Press 1 to check standard S1, it should read C = 71.09, H = 6.71 and N = 10.36. Press “parameters” to return to standby mode.

7. Select “auto run” and select option 4. Press option 1 to reset the display. Turn the carousel in a clockwise direction till the number 1 hole is over the sample intake.

8. Run a series of blanks. While in the “auto run” mode press 1 the required number of times. Three dummy and seven sample blanks (folded empty capsules) are recommended. Press “start” to commence analysis. Check that blank values stabilise and become reproducible. If blanks do not stabilise or are not reproducible continue running blanks until this is achieved.

9. Run three K factors. Press 2 for K factor, then press one for standard S1 and type in the weight of K-factor used. Use 1.5 to 2 mg of acetanilide as a K factor. The K factors should be reproducible.

10. Use 1.5 to 2 mg of acetanilide as a sample to check the accuracy of the calibration. Press 3, then type in a sample identification (eg acet) using the code outlined in Table 3. Press “enter” and type in the weight of acetanilide used. The values obtained should be close to 71.09 % carbon, 6.71 % hydrogen and 10.36 % nitrogen.

Table 3. Numerical codes for keypad entry of characters.

Character	**Keypad entry**	**Character**	**Keypad entry**
(space)	.00	N	.14
A	.01	O	.15
B	.02	P	.16
C	.03	Q	.17
D	.04	R	.18
E	.05	S	.19
F	.06	T	.20
G	.07	U	.21
H	.08	V	.22
I	.09	W	.23
J	.10	X	.24
K	.11	Y	.25
L	.12	Z	.26
M	.13		

11. Run a standard reference material in the same way as a further check on the accuracy of the analysis. In the case of sediment samples 9 - 11 mg of estuarine sediment is appropriate.

12. To run samples follow the same procedure. Press 3, enter a sample identification and enter the weight of the sample used (9 - 11 mg for sediments). Alternate between an acetanilide sample and standard reference material every ten samples to monitor the accuracy of the analysis.

Note: It is essential to have enough samples entered to ensure the machine does not catch up and stop. The analyser runs ten samples per hour so ensure the number of samples entered allows enough time to finish and shut down the analyser at the end of the day.

If the nitrogen values begin to increase exponentially above expected levels, the reduction tube needs replacing. A reduction tube will analyse between 200 and 300 samples. See laboratory services staff for maintenance. Repeat the samples analysed immediately before the reduction tube expired to ensure that accurate results have been obtained.

Calibrating the AD-4 auto balance

1. The balance should be calibrated before use. The procedure for calibration is outlined below.

2. Remove capsules from sample and reference pans and close balance doors. Lower pan arrests.

3. Press the “autotare” button and wait until integration (INT) is complete.

4. Press the “range” button until 200 mg appears on the left display. It may be necessary to activate the range key by pressing the “blue” key and typing 99 followed by pressing the “blue” key again.

5. Raise the pan arrests, open the door and using forceps place a 100 mg calibration weight on the front pan. Close the doors and lower the pan arrests.

6. Type 100.00 on the numeric key pad and press the “calib” button. Calibration is complete when “INT” symbol disappears from left display.

Operating the AD-4 auto balance

1. Select a tin capsule using the offset forceps and place it on the front balance pan. Close the balance door and release the pan. Press the “autotare” button and wait until the “INT” symbol on the display disappears. Raise the pan to arrest the balance.

2. Open the balance door and remove the capsule using the offset forceps. Using a small spatula estimate around 10 mg of sediment and place it into the capsule. Place the capsule back onto the balance and lower the pan. Check that the display shows between 9 - 11 mg. Arrest the balance and remove the capsule. Add or remove sediment until the required amount is reached. *Clean up any spills with a small brush.*

3. When the desired amount of sediment is reached (9 - 11 mg) the capsule is folded using two pairs of flat forceps so the sediment is contained in a small parcel. Care should be taken not to trap any air in the parcel or to rupture the capsule. To ensure the capsule is not ruptured, drop the parcel several times onto the black tile and scan for escaping sediment.

4. Place the folded capsule back onto the front pan of the balance and close the balance door. Lower the pan and record the first stable reading.

Shutting down the analyser

1. After completion of the day,s run the machine will be in “standby” mode. Press the “diagnostics” button. Select option 2, “gas” and press 2 for the “valve” option. Enter code 4 (valve D) and press 1 to select “on” (to open). Enter code 5 (valve E) and press 1 to select “on”. Wait 15 seconds and press the “diagnostics” button to exit.

2. Lower the analyser temperatures. Press the “parameters” button and select option 10. Select 1 for “on” and enter the date at the prompt. Enter a time 1 minute past the current time at the next prompt. Set the combustion temperature to 500°C and the reduction temperature to 200°C.

3. Set the wake up option as already described for the next analysis day.

4. Lower the helium pressure to 4-5 psi.

5. Fill in log book details. Count all blanks (dummy and sample), K factors, standards and samples in the number of analyses.

Total Organic Carbon

Background

Organic carbon in marine waters arises principally from living material. The amount of organic carbon that accumulates in marine sediments is controlled by three factors, the production of marine organic matter, preservation of organic matter and the supply of terrigenous organic matter. Primary production in marine systems is predominantly controlled by availability of nutrients, so a rise in nutrients due to factors such as upwelling and areas of high fluvial nutrient input may be expected to exhibit higher rates of organic matter production. The preservation of organic matter in the sediment is determined by a balance of breakdown and sedimentation rates. The oxygen content, bulk sedimentation rate, amount of bioturbation, resuspension and the composition of the organic matter will all contribute to this equation. The volume of terrigenous organic matter reaching the marine environment is determined by run off, as a result coastal areas off river mouths receive the greatest volume of terrigenous organic matter (Stein 1991).

The method adopted for this analysis is described in Sandstrom *et al.* (1986). This method was developed specifically for the analysis of organic carbon in calcareous reef sediments. Carbonates are removed from dried sediment samples by acidification using phosphoric acid. Liberated CO_2 is removed by stripping with oxygen. Samples are injected directly into a catalytic combustion tube at 950°C. The carbon present in the sample is oxidised to CO_2 gas which is carried into an infrared detector in a stream of oxygen carrier gas.

Sandstrom *et al.* (1986) determined that this method is quicker and less laborious than other similar techniques while still providing comparable accuracy (better than ± 2%) and precision (better than ± 2 mg organic carbon/g of sediment). The advantage of this method is that it employs an instrument that simultaneously measures dissolved and particulate organic carbon fractions.

Equipment

- Beckman™ Model 915-B Tocamaster™ TOC analyser
- Glenco™ Model DP-810 digital programmer
- ICI instruments™ DP-600 dual pen chart recorder
- MGW Lauda™ RM3 refrigeration system
- Stirrer
- Digital balance
- 50 µl capacity auto syringe
- Glass sample vials

- Sparge tops
- 5% $^{V}/_{V}$ phosphoric acid
- 10,000 ppm organic carbon stock solution (2.125 g of KHP in 100 ml of H_3PO_4)
- Blank
- Standard reference material
- Auto dispenser
- Stop watch
- Spatula

Procedure

Starting up the analyser

1. Turn on the TOC analyser, the chart recorder, the stirrer and the refrigeration unit at the power board. Press the red "TC power" and "IR adjust" buttons on the front of the TOC analyser. Turn on oxygen flow at the cylinder. *Oxygen flow should be around 300 ml/min, it is monitored on the display on the upper right and altered using the dial just below the display*.

2. Allow 45 minutes for the furnace temperature to stabilise (around 950°C). Furnace temperature can be monitored by pressing the yellow "furnace temperature" button. The temperature on the refrigeration unit should also be stable at approximately 3°C.

3. Prepare 25, 50 and 100 ppm standards. Take an aliquot (Table 4) of the 10,000 ppm stock solution and place into a 100 ml volumetric flask. Make up to the mark using 5% H_3PO_4. The blank is 5% H_3PO_4.

Table 4. Aliquots of 10,000 ppm stock solution used when making up standards.

Amount of stock solution	Concentration of standard
0.25 ml	25 ppm
0.5 ml	50 ppm
1.0 ml	100 ppm

1. All samples and standards must be sparged with oxygen to remove carbon dioxide. A sparge cap is inserted into the sample vial. The sample vial with sparge cap is then screwed into one of the slots on the lower left of the analyser. Switch the digital programmer on to single run. Press the "reset" button to begin sparging. Ensure that gas is bubbled through each tube, if gas does not flow then loosen the tube and reseal. Each tube is sparged for seven minutes.

2. Press the red “auto zero” button on the front of the analyser and turn off the IR adjust by pressing the yellow “escape” key. Turn on the chart recorder. The reading on the right hand side of the display should read zero and the pen of the chart recorder should be at the base line. Clear any old calibration curves from the machine’s memory by pressing the blue “ranges” button and the white “reject” button.

3. To calibrate the analyser press the blue “calibration” button, followed by the yellow “TC” button. Next type the concentration of the highest standard (100 ppm) using the numeric key pad and press enter. Type in the injection volume (45 µl) and press enter.

4. Inject the full standard (100 ppm organic carbon in 5% $^{V}/_{V}$ H_3PO_4). Place the needle of the auto syringe into the 100 ppm standard pull the plunger up slowly to half way and release it to expel any air bubbles. Pull the plunger up fully, ensuring it locks into place. Check to make sure there are no air bubbles in the syringe.

5. Insert the needle into the TC port and release the syringe by pressing the silver button on the side. Press the “injection” button on the front of the machine and ensure it registers on the display. Start the stop watch.

6. The MVS reading on the right hand side of the display will increase as will the pen on the chart recorder until a reading is taken and displayed on the left of the display under the number 1. The reading should be around 90 mV. If the reading is not in the desired range then the sensitivity or injection volume may be altered accordingly.

7. Continue injections until at least two consistant results have been achieved. There is room to store the results of four injections on the display. Subsequent injections will result in early results being lost. Results may be rejected by pressing the corresponding number on the numeric key pad and pressing the “reject” key.

8. Once consistant readings have been obtained and other results have been rejected press the yellow “calculation” button to give the mean reading, the standard deviation and the percentage error. Press “enter” and repeat the process for the 50 ppm (50 ppm organic carbon in 5% $^{V}/_{V}$ H_3PO_4) standard and the blank (5% $^{V}/_{V}$ H_3PO_4) to complete the calibration.

Analysis

1. Run the 25 ppm and 50 ppm standards as samples at the start of the analysis to check the calibration. Alternate between injections of the 25 ppm and 50 ppm standards every 10 to 15 samples to check for drift. Incorporate a standard reference material such as estuarine sediment into the analysis.

2. Weigh 0.1 ± 0.002 g of sediment into a sample vial and add 20 ml of 5% $^{V}/_{V}$ phosphoric acid. Sparge the sample with oxygen for seven minutes to drive off any dissolved CO_2. Place a small magnetic stirrer bar into the bottom of each vial, place the vial onto a magnetic stirrer to ensure the mixture is homogenous.

3. Press the blue "sample" button followed by the yellow "TC" button to set the machine to read samples. Enter 100 ppm as the full standard and 45 µl as the injection volume (ie full standard and injection volume should be the same for calibration as sample analysis). Enter 1 as a dilution factor and 1 as the sample number. Inject the sample in the same way as the standards, ensuring it is well stirred.

4. When consistant readings have been obtained press the yellow "calculation" button and record the mean, variance and percentage error. Press "enter", type 1 as the next sample number and follow the same procedure for the next sample.

Shutting down the analyser

1. On completion of the analysis press the yellow "escape" button to get out of sampling mode. Switch off the auto zero by pressing the red "auto zero" button. Press the red "IR adjust" button. Switch of the furnace by pressing the red "TC" power button. Switch off the chart recorder, the digital programmer and the magnetic stirrer. Turn off the gas at the cylinder.

2. Wash sample vials, sparge caps and stirrer bars in distilled water. Dry vials in a 80°C oven.

Dissolved Organic Carbon

Background

Total organic carbon is made up of a dissolved and a particulate component. Dissolved organic carbon can be determined after separating the dissolved and particulate components by filtering. In this case a 0.4 µm filter paper is used. Dissolved organic carbon concentrations exceed particulate organic carbon concentrations in almost all situations (Furnas 1992). In times of high river flow, particulate organic carbon may become dominant in inshore plume regions (Chapman and Kimstach 1992).

Particulate and larger dissolved organic molecules are susceptible to degradation and mineralisation. This is generally carried out by bacteria and fungi (Furnas 1992). The products of breakdown may be released into the water column across the sediment - water interface, via the sediment pore water. Dissolved organic carbon is an important component of tropical microbial food chains (Boto *et al.* 1989).

In this procedure pore waters and samples for measuring carbon fluxes are collected filtered and stored acidified at approximately 4°C. The samples may then be analysed on a total organic carbon analyser, set up to analyse organic carbon in seawater samples. Acid is added to the samples to convert all carbonates and bicarbonates to free CO_2 which is sparged off using the carrier gas. An aliquot is then injected into a combustion tube at 680°C where the remaining carbon (the organic fraction) is converted to CO_2. This CO_2 is then carried off to an infrared detector in a stream of carrier gas.

Equipment

- Shimadzu™ Model TOC-5000 Total Organic Carbon analyser
- Shimadzu™ Model ASI-5000 Auto Sampler
- Large Glass sample vials
- Small Glass sample vials
- 2 N HCl
- 1000 ppm organic carbon stock solution (0.2125 KHP in 100 ml of H_20).
- Blank
- Micropipette
- Milli-Q™ Water

Procedure

Starting up the analyser

1. After collection, the sample (approx 10 ml) should be acidified (100 µl of conc HCl) and refrigerated until analysis.

2. The day before analysis set a time for the furnace to start heating up. From the "main menu" select option 9 "auto sampler" and press enter on the numeric keypad. Press F1 for "next". In "ASI conditions" set the "auto start time" to start the machine the next day . *Allow about thirty minutes to allow the furnace to heat up*. Press F2 to go the "sample measurement" screen. Press F2 again ford "main menu". Turn off the screen (F6). Pressing any key turns screen on again. *Note that the auto start time defaults to current time when this screen is opened so once time is set do not return to this menu*.

Calibrating the analyser (when necessary)

1. Make up 3 appropriate standard solutions. *Concentrations will depend on the concentrations of organic carbon present in the samples. Suggested concentrations may be 2,5 and 10 ppm for flux samples and 5, 10, and 20 for pore water samples.* Transfer the required aliquot (Table 5) of a stock solution (1000 ppm) into a 100 ml volumetric flask using a micro pipette, then add 1 ml of 2N HCl and dilute to the mark. Dilute 1 ml of 2N HCl to 100 ml in a volumetric flask for use as a blank.

Table 5. Aliquots of 1000 ppm stock solution used when making up standards.

Amount of stock solution	Concentration of standard
0.1 ml	1 ppm
0.2 ml	2 ppm
0.5 ml	5 ppm
1.0 ml	10 ppm
2.0 ml	20 ppm

2. Transfer some of the blank into a large sample vial to approximately 75% capacity. Place the vial into the carousel in the "S1" position. Fill another vial with the first standard and place into the "S2" position, continue until all standards are dispensed.

3. In the "main menu" select option 1 "calibration". Set the calibration curve number in "Cal Curve F#". Set the "Type" as 1 (TC). Enter the four standard solution concentrations in the order loaded (step 2). Set the "Range" to x1 for dilute standards (to 10 ppm) and x5 for higher concentrations. Set the "Inj Vol" to 100 µl, the "No of Injects" to 4 and the "Max No of Inj" to 6. Set the "Sparge Time" at 10 minutes.

4. Press the "F1" key for "Next" and press "Start". *The instrument samples each standard in turn and derives a standard curve. It is stored under the calibration curve number selected earlier.*

Re calibration is not required before each run. Standards are included into each run to check calibration.

Analysis

1. Fill four small sample vials (75% full) with super-Q water and place them in the spaces labelled one to four on the carousel. *This allows the machine to settle before analysis.* Fill the next tube (75%) with blank and place into space five. Place each of the standards into spaces six to eight. *Running standards allows the calibration to be checked.*

2. Fill a vial (75% full) with the first sample and place into space nine. Repeat nine more times before adding two blanks and two mid range standards to check for baseline drift. Continue adding samples, blanks and standards until the carousel is full or all samples have been loaded. *Make sure the sample identification and corresponding carousel position are noted during loading.*

3. Ensure that sample number 1 is in the correct position to be the first sample taken. Place the lid over the carousel.

4. Fill the wash bottle with Milli-Q™ water.

5. From "main menu" select option 9 "auto sampler" to bring up the "sample measurement" screen.

6. Select run number one. Select type "4" for NPOC analysis. Enter the space the first sample is inserted under "IS" (usually 1) and the space the last sample has been placed under "FS" (Max 78). Enter the number of the stored calibration curve under "F1". *This can be checked in the main menu under selection 4 "calibration curve file list"*. The range (RG) is as used in calibration. The injection volume is also the same as that used in calibration. The number of injections of each sample and the maximum number of injections can be set, use 3 and 5 respectively. Press "next" (F1) to bring up the "ASI conditions" screen.

7. Set the "auto add acid" option to off (2). *Samples are acidified on board at the time of sampling.* Set the "auto start time" for the next day's analysis. Press "next" (F1).

8. Press the "start" button to begin the run.

Shutting down the analyser

1. After the run is completed, remove sample carousel from auto sampler and was vials with Milli-Q™ water. Place vials upside down in a tray lined with paper towel and put into an 80°C oven to dry. Remove data print out from machine and set start up for next day if required.

DATA MANAGEMENT

It is important that effective data management techniques be employed to ensure that only complete, high quality data is entered into the database. The structure and function of the long-term-monitoring data base is outlined in Baker (in Prep).

Field

The following procedure should be implemented to ensure data quality assurance.

1. Collect relevant field data before sampling. Field data include date, time, site, location, depth, sample identifications, weather conditions and any comments that need to be made.

2. Clearly label all samples in a permanent manner before storage. Sample identification numbers for sediment data consist of a two letter trip code followed by a three digit site number. Site numbers start at 801 through to 818. Sediment and pore water samples are also labelled with depth increment and replicate number. Flux chamber samples are labelled with replicate number and time interval of sampling.

3. Immediately after sampling ensure the samples are stored together under appropriate conditions and in a suitable container.

4. Enter field data into a computer. Data entry software has been developed using Microsoft Access™ and should be installed onto any computer taken into the field.

5. Back up data onto disc.

Office

After field trip or on completion of data analysis check all data and add to the main data base as follows:

1. Print out entered data (field data or data from chemical analysis entered into a text file through either Microsoft Access™ or Excel™). Check print out against raw data sheets.

2. Correct any errors in data file and save final version on disc.

3. Submit corrected data files to database manager for inclusion into Oracle™ data base.

4. File raw data for future reference.

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