
Standardized sampling protocols for verifying mid-ocean ballast water exchange.

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by

Kate Murphy¹, Gregory Ruiz¹, Mark Sytsma²



Address for Correspondence:

¹ Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, Maryland 21037, USA

² Center for Lake and Reservoir Study, Portland State University, Portland, Oregon 97207-0751, USA

Contents

1	Introduction.....	6
2	Ships, Tanks and Sampling Designs.....	7
2.1	Representative Sampling.....	7
2.2	Tank Configurations and Access Ports.....	7
2.2.1	Hatches and Manholes.....	8
2.2.2	Sounding pipes.....	8
3	Ships and Contaminants.....	11
4	Ballast Water Sampling Apparatus.....	13
4.1	Discrete Samplers versus Profiling Instruments.....	13
4.2	Niskin Bottles.....	13
4.3	Syringe Samplers.....	14
4.4	Pumps.....	14
4.4.1	Power supply.....	14
4.4.2	Capacity.....	14
4.4.3	Performance.....	15
4.4.4	Materials.....	15
4.4.5	Air Hose Couplings.....	15
5	Ballast Water Sampling Protocols.....	17
5.1	Diaphragm Pump Configuration.....	17
5.1.1	Overview.....	17
5.1.2	Equipment Specifications.....	17
5.2	Salinity Sampling Protocol.....	18
5.2.1	Overview.....	18
5.2.2	Sampling Apparatus.....	18
5.2.3	Procedure.....	19
5.3	Trace Element Sampling Protocol.....	20
5.3.1	Overview.....	20
5.3.2	Sampling Apparatus.....	20
5.3.3	Equipment Specifications.....	21
5.3.4	Products.....	22
5.3.5	Procedure.....	22
5.3.6	Sample Log.....	24
5.3.7	Sample Delivery to Analytical Laboratories.....	24
5.4	Colored Dissolved Organic Matter (CDOM) Sampling Protocol.....	25
5.4.1	Overview.....	25
5.4.2	In-situ CDOM Fluorometers.....	25
5.4.3	Sampling Apparatus.....	25
5.4.4	Equipment Specifications.....	26
5.4.5	Products.....	26
5.4.6	Procedure.....	26
5.4.7	Sample Log.....	28
5.4.8	Sample Delivery to Analytical Laboratories.....	28
5.5	Radium Sampling Protocol.....	29
5.5.1	Overview.....	29
5.5.2	Sampling Apparatus.....	29
5.5.3	Equipment Specifications.....	30
5.5.4	Products.....	31
5.5.5	Procedure.....	31

5.5.6	Sample Log	32
5.5.7	Sample delivery to Analytical Laboratories	32
5.6	Blank Sampling Protocol.....	32
5.6.1	Overview	33
5.6.2	Sampling Apparatus	34
5.6.3	Products.....	34
5.6.4	Procedure.....	34
5.7	Ship-side Sampling Protocol	35
5.7.1	Overview	35
5.7.2	Procedure.....	35
5.8	References	36

List of Tables

Table 1: Ships and contaminants	11
Table 2: Materials compatibility table.	12
Table 3: Example log book entries for trace element samples.....	24
Table 5: Example log book entries for CDOM samples.....	28
Table 6: Example log book entries for radium samples.....	32

List of Figures

Figure 1: Ballast tank access locations on a bulk cargo ship.....	9
Figure 2: Common ballast tank configurations on tanker ships.....	10
Figure 3: Niskin bottle sampler.....	13
Figure 4: Syringe sampler.....	14
Figure 5: Types of air hose couplings commonly encountered on ships.....	16
Figure 6: Diaphragm pump set-up for ballast water sampling.....	17
Figure 7: Trace Element Pump Sampling Apparatus.....	21
Figure 8: Trace Element sampling by Syringe Sampler.....	21
Figure 9: CDOM pump sampling apparatus.....	26
Figure 10: Radium Pump Sampling Apparatus.....	29
Figure 11: Conceptual diagram of pre-blank and blank samples.....	33
Figure 12: Preventing CDOM and trace element bottle breakages during freezing.....	34

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Cover photo: Monaca Noble is collecting samples on the deck of the *MV Asahi Sunrise*.

1 Introduction

This document summarizes protocols used for sampling ships' ballast water in order to quantify concentrations of trace elements, colored dissolved organic matter (CDOM) and radium isotopes. The protocols described in this document were first implemented to verify mid-ocean ballast water exchange (BWE) by Murphy *et al.* (2001).

Due to a requirement for this study that it be possible to sample ballast tanks while at sea, these protocols have been tested exclusively on wing ballast tanks. It is expected that they are readily transferable to other ballast tanks accessed via the deck (Cargo Holds and Fore/Aft-peak tanks). However, significant protocol modifications may be necessary to enable sampling of double-bottom tanks.

Our goal in publishing these protocols is to provide enough detail to allow others to replicate our methodology, thus facilitating data accumulation and allowing comparisons across different geographic regions and research laboratories during future verification programs.

2 Ships, Tanks and Sampling Designs

2.1 Representative Sampling

The over-riding goal when sampling a ship's ballast tank is to obtain enough samples to accurately and sufficiently characterize the ballast water. Replicate profiles obtained from a particular location in the tank can yield precise measurements at that location. However, it may be argued that replicate profiles taken from a single location under-represent the true variability in the tank by failing to account for spatial differences.

“Representative sampling” is a statistical term used to describe the practice of obtaining samples that provide an unbiased estimate of a population. Non-representative ballast water sampling can occur when the number of samples collected is too few to describe the natural variability in the ballast tank, or when a disproportionate number of samples are obtained from regions of a ballast tank that differ significantly from other regions of the tank. In this case, there exists the risk that any non-compliance determination could be discounted (i.e., either in a scientific venue or a court of law), on the basis that measured concentrations were uncharacteristic.

If the distribution of tracers in a ballast tank is unknown prior to sampling, measurements should be obtained from more than one location in the tank (e.g. forward and aft manholes) and at more than one depth (particularly if the tank is stratified). Where possible, samples should be collected from more than one ballast tank. It is far better to obtain more samples than one intends to analyze, than to collect too few or atypical samples, particularly if a ship appears not to have performed ballast water exchange. Any shortcuts or lack of due diligence on deck may compromise the quality and utility of the resulting information.

2.2 Tank Configurations and Access Ports

In theory, ballast tanks can be accessed from deck via manholes, hatches, vents and sounding pipes (Figure 1). In practice, a subset of these options are often unavailable on a target ship: manholes may be under pressure or obstructed by cargo, vents may be closed with wire mesh and hatches or sounding pipes may be absent.

The most difficult tanks to sample quantitatively are those in which access is severely restricted by design or safety issues. Because cargo holds can usually be accessed to their full depth through the open hatch, they are generally more amenable to representative sampling than are wing or double-bottom tanks. It is often impossible to access the entire depth profile of a wing tank except directly below a manhole, while in many cases, ladders and other tank structures below manholes prevent access to all but the top few

meters (Figure 2). Pre-inspections of empty ballast tanks, and/or custom-made sampling equipment may be highly beneficial to representative sampling efforts. While the best information is always obtained by visual inspection, design plans showing the dimensions of the ballast tanks provide a useful overview of tank design, and may alert you to problems ahead of time.

If it is not possible to view empty ballast tanks prior to sampling, samples should be taken from as many tanks, depths and locations as are feasible, while design plans showing the dimensions of the ballast tanks should be obtained from the ship. Mark all sampling positions on the design plans - this information may be needed to interpret the resulting data.

2.2.1 Hatches and Manholes

In most cases, tanks can be accessed by at least one manhole/hatch of > 30 cm diameter. In some cases, there will be two or three access points of this type. Access via a manhole will usually require the removal of 10 – 30 bolts via wrench or air gun. Hatches are opened by unscrewing a single large wing nut that prevents the lid from swinging open.

2.2.2 Sounding pipes

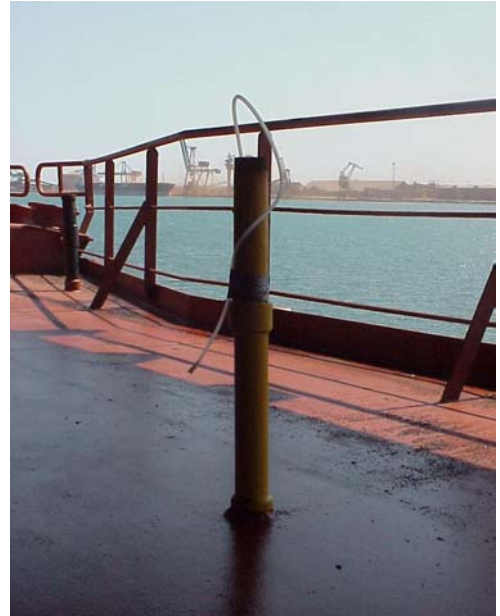
Sounding pipes are narrow metal tubes that connect a tank to the deck. Their purpose is to guide a sounding tape while it is lowered to estimate the level of fluid in the tank. The sounding pipe may be a continuous tube insulated from the rest of the tank except at the bottom end, or it may have perforated sections that encourage exchange between the pipe and surrounding ballast water. Sounding pipes may be straight or have bends that could prevent the passage of an instrument larger than the weight at the end of a sounding tape. Usually, the exact configuration of a sounding pipe will not be known prior to sampling.

Until proven otherwise, sounding pipes should be treated as specialized micro-environments that are probably not representative of the remainder of the ballast tank. For this reason, we do not recommend collecting BWE verification samples from sounding pipes, except to supplement other tank samples or where there are no alternative access locations. Should it be necessary to sample ballast tanks via sounding pipes, Dodgshun and Handley (1997) of the Cawthron Institute in New Zealand, have published a procedure for using impeller and inertia pumps to obtain water from sounding pipes.

Discussions in the remainder of this document regarding apparatus, protocols and contaminants are also relevant to sampling via sounding pipes.



A. Manhole and vent



B. Sounding pipe, with pump tubing inserted



C. Hatch

Figure 1: Ballast tank access locations on a bulk cargo ship.

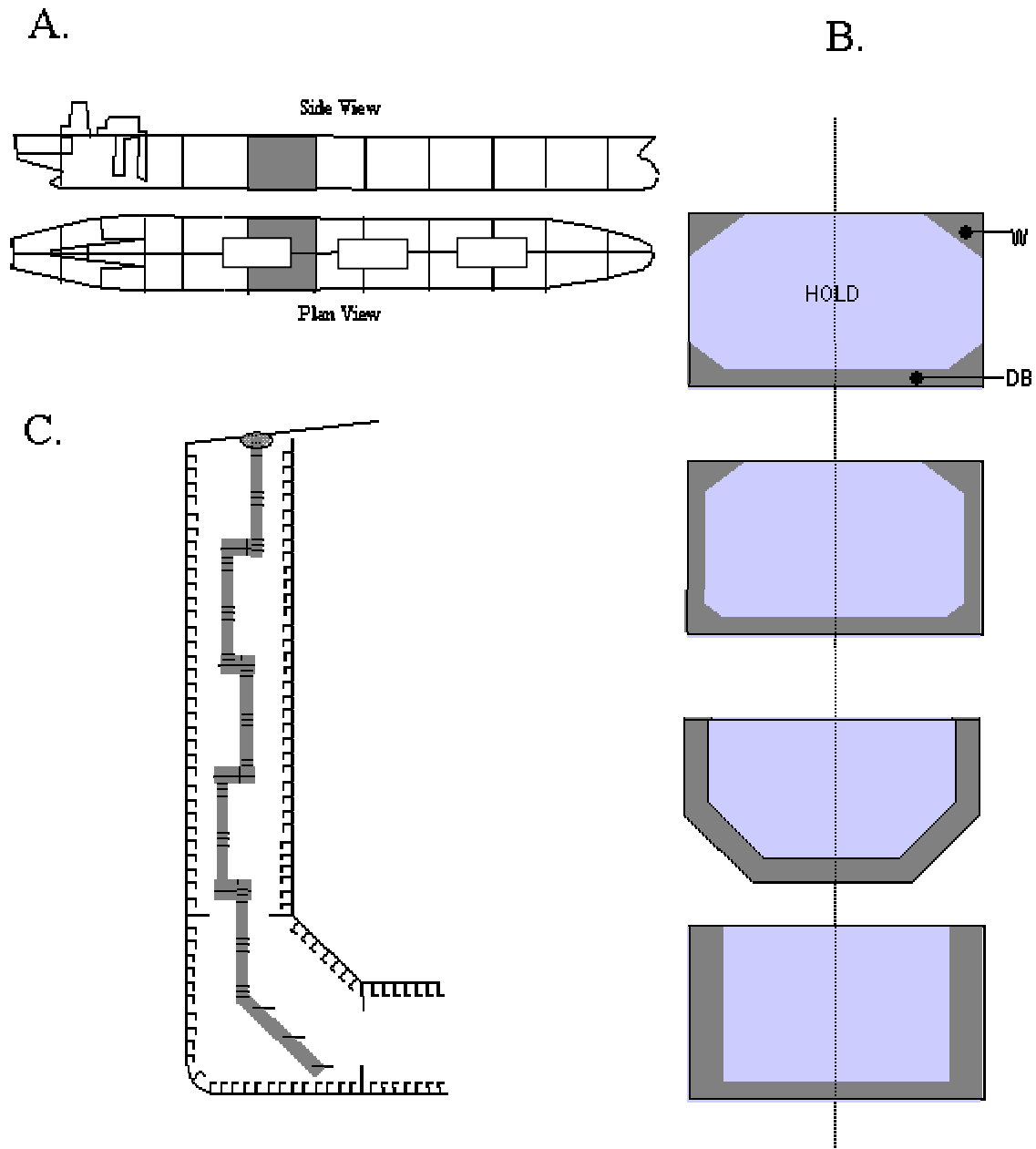


Figure 2: Common ballast tank configurations on tanker ships. A) Paired ballast tanks flank the central cargo holds on either side of the vessel, B) ship cross-sections, showing several possible tank configurations (W = wing tank, DB = double bottom tank), C) ballast tank cross-section: on some vessels, staircases and platforms (shaded) present significant obstacles to ballast water sampling beneath manholes and hatches.

3 Ships and Contaminants

All exchange verification techniques require use of strict protocols in order to minimize the chance of unintentionally contaminating the samples during collection. Ships are inherently biologically and chemically complex environments, in which it is relatively difficult to obtain ‘clean’ samples. For this reason alone, it is well worth the effort and cost to develop specialized sampling devices which minimize the number of sampling steps and hence the opportunity for contamination.

Potential sources of contamination on the vessel include the ship structure and cargo, greases, fuels, dirt and dust (Table 1). Aerosol contaminants may be a significant problem, particularly while the cargo is being shifted and on windy days. At all times, care should be taken to protect samples from aerosol contaminants. Care must also be taken to prevent samples from contacting human skin. Non-talc, chemical resistant gloves (e.g. polyethylene, Fisherbrand 11-394-100A), should be worn by persons handling CDOM and Trace Element samples.

Table 1: Ships are sources of a variety of contaminants that can contribute error to measurements of the potential verification tracers discussed in the remainder of this document. In the table below, sources of contamination are listed along with the tracers that are likely (Y) or unlikely (N) to be impacted by each contaminant.

Contaminant Source	Potential Verification Tracer		
	Trace Elements	CDOM	Radium
Clean metal structures	Y	N	N
rust	Y	N	Y
fuels	Y	Y	N
Aerosols	Y	Y	N
dust	Y	Y	Y
sediments	Y	Y	Y
Organic matter	Y	Y	N
Human hands	Y	Y	N

The risk of contamination by various materials, in terms of their suitability for trace elements, CDOM and radium sampling, is summarized in Table 2. Note that even where a material is considered suitable, it still needs to be thoroughly cleaned before use. Furthermore, the amount of time a sample stays in contact with any materials other than its storage container, and the number of processing steps, should always be kept at a minimum. Fluorescent materials leaching from new plastic tubing tend to decrease over time. Consequently, one should ensure that plastics used for CDOM sampling are both cleaned and well-flushed prior to use.

Table 2: Materials compatibility. The compatibility of a range of materials with tracer (trace element, CDOM and radium) sampling, in terms of the likelihood that the material will contribute contaminants, are indicated below. Materials are considered suitable (Y), unsuitable (N) or of unknown suitability (-). Materials that should be restricted to short-term exposure applications (e.g. Niskin, pump components, hoses etc.) rather than prolonged exposure (e.g. sample storage bottles) are further identified by the symbol (*).

Materials	Trace Elements	CDOM	Radium
Synthetics / Plastics			
nylon	N	Y	Y
tygon	N	N	Y
HDPE	Y	Y*	Y
Teflon - FEP	Y	Y	Y
Teflon - PTFE	Y*	Y	Y
polyethylene	Y	Y*	Y
polycarbonate	Y	Y*	Y
polysulfone	Y	Y*	Y
polypropylene	Y	Y*	Y
polyvinylchloride	N	Y*	Y
highly colored plastics	N	N	Y
Silicone	Y	N	Y
Buna-N	N	N	Y
Metal			
stainless steel	N	Y	Y
titanium	N	Y	Y
other metals	N	-	-
Glass			
Pyrex	N	Y	Y
Kimax	N	Y	Y
Vycor	N	Y	Y
Paper cap liners	N	N	Y
Methacrylate	N	-	Y
Rubber	N	N	N
Ultrapure quartz	Y	Y	-
Clean human hands	N	N	-
Latex gloves	Y	N	Y
Polyethylene gloves	Y	Y	Y

4 Ballast Water Sampling Apparatus

4.1 Discrete Samplers versus Profiling Instruments.

Ballast water samples may be divided into two types – discrete and continuous. Discrete samples are collected from a defined position in the ballast tank at a single point in time. Continuous (or integrated) samples are collected over a longer period of time, or over a wider area. Niskin bottles and syringe samplers collect discrete samples from a given location and depth; profiling instruments, such as Hydrolab and YSI multi-probe instruments, collect repeated measurements at programmed intervals while the instrument is mounted in the tank or drawn through the water column. Pumps can be used to collect discrete or integrated samples, depending on the size of the sample and whether samples are drawn from a fixed or changing depth.

Where available, profiling instruments are preferable to discrete samplers, since they give the greatest quantity of data per unit effort, including spatial and/or temporal concentration gradients (e.g. increasing salinity with increasing depth), should these exist. Since profiling instruments are not yet available for most tracers of interest (e.g. trace elements), enough discrete samples should be collected to encompass the range of conditions present in the ballast tanks (e.g. deep and shallow samples).

4.2 Niskin Bottles

Niskin bottles are rigid PVC tubes, designed to collect a “grab” of water from a discrete depth in the water column. Just prior to deployment, the ends of the tube are arranged to remain open until a catch is released, at which time they snap shut. The bottle is lowered in the water to the desired depth, the catch is triggered by dropping a weight called a “messenger”, then the Niskin bottle with its enclosed sample is retrieved. The sample is drained from the Niskin via a small nozzle.

Niskin bottles are available in a range of sizes. For the purposes of sampling ballast water, a Niskin bottle of volume 1.7 L (full weight ~ 9 lb) should be sufficiently large to collect enough water for several samples. Niskin bottles are suited to CDOM sampling, but may be difficult to keep clean of trace elements.



Figure 3: Niskin bottle sampler.

4.3 Syringe Samplers

A messenger-activated syringe sampler can be used to collect small volume (~ 60 mL) discrete samples. The sampler is lowered into the tank and the messenger activated, causing water to be drawn into a disposable plastic or re-usable glass syringe. The syringe is removable as one unit for simple transfer or storage.

The principal advantage of syringe samplers is that contamination risk is reduced due to the decreased number of handling steps and decreased area of apparatus exposed to the sample. Moreover, samples can be taken through openings as small as two inches. Plastic syringe samplers are particularly suited to trace element sampling.



Figure 4: Syringe sampler.

4.4 Pumps

Pumps are highly suited to intensive ballast sampling operations. Such operations may necessitate repeated sampling in a tank, access to regions of tanks that are not normally accessible from deck, and/or the collection of a large volume of water impractical to obtain by other methods. In selecting a pump, important considerations include the type of materials in contact with the fluid in the pump, distance from the pump to the water level, and power supply.

4.4.1 Power supply

Pumps suited to ballast tank sampling are typically powered by gas, electricity, batteries or air. Air driven pumps are intrinsically safe - a feature that is required on some types of vessel (e.g. oil tankers). They are also relatively flexible - most ships have air available on deck - and inexpensive. Disadvantages of air-driven pumps are that air supply outlets on deck may be far from the sampling location, and while air hoses are long, heavy, unwieldy, and may be in short supply. Electrical pumps often require use of transformers and adaptors because electricity supplies vary greatly across ships. Gas and battery-powered pumps may be suitable for ballast water sampling, although they are not usually permitted on ships with flammable cargo or in confined spaces, due to the risk of sparking.

4.4.2 Capacity

Pumps can be purchased that are capable of delivering the full spectrum of flow rates from milliliters to gallons per minute. A low capacity pump (flow rate 0.3 – 3 Ga./min) was found to be suitable for radium, trace element and CDOM sampling, since sample collection requires low flow rates. If the intention is to

collect or store large volumes of ballast water, it is advisable to also have available a pump capable of higher flow rates.

4.4.3 Performance

The performance of a pump is a function of both its capacity and how much work is required to lift the water from the ballast tank to the deck. This depends upon the distance between the surface of the water and the outlet of the pump tubing (head), and how much of that distance the water must travel under suction (suction lift) versus the distance the water is pushed by the pump (dynamic head). Pumps operating from deck are limited by their suction lift capacity, which typically vary from just a few feet to a few meters. This renders them unsuitable for deck-sampling of partially full or double-bottom tanks. Submersible pumps are often capable of elevating water to much greater heights and are better suited to sampling in these situations.

4.4.4 Materials

Pumps, hoses and fittings can be obtained with all-plastic parts, all-metal parts or a combination of metal and plastic parts. Plastic pumps are recommended for collection of radium and trace element samples. Plastic pumps can be also used to collect CDOM samples providing that the plastic does not leach significant quantities of fluorescent compounds. In diaphragm pumps, internal parts (e.g. diaphragms) may be made of a range of materials, including rubber derivatives (not recommended), Teflon® (suitable but very expensive), Wil-flex® (lower cost alternative to Teflon). Since plastic products are being developed continually and manufacturers vary in their choices of materials, the suitability of any given pump for CDOM or trace element work may be difficult to predict. The best way to address this uncertainty is to test products in the laboratory prior to use, and collect blanks as well as ballast water samples (Section 5.6).

4.4.5 Air Hose Couplings

Air supply lines that can be used to power air-driven pumps are generally found at regular intervals on deck, typically between or alongside cargo holds. Each ship usually uses a single type of air-hose coupling on deck, however, different ships may favor different types of couplings. If the coupling type is unknown prior to boarding a vessel, a variety of connector and adapters should be carried on board to allow access to air supplies under all potential scenarios.

Note that air pressure on deck may be less than what the pump is rated for, causing the pump to operate at lower flow rates than specified in technical data sheets.

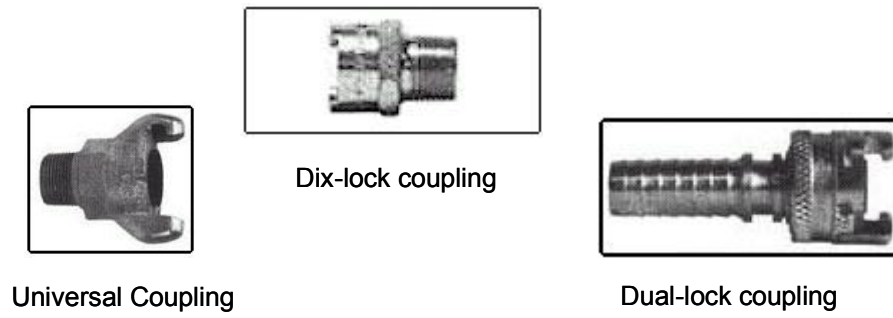


Figure 5: Types of air hose couplings commonly encountered on ships.

5 Ballast Water Sampling Protocols

5.1 Diaphragm Pump Configuration

5.1.1 Overview

As an example for obtaining samples, this section describes the configuration and operation of an air-driven diaphragm pump (Wilden Pro-flo, P.025) used extensively by Murphy *et al.* (2001) for ballast water sampling. Trace element, CDOM and radium protocols were implemented by connecting the appropriate appendages to the outlet hose of the pump, as described in Sections 5.3- 5.5.

5.1.2 Equipment Specifications

- Air filter / regulator
Example: Master / pneumatic filter-regulator (CFR55-1-E5)
- Diaphragm pump
high capacity (flow rate > 10 Ga. / min)
Example: Wilden Pro-flo Air operated 1/2" double diaphragm pump P .05
low capacity (flow rate 1-2 l / min)
Example: Wilden Pro-flo Air operated 1/4" double diaphragm pump P .025

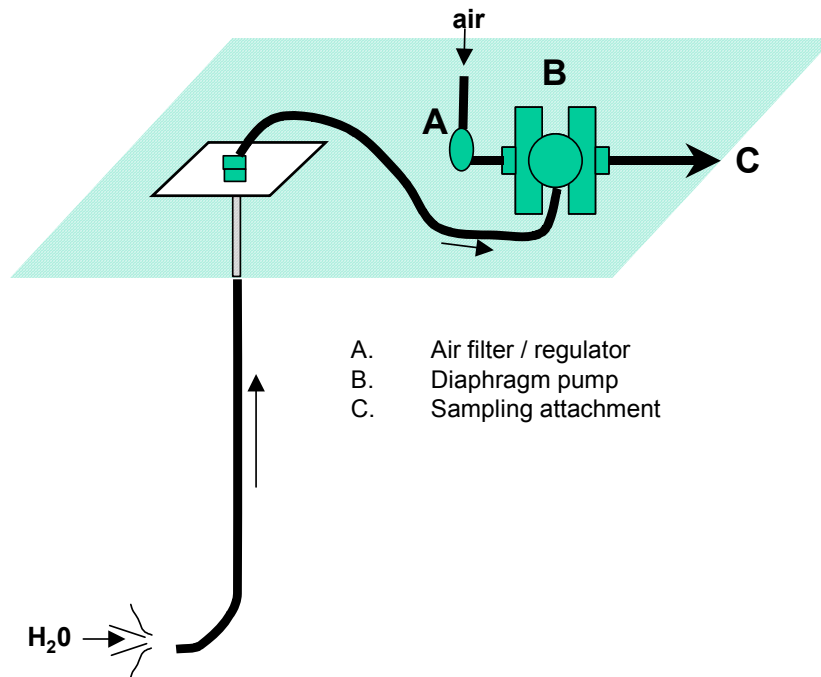


Figure 6: Diaphragm pump set-up for ballast water sampling.

5.2 Salinity Sampling Protocol

5.2.1 Overview

Mid-ocean salinities are generally stable and well defined: in the North Atlantic, surface ocean salinities are typically around 35-36 ppt. They are slightly lower in the North Pacific (32-33 ppt). In contrast, many coastal ports are characterized by fresh (< 0.5 ppt) or brackish water (0.5 -17 ppt).

While many ports are less salty than the open ocean, coastal regions with little river or rain input can exhibit salinities similar to in the open ocean. It is for this reason that salinity measurements alone are insufficient for verifying mid-ocean ballast water exchange. However, since salinity measurements are simple to perform and may quickly reveal the contents of a ballast tank to be fresh or brackish coastal water, they are a critical part of any ballast water sampling program.

5.2.2 Sampling Apparatus

Salinity can be measured conveniently in real-time using a variety of readily available instruments designed for profiling applications. Many such instruments combine salinity measurement with other water data, including depth, temperature, pH and dissolved oxygen.

We recommend the use of multi-probe instruments capable of measuring dissolved oxygen, since oxygen measurements may assist in the interpretation of CDOM and trace element data. To minimize the risk of getting the instrument caught in the tank, ensure that it is of streamlined design and symmetrical around the rope or cable used to lower it. Two examples of suitable salinity meters (salinometers) are as follows:

- **YSI Environmental DO, Conductivity, Salinity, Temperature Instrument (YSI-85)**
This instrument measures dissolved oxygen, conductivity, salinity and temperature simultaneously. A sensor on the end of a cable (10 – 100 feet) is lowered in the water while the operator reads the measurements from a handheld digital display. This instrument is easily handled and suitable for profiling applications.
- **Hydrolab Minisonde 4a Water Quality Multiprobe**
This programmable instrument measures depth, dissolved oxygen, conductivity, salinity and temperature at pre-programmed intervals and logs the results in a data file that can be later downloaded to a computer. A digital readout can be purchased for real-time data display. This instrument is suitable for profiling and long-term monitoring applications.

Instruments should always be calibrated using manufacture's recommendations prior to use. Calibration checks should be done following use.

5.2.3 Procedure

Before lowering an expensive instrument into a hatch or sounding pipe for the first time, always verify that the instrument will have unobstructed passage for the length of the profile. To do this, first lower a “profiling dummy” of length and width no less (and preferably greater) than that of the actual instrument. Note your body position and the position of the cable during lowering, because you will want to repeat this precisely with the real instrument. Note the position (depth) whenever you hear clanging noises or feel tugs on the line – these could indicate obstructions that present a real risk of snagging.

If you feel there to be a significant risk of snagging the instrument in the ballast tank, abandon the profiling attempt or limit it to the upper portion of the tank, if you know this to be obstruction-free. Alternatively, deploy a back-up instrument that you can afford to lose. Such an instrument should be small, self-contained and lowered on a rope or fishing-wire cable. It should not be connected to a digital display. If you get a backup instrument stuck in the tank, you will need to cut it loose, or preferably, tie it to a ladder so that the crew can retrieve it at a later date.

To take measurements using a salinity-profiling instrument (SPI), first mark 1-m increments on the cable, then:

- Lower the SPI until the sensors sit 1 meter below the water surface. Record depth, salinity, and other sensor readings (e.g. DO, pH, etc.).
- Lower the SPI until it just touches the bottom of the tank, or the bottom of the intended profiling region as determined by the “profiling dummy”. Record depth, salinity, and other sensor readings.
- If the salinity varies by LESS THAN 1 ppt (or 1 psu) between the top and bottom of the tank, begin raising the SPI, stopping to take further readings at ~ 5 m intervals.
- If the salinity varies by MORE THAN 1 ppt (or 1 psu) between the top and bottom of the tank, begin raising the SPI, stopping to take further readings at ~ 2 m intervals.
- If you notice an abrupt change between two consecutive readings (readings differ by more than 10 percent), lower the SPI by 1 meter and take readings there also. Note the approximate depth at which the abrupt change occurs – this indicates that there is more than one distinct water mass in the tank (the tank is stratified). You will want to take any discrete samples from each water mass in a stratified ballast tank.

5.3 Trace Element Sampling Protocol

5.3.1 Overview

Many elements, including metals, exhibit pronounced onshore-offshore concentration gradients which reflect their terrestrial origin. Trace elements enter waterways after leaching naturally from rocks and soil, or in elevated concentrations associated with industrial sources. Particularly common in nearshore waters are the constituents of steel, brass and bronze (Fe, Ni, Zn, Cu, Al). In localized regions, high concentrations of silver (Ag) in seawater are found in association with sewage outfalls and the jewelry industry. Main coastal sources for Manganese (Mn), Barium (Ba) and Thorium (Th) are riverine inputs (desorption from minerals), groundwater input (seepage through sediments) and atmospheric deposition of dust.

Trace element samples are extremely easy to contaminate, consequently, all sampling materials and apparatus should be left sealed in plastic bags until needed, handled as cleanly as possible and returned to sealed bags after use. Hands that have touched any potential source of contamination (any metal objects, anything that has come into contact with metal surfaces) should never come near the open ends of the sampling hose or the sample bottles and lids. Non-talc polyethylene gloves are a good precaution, however, even these can quickly become dirty on a ship, and will not alone prevent the transfer of contaminants.

It is imperative that the open ends of the pump tubing (inlet and outlet) remain as clean as possible. Whenever the tubing connected to the pump is not submerged in the ballast tank it should be bagged up such that the system (tubing and pump) is closed to the outside environment. Utmost care should be taken to avoid brushing the inlet or outlet against the deck or the walls of the ballast tank. Similarly, never allow the outer surfaces of syringes or hoses to come into contact with the inside of the sample bottles.

5.3.2 Sampling Apparatus

Trace element samples should be collected using a syringe sampler or plastic pump. Sampling by Niskin bottle is not recommended because Niskin bottles are difficult to clean.

To obtain trace element samples by pump, the pump apparatus of Figure 6 should be connected to the apparatus of Figure 7. Samples can also be obtained using a syringe sampler (Figure 4 and Figure 8),

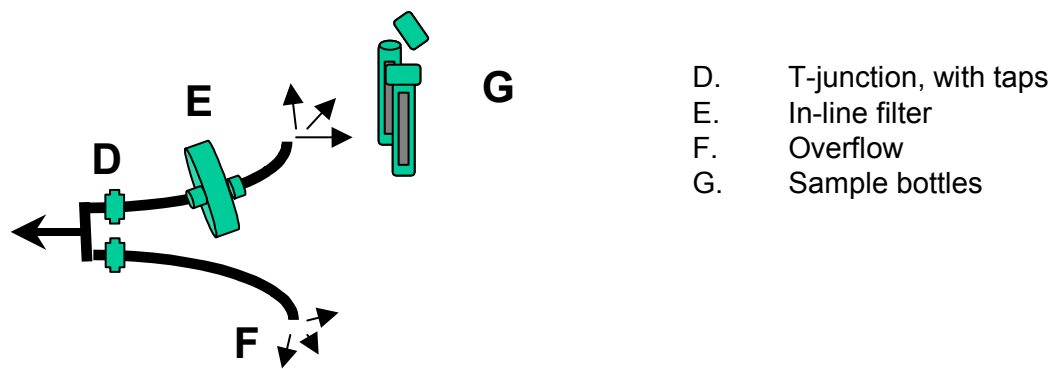


Figure 7: Trace Element Pump Sampling Apparatus.

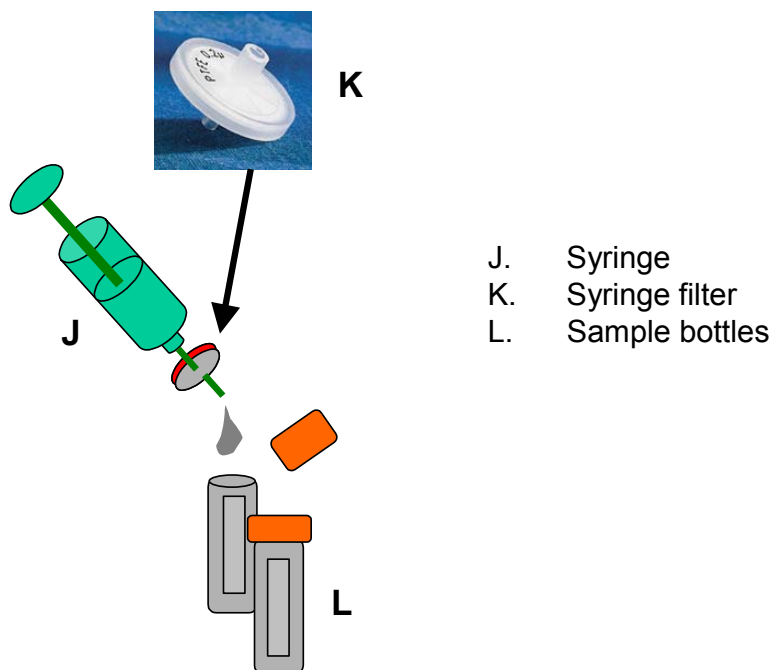


Figure 8: Trace Element sampling by Syringe Sampler

5.3.3 Equipment Specifications

- In-line filter for pump sampling
Example: Osmonics Inc.: Memtrex™ CMMP, 0.45 μm
- Syringe sampler
Example: General Oceanics, Model No. 1050.
- Disposable syringe

Example: 20 ml polypropylene syringes (Fisher Scientific: NC 9374 494).

- Syringe Filters (0.22-0.45 μm)

Example: 25 mm syringe filters with 0.45 μm supor membranes (Fisher Scientific: 09-731-124).

- Sample Tubes: HDPE conical bottom centrifuge tubes

Example: 50 ml polypropylene centrifuge tubes (Fisher Scientific: 05-538-55).

5.3.4 Products

The objective is to obtain ~10 mL of filtered ballast water in each sample bottle (test tube), according to strict cleanliness protocols. Following collection, the samples are frozen then shipped to the laboratory for ICP-MS analysis.

5.3.5 Procedure

5.3.5.1 Preparation of sampling apparatus

Before setting out for the ship, ensure that any materials that will come in contact with the water sample (i.e. pumps, hoses, syringes, filters and sample bottles) are trace-clean, and protected from the elements inside fresh zip-lock plastic bags. Cleaning is performed on-shore in properly-equipped laboratories, by soaking materials in 1 mol L⁻¹ HCl (reagent grade) at 60 °C for at least 24 h prior to use. Following acid leaches all materials should be rinsed thoroughly (5 times) with distilled, deionized H₂O and left to dry in a Class 100 laminar flow bench.

The cleanliness of the sampling apparatus (at least in regard to the metals of interest, e.g. Ba, Mn, Mo, P, U, V) should be verified using the sample blank procedures described in Section 0.

5.3.5.2 Ship-board Sampling

Collection of trace element samples requires extremely careful handling to prevent the samples from becoming contaminated.

The general procedure is as follows:

- Collect trace element **blanks** according to the procedure of Section 0.
- Collect trace element **samples** according to the procedure below.
- Affix a waterproof label to the sample test tube, and write the sample ID number directly on the test tube using a permanent marker.

- Seal sample bottles with parafilm and place samples in individual zip-lock bags. Place bagged samples together inside a larger plastic bag.
- Fill out the sample log. Be sure to note any problems experienced while collecting the sample, in particular, bad weather conditions (wind or rain).
- Carefully retrieve sample hoses, cap their ends with parafilm and place the hoses in clearly marked zip-lock bags

Trace element sampling by pump:

If the pump system is arranged so that it is not necessary to handle the pump or hose during sampling, then sampling can be performed easily by one person.

1. Turn on the pump and flush at least 10 L water through the sampling system and overflow outlet. Now adjust the taps to allow at least 2 L of water to flow through the in-line filter.
2. Using filtered water, carefully fill a centrifuge tube to the 2/3 mark, taking care never to touch the inside of the tube or its cap.
3. Fasten the lid tightly, then bag and freeze the sample.

Trace element sampling by syringe:

The procedure described below for deploying the syringe sampler requires two people to avoid someone having to manipulate the sample bottle with only one hand. The first person (A) should concentrate on operating the syringe, while the second (B) need be concerned only with the handling of the sample bottle. Person B must be careful not to touch the rim of the centrifuge tube (with or without gloves).

1. Load a clean syringe in to the sampler according to the manufacturer's instructions.
2. Lower the syringe sampler (Figure 4) to the correct depth and trigger the messenger to obtain a syringe full of water. Retrieve the device.
3. Carefully remove the syringe, then without removing the filter from its zip-lock bag, screw a filter on to the end of the syringe (Figure 8).
4. Remove the filter (now attached to the syringe) from the bag, and gently push approximately 1 mL of ballast water through the syringe. This water is for flushing purposes only and is discarded.

5. Without touching the inside of either the sample bottle or its lid, carefully remove the lid from the centrifuge tube. Without overfilling the centrifuge tube (i.e., allowing approx. 1/3 air-space by volume), empty the contents of the syringe into the centrifuge tube.
6. Fasten the lid tightly, then bag and freeze the sample.

5.3.6 Sample Log

Table 3 shows an example log-book entry for two hypothetical samples. All fields must be filled out each time a sample is collected.

Table 3: Example log book entries for trace element samples.

Sample ID-No.	Method	Date	Depth	Filter	Blank Id-No.	Notes
Met-511	Syringe	1/Jan/03	1 m	0.22 µm	B1-0353-11	As per protocols
Met-512	Pump	1/Jan/03	10 m	0.22 µm	B1-0353-12	As per protocols

5.3.7 Sample Delivery to Analytical Laboratories

Trace element samples should be frozen to prevent microbial activity that may alter the chemistry of the sample. If it is not possible to freeze the samples, they should be kept cold until they arrive at the laboratory. Frozen samples will not expire, so these may be accumulated and shipped to the laboratory in bulk.

5.4 Colored Dissolved Organic Matter (CDOM) Sampling Protocol

5.4.1 Overview

Fluorescence of colored dissolved organic matter (CDOM) has been used as a sensitive and specific tracer of natural and anthropogenic compounds in the environment for many years. Most CDOM in coastal margins is of terrestrial origin, and is chemically distinct from CDOM produced insitu. Spectral properties, including fluorescence intensity and the positions of peaks in excitation and emission wavelengths vary with organic matter source and type. Riverine and marine samples can be distinguished on the basis of CDOM, as can contributions from petroleum hydrocarbons, microbial growth, and other specific sources.

Fluorescence can be measured in-situ using field fluorometers or with more complex lab-based instrumentation. While the goal is to eventually measure CDOM in ballast tanks using in-situ instruments, the best possible configuration of such instruments is still to be determined. Since laboratory equipment is currently able to perform more intensive CDOM analyses than in-situ instruments, we currently advocate collection of discrete samples for laboratory analysis. This will help inform the development of in-situ instrumentation.

While plastic can be used to collect CDOM samples, plastic contains carbon and is a potential source of contamination. Glass fiber filters are commonly used to filter CDOM samples from coastal environments, however these are also potential source of low level CDOM contamination, and should be flushed prior to collecting samples.

5.4.2 In-situ CDOM Fluorometers

Instrumentation and protocols for in-situ CDOM measurements are still to be established. A generalized protocol follows:

- Calibrate and optimize field instrument for detection of CDOM.
- Measure the CDOM profile in the ballast tank in a manner similar to that described for salinity.
- Determine compliance by comparing output with pre-determined standards.

5.4.3 Sampling Apparatus

To obtain CDOM samples for laboratory analysis, the pump apparatus of Figure 6 should be connected to the apparatus of Figure 9. Other than the choice of sample bottle, the apparatus and procedure as identical to that used for collecting trace elements.

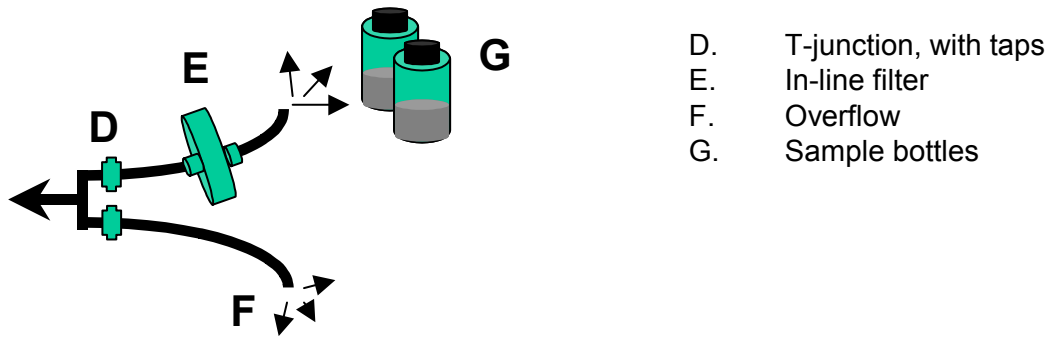


Figure 9: CDOM pump sampling apparatus.

5.4.4 Equipment Specifications

- In-line filter (0.22-0.45 μ m)
Example: Memtrex™ CMMP, 0.45 μ m (Osmonics Inc.)
- Cleaned 120 mL amber glass storage bottles with teflon lined caps
Example: 120mL Amber Boston Round (Fisher Scientific: 03-320-4B)

5.4.5 Products

The objective is to obtain ~ 100 mL of filtered ballast water in each sample bottle, according to strict cleanliness protocols. Following collection, the samples are frozen then shipped to the laboratory for analysis by Emission Excitation Matrix Spectroscopy (EEMS).

5.4.6 Procedure

5.4.6.1 Preparation of Sampling Apparatus

Any materials that will come in contact with the sample (i.e. pumps, hoses, syringes, filters and sample containers) must be clean of grease, organic matter and other fluorescent materials. Cleaning should be performed in the laboratory, prior to setting out for the ship, as follows:

Glass Fiber Filters:

- Bake at 400° C for 5-12 hours, then pack in aluminum foil inside zip-lock bags.

Glass Sample Bottles (caps removed):

- Wash with glassware detergent.
- Rinse x 2 with tap water, to remove detergent.

- Rinse x 3 with with distilled, deionized H₂O (e.g. Milli-Q).
- Bake bottles at 450° C for 8-24 hours.

Teflon Bottle Caps:

- Rinse x 2 with MilliQ water (do not soak).
- Rinse x 1 with HPLC grade methanol (do not soak).
- Dry in 30-35° C oven until methanol evaporates (~ 1 hour).

The cleanliness of the sampling apparatus should be verified using the sample blank procedure described in Section 5.6.

5.4.6.2 Ship-board Sampling

Connect the pump inlet hose to pre-installed hoses in the ballast tank, or otherwise to the desired sampling location. Connect the pump to the power supply. Use apparatus arrangement of Figure 6 and Figure 9.

The general procedure is as follows.

- Collect CDOM **blanks** according to the procedure of Section 5.6.4.
- Turn on the pump and flush at least 10 L water through the sampling system and overflow outlet. Now adjust the taps to allow at least 2 L of water to flow through the in-line filter.
- Carefully remove the cap from the sample bottle and rinse the bottle three times with approx. 20 ml of water.
- Taking care not to touch the inside of the cap or bottle, fill the sample bottle with filtered ballast water to below the shoulder. Do not overfill the bottle, or else it may break during freezing.
- Ensure bottle caps are tight, then further secure them with teflon tape.
- Store the samples in light-proof Styrofoam boxes. Do not allow them to sit around before re-fridgerating or freezing, as heat will cause the samples to deteriorate. Freeze, and ship the samples to laboratory according to instructions in Section 5.4.8.

5.4.7 Sample Log

Table 3 shows an example log-book entry for two hypothetical samples. All fields should be filled out each time a sample is collected.

Table 4: Example log book entries for CDOM samples.

ID-No.	Method	Date	Depth	Filter	Blank Id-No.	Notes
CDOM-611	Pump	1/Jan/03	1 m	0.45µm	Bl-0363-11	As per protocols
CDOM-612	Niskin	1/Jan/03	10 m	0.45µm	Bl-0363-12	As per protocols

5.4.8 Sample Delivery to Analytical Laboratories

Samples should be stored in light-proof Styrofoam boxes, with sufficient padding to prevent breakage. CDOM samples are sensitive to post-collection handling and storage and should be analyzed as soon as possible after collection. It is important to note that exhaustive tests for the effects of storage time and handling on natural CDOM fluorescence characteristics are yet to be carried out. While many researchers have reported no change in fluorescence characteristics over short (less than 30 day) time periods, it is unclear whether this finding is widely applicable. It is thus critical that the potential for sample degradation during storage is taken into account using appropriate scientific methods (including replication, randomization and scientific controls).

With these caveats in mind, we recommend that if the samples will be analyzed in the next 30 days, they are refrigerated and shipped (express) to the laboratory responsible for their analysis. If they will not be analyzed in this time frame, they should be immediately frozen. Frozen samples must be packed in a way that will ensure that they do not thaw in transit. To prevent their arrival while the laboratory is unattended, frozen samples should be shipped overnight in the early part of the week. Analysis of frozen samples must account for possible loss of fluorescence during storage.

5.5 Radium Sampling Protocol

5.5.1 Overview

Two types of samples (short- and long-lived isotopes) are used to fully characterize radium in seawater. Because of the large volume of water required for collection of samples for analysis of long-lived isotopes, extraction of radium onto filters is easiest performed on deck using a pump. In the interests of efficiency, the pumping system should be organized such that the two types of samples are collected simultaneously:

1. Long-lived isotopes and Activity Ratios: ^{226}Ra and ^{228}Ra
2. Short-lived isotopes: ^{223}Ra and ^{224}Ra

It is not necessary for an operator to be present during the entire pumping process provided that the pump is appropriately secured and automated.

5.5.2 Sampling Apparatus

To obtain radium samples by pumping directly from the ballast tanks via a flowmeter/accumulator, the pump apparatus of Figure 6 should be connected to the apparatus of Figure 10a.

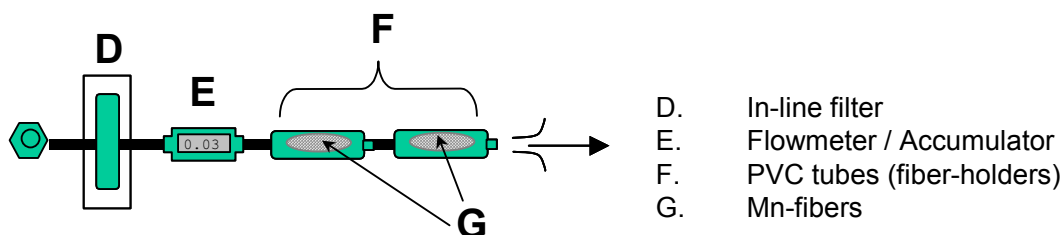


Figure 10a: Radium Pump Sampling Apparatus (using a flowmeter / accumulator for volume standardization).

If volumes are to be standardized using a reservoir instead of a flowmeter/accumulator, use the arrangement of Figure 10b.

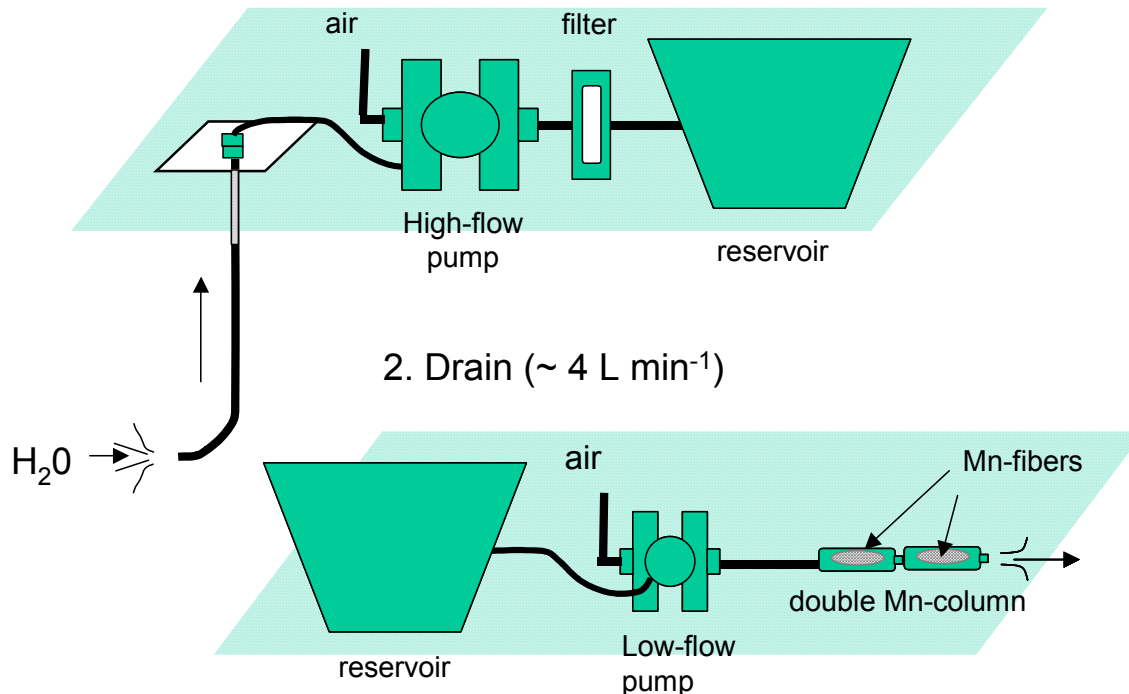
1. Fill ($\sim 20 \text{ L min}^{-1}$)

Figure 10b: Radium Pump Sampling Apparatus (using a reservoir for volume standardization).

5.5.3 Equipment Specifications

- In-line filter:
Example: Cole-Parmer Polycarbonate inline filter holder (29828-00) with spun polypropylene filter (01509-15)
- EITHER:
 - Flow meter / accumulator
Example: Cole-Parmer Electronic Flowmeter/Accumulator (05610-60)
 - ALTERNATIVELY: A 55-80 gallon plastic drum or bag for volume standardization, and a 2-gallon plastic carboy, for flow rate determination.
- Mn-columns:
Assembled in the laboratory of W.S. Moore from readily-available PVC parts.
- Mn-fiber:
Produced in the laboratory of W.S. Moore using a published procedure (Moore 1973, 1976).

5.5.4 Products

Each ballast water-soaked MnO₂-coated fiber constitutes a sample. These are stored in separate zip-lock bags and shipped to the laboratory for analysis.

5.5.5 Procedure

5.5.5.1 Preparation of Mn-fibers

1. Obtain Mn-O₂ coated fibers (Mn-fiber) from an approved source. Fibers are stored in individual zip-lock bags.

5.5.5.2 Control of Volumes and Flow Rates:

Flow rates and sample volumes can be standardized by a range of methods. The only crucial detail is to construct system that allows a known quantity of filtered ballast water to flow through the Mn fiber at a relatively constant low flow rate (max 4 L min⁻¹).

A very simple system might involve first filling a large drum (at least 55 gallon) with pre-filtered water. This could be done relatively quickly at a high flow rate, depending upon the specifications of the in-line filter. Next the water is pumped slowly out of the reservoir and through the sample columns containing the Mn-fibers. The flow rate is checked periodically by measuring the time taken to fill measuring jug with the effluent. Prior to using a drum or carboy for the first time, the volume of the container is determined accurately and thereafter, the container is filled to the same level each time.

A more sophisticated system might involve pumping directly from the ballast tank through a flowmeter/accumulator that records the flow rate at any instant and the total volume through the meter. In this case, the need for the drum and measuring jug would be eliminated. At this time, however, we are unaware of any reasonably-priced flowmeter/accumulators that can accurately keep track of volumes or flow rates at the 3-4 L/min (variable) flow rates required for this procedure.

A system for pumping directly from the tank using a flowmeter/accumulator is described below. It can be simply adapted for the alternative case that ballast water is pumped or drained from a drum, using the drum and carboy in place of the flowmeter.

5.5.5.3 Ship-board sampling

1. Connect the pump inlet hose to pre-installed hoses in the ballast tank, or otherwise to the desired sampling location. Connect the pump to the power supply. Use apparatus arrangement of Figure 6 and Figure 10.

2. Turn on the pump and flush ballast water through the sampling system water line for 2-5 minutes.
3. Turn off pump and set flowmeter / accumulator volume to zero.
4. Place a clean, dry Mn-fiber in each sample column. Connect these in series to the source of filtered ballast water.
5. Turn on pump. Adjust the air supply until the flow rate through the sample column is between 3 - 4 L min⁻¹.
6. Pump no less than 55 Ga. of ballast water through the filter column, then shut off the pump and remove the Mn-fibers. Place the first Mn-fiber in a zip-lock bag, squeeze out excess water, then label the bag, identifying that the sample is from the first column of the series. Place the second Mn-fiber in a zip-lock bag, squeeze out excess water, then label the bag, identifying that the sample is from the rear column of the series. Place each pair of bags in a single, larger bag that is clearly labelled.
7. Write the Sample id, type, volume and date on the zip-lock bag in indelible ink.
8. Fill out the sample log. Be sure to note the accumulated volume displayed by the flowmeter (alternatively, record the known volume of the drum).

5.5.6 Sample Log

Table 5 shows an example log-book entry for two hypothetical samples. All fields must be filled out each time a sample is collected.

Table 5: Example log book entries for radium samples.

ID-No.	Type	Depth	Date	Collection time		Volume (liters)	
				Start	Stop	Measured	Flowmeter
Ra-123	Radium	1m	1/Jan/03	0800	0900	-	199.5
Ra-124	Radium	10m	1/Jan/03	1000	1100	206.4	-

5.5.7 Sample delivery to Analytical Laboratories

Radium samples are time-sensitive, and must be analyzed as soon as possible after collection. At the end of the voyage, all samples, together with copies of log sheets, should be shipped overnight in a padded envelope to the processing laboratory. Radium samples are not hazardous and do not require any other special handling. For mailing purposes, they can be described on the package as ‘scientific samples’.

5.6 Blank Sampling Protocol

5.6.1 Overview

Sample “blanks” are used to account for contamination introduced during the sampling process. They play an important role in quality control, particularly in the case of easily contaminated tracers, such as metals and CDOM. Blanks are necessary if there is a risk that samples will be exposed to contaminants affecting their chemical composition as a result of being removed from their original environment. In the case of ballast water sampling, a contaminated sample will likely have higher metal and/or CDOM concentrations than does the water in the ballast tank. This may lead to a situation in which a vessel that underwent mid-ocean exchange appears not to have done so.

Contaminants due to the sampling process are quantified by subjecting an ultra clean solution, known to have very low levels of contaminants, to the same sampling procedure as the ballast water samples. The levels of contaminants measured in these “blank” samples are then used to define a baseline that is subtracted from the levels measured in the ballast water samples. This provides verification that tracers measured in ballast water samples originated in the ballast tanks rather than in the sampling apparatus.

Blank samples require ultra-clean water (ucH₂O), such as water treated under a Milli-Q® system, which has been stored in specially prepared containers. If there is any doubt that the ucH₂O is ultra-clean prior to sampling, a sample of the ucH₂O should be collected (“pre-blank”) so unintended contaminants in the ucH₂O can be subtracted from the blank measurements (Figure 11).

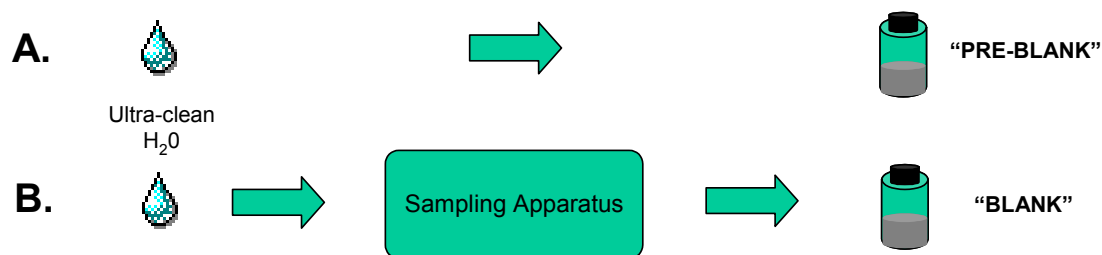


Figure 11: Conceptual diagram of pre-blank and blank samples.

5.6.2 Sampling Apparatus

Sampling apparatus and specifications are identical to that required for ballast water sampling for either trace element (5.3.2) or CDOM (5.4.3) tracers.

In addition to the usual sampling equipment, a reliable supply of ultra-clean water is required. This water should be stored in containers made of plastic (trace element blanks) or glass (CDOM blanks). Containers should be modified with internal tubing and/or spigots so that it is possible to extract the water without inserting any objects (e.g. pump tubing) that may have unclean surfaces.

5.6.3 Products

The objective is to obtain samples of filtered ultra-clean water, according to the same protocols used to collect ballast water samples. Following collection, the samples are shipped along with the ballast water samples to the appropriate analytical laboratory for analysis.

5.6.4 Procedure.

- Sample blanks via pump should be taken on deck immediately prior to ballast water sampling.
- Sample blanks via syringe should be collected in the laboratory as part of a quality control procedure.
- Collect two (2) replicate pre-blank samples, if required.
- Flush sampling apparatus with at least ten volumes of ucH₂O.
- Collect two (2) replicate blank samples for each analysis type (trace elements or CDOM).
- If samples are to be frozen, take particular care not to overfill sample bottles, since pure water expands more than does seawater when frozen (Figure 12). Store upright during freezing to minimize contact between the sample and the bottles caps.

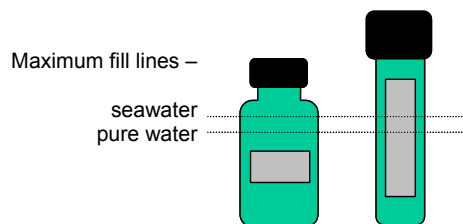


Figure 12: Preventing CDOM and trace element bottle breakages during freezing.

5.7 Ship-side Sampling Protocol

5.7.1 Overview

While a ship is sailing, it is possible to obtain samples of ambient water by accessing the engine cooling pipe system. Water is circulated constantly from the outside of the vessel, through the engine cooling system and out again. The system should be accessed as near as possible to the point of entry of the seawater, i.e., near the sea-chest in the engine room. A less-convenient alternative is to obtain samples on deck via a fire hose, which also taps water from originating from outside of the ship. Accessing pipes in the engine-room is preferable, since seawater is ejected from fire hoses at high pressure, requiring a separate collection step to obtain water which can be subsequently filtered according to normal protocols.

5.7.2 Procedure

5.7.2.1 Fire hose

- Confirm that the fire hose is supplied with clean, untreated ambient water.
- Stabilize the fire hose by tying it to the railing of the ship.
- Ask a crew member to flush the hose for at least 30 minutes.
- Measure and record salinity and temperature of water supplied by the hose.
- CDOM: Fill a clean amber glass container with water from the hose.
- Trace Elements: Fill a clean plastic container with water from the hose.
- Radium: Fill a clean plastic 55-gallon drum with water from the hose.
- Apply normal trace element, CDOM or radium protocols to the containers of ambient water.

5.7.2.2 Engine Room

- Ask a ships' engineer to direct you to a tap which accesses clean, untreated ambient water.
- Flush ~ 4 Ga. of water through the tap, collecting the waste in a bucket.
- Adjust the flow from the tap to a trickle so that trace element and CDOM filtration can be performed under low pressure.
- Measure and record salinity and temperature.
- Trace Elements: Filter water directly from the tap, then proceed according to the protocols of Section 5.3.
- CDOM: Filter water directly from the tap, then proceed according to the protocols of Section 5.4.
- Radium: Fill a clean plastic 55 Ga. drum with water from the hose, then filter the stored water according to the protocols of Section 5.5.

5.8 References

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