Rosette water sampling

R/V Dr. Fridtjof Nansen

EAF-Nansen Programme

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Introduction

In support of EAF-Nansen Programme Themes 9 and 10, this protocol describes the different methods for collecting the various water samples on board R/V Dr. Fridtjof Nansen from the rosette water sampler. The steps described here are specific to the equipment on board R/V Dr. Fridtjof Nansen but can be modified for use in other laboratories as long as differences in equipment are considered.

Equipment

- SBE 32 Carousel Water Sampler (12, 24 or 36 bottles)
- 10 litre PVC Sample Bottles (with air vent and spigot disk lock)
- Silicone Tubing
- Dissolved Oxygen
  - 125 ml Pyrex glass “Iodine titration” flasks with matching stopper and calibrated volume
- pH and Total Alkalinity
  - 250 ml borosilicate glass bottles
- Chlorophyll a
  - 250 ml HDPE Bottles, amber, narrow neck, with screw cap
  - 1 L HDPE Bottles, amber, narrow neck, with screw cap
- Dissolved Nutrients
  - 20 ml plastic scintillation vials
- Salinity
  - 250 ml Type III glass bottle with plastic insert and bakelite screw cap

Overview

At each hydrographic station where water samples are to be collected, the Instrument engineer and crew will deploy the rosette water sampler from the CTD hangar. The rosette will descend in one continuous motion until it reaches the bottom of its deployment. Beginning at that bottom depth, the rosette will stop for 30-60 seconds before the Instrument engineer closes the bottle. This prevents the wake of the rosette from affecting the water inside the bottle and gives us the best water sample possible at the desired depth. This process is repeated at the additional predefined depths during the upcast until the rosette has reached the surface. Before any sampling can take place, the Instrument engineer must come down to the hangar, deliver a .btl file data sheet, and rinse off the CTD sensors.

Careful and attentive water sample collection is vital for acquiring high quality hydrographic samples. Water sample measurement data are unreliable if the water samples are not collected well. Rosette water sampling always begins by collecting from the deepest sample first, which will always be in Bottle 1. When collecting from a bottle for the first time, the Gas Sensitive Samples will always be sampled first. Whoever is collecting the Gas Sensitive Samples has the added responsibility of Checking for Leaks right before they collect the sample. Once the air vent is opened and sample collection has begun, it is not necessary to close the air vent.

Rosette Bottle Sample Log

Before the rosette water sampler reenters the hangar, fill out the Rosette Bottle Sample Log in its entirety. Write down all the different sample bottle numbers used for each water parameter for each rosette bottle. If a rosette bottle is found to be leaking, cross out the row on the sample log, note it in the comments and move on to the next depth's assigned sample bottles. Do not use the sample bottles that were assigned to the leaking bottle. Doing so can and has caused mix ups in sample identification.

R/V Dr. Fridtjof Nansen - Rosette Bottle Sample Log

Survey:	Station:	Area:	Samplers:
2020403	0010	Bergen, Norway	FN, DGC, MC

Latitude:	Longitude:	Echodepth:	Date:	Time:
60.4°N	5.3°W	100	06.06.2020	12:00

Depth	Rosette Bottle	Oxygen (Flask #)	pH/TAlk (Bottle #)	Nutrients (Sta-Bot)	Chloro a (Sta-Bot)	Salinity (Bottle #)	Comments
95	1	323	13	0010-01	0010-01	1	
75	2	212	14	0010-02	0010-02	2	
~~50~~	~~3~~	~~458~~	~~15~~	~~0010-03~~	~~0010-03~~	~~3~~	Bottle leaked
30	4	112	16	0010-04	0010-04	4	
20	5	365	17	0010-05	0010-05	5	
10	6		18	0010-06	0010-06		
5	7		19	0010-07	0010-07		
	8						
	9						
	10						
	11						
	12						

Figure 1. Rosette Bottle Sample Log that notes that Bottle 3 was found to have a leak and was therefore not sampled.

Checking for Leaks

Immediately before we collect the first sample from a rosette bottle, it is very important to check for leaks **before** opening the air vent. Once the air vent is opened, it is impossible to know whether that rosette bottle has a subtle leak. Right before that first sample is collected, open the white spigot at the bottom of the bottle by aligning the spigot disk hole with the tiny metal rod and pushing in. If a continuous dripping or stream of water comes out of the spigot, that means there is a leak and/or the bottle lids did not close properly. **Do not collect samples from a leaking bottle (for any parameter).** That bottle has been exposed to air and/or has a mixture of water that does not represent the water at the predefined depth where it was closed. Therefore, a measurement value from a leaking bottle is not useful because the exact location of where that water comes from is uncertain. Cross out that row on your Rosette Bottle Sample Log and proceed to the next rosette bottle. When you call the Instrument engineer (by dialing 303) to notify them that you are finished collecting samples, also tell them about the leaking bottle so they can investigate and fix the leak or replace the bottle. **Always document leaks on the Rosette Bottle Sample Log. Document how often this happens and to which rosette bottle. If it happens often to the same rosette bottle and the Instrument engineer has not replaced the bottle, ask them to replace it.**

If no leaks are detected, close the spigot by pulling it out towards you. We are now ready to collect our first sample.

Gas Sensitive Samples

Dissolved oxygen and pH samples are extremely gas sensitive, meaning that their values will change the longer they are exposed to the surrounding atmosphere. These samples must be collected first and immediately after the Instrument engineer is finished rinsing the CTD sensors. Otherwise, the samples can be compromised. When oxygen samples are being collected, oxygen will be collected before pH (same bottle as total alkalinity). When oxygen is not being collected, the pH/TAlk samples will be collected first.

Opening a Rosette Bottle

When we are ready to open the rosette bottle to collect our first sample, we begin so by opening the white air vent near the top of the bottle by turning it counter clockwise. Hearing the sound of pressure releasing from the bottle is common. We will get into the details of collecting each sample parameter later on but since we will almost always start with oxygen or pH, we can begin with these steps:

1. Before opening the spigot, attach a silicone tube to the spigot.
2. Insert the other end of the tube to the bottom of the dissolved oxygen or pH/TAlk bottle.
3. Open the spigot (as you did when you checked for leaks) and follow the steps below in the Dissolved Oxygen or pH and Total Alkalinity sections.
    - It is not necessary to use a silicone tube for the other samples.

Dissolved Oxygen

All dissolved oxygen flasks are numbered with a matching ground glass stopper because the volume for each pair has been precisely measured at 20°C for sample size conversion calculation. A mismatching pair will have an unknown volume and therefore an unknown sample size, resulting in a useless sample. Oxygen flasks can be found in the wooden boxes in the CTD hangar.

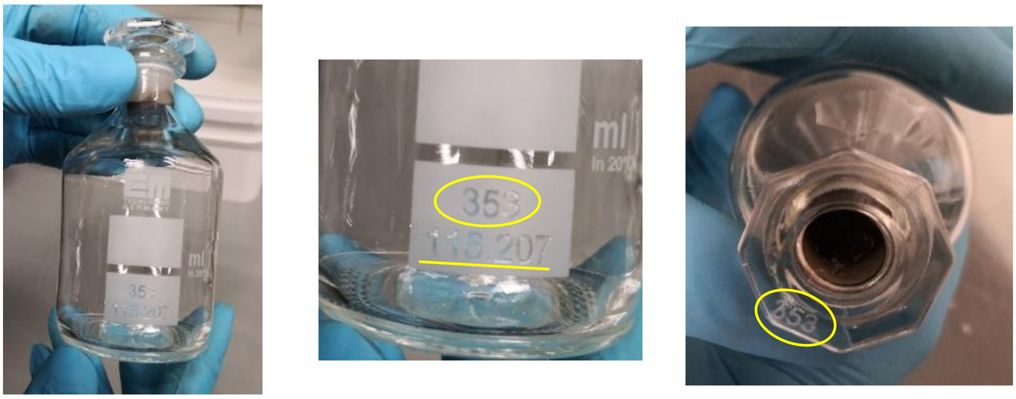


Figure 2. Calibrated dissolved oxygen flask with matching stopper number. The volume at 20°C is shown on every flask.

1. Put on gloves.
2. Checking for Leaks and remember the steps for Opening a Rosette Bottle.
3. With the tube inserted to the bottom of the bottle, open the spigot.
4. As the flow begins, pinch the tube repeatedly and shake the tube to remove air trapped in and on the tube.
5. Once the bottle is ~30% filled, pinch or bend the tube to stop the flow.
6. Remove the bottle from the tube, swirl the seawater inside vigorously and pour the rinse water over the stopper (in your other hand) and sampling tube.
7. Repeat Steps 4 – 6 two more times.
8. On the fourth fill, overflow the bottle 100%.
    - For example: If it takes five seconds to fill the flask, then overflow the sample for five seconds for a 100% overflow.
    - One can rinse the stopper with the overflow water.
9. Remove any bubbles by pinching the tube repeatedly and shaking the tube.
10. Allow the water flow to continue and slowly remove the tube from the flask so the flask is filled to the rim so absolutely no air bubbles are left inside.
11. Carefully, carry the filled flask and stopper to the Winkler chemicals
12. Insert the Winkler A tip ~1 cm into the flask and dispense 1 ml of Winkler A
    - Sample will spill since the flask is full. This is okay.
13. Insert the Winkler B tip ~1 cm into the flask and dispense 1 ml of Winkler B
    - Sample will spill since the flask is full. This is okay.
14. Slowly and carefully, put on the stopper in a manner that prevents air bubbles from being trapped underneath.
15. There should be no head space and no bubbles in the flask when the stopper is fixed tightly. Hold tightly onto the flask and stopper and shake for 5 seconds.

16. When all samples have been collected, place the samples dark in the same room where the analyses will take place.
17. After 30 minutes, shake the samples again to ensure the reagents are well mixed.
18. After two hours, the samples ready for analysis.
    - As long as the seal integrity is maintained, samples can be stored for many days without any noticeable change in concentration (Langdon, 2010)

pH and Total Alkalinity

A sample for pH and total alkalinity will be collected in a single 250 ml borosilicate glass bottle using a silicone tube. Sub-samples will be taken from this 250 ml sample for the individual analyses of pH and total alkalinity. These bottles are located in the wooden OA boxes in the CTD lab. Make sure the blue ring is on the glass bottle (lip facing up) before sampling.

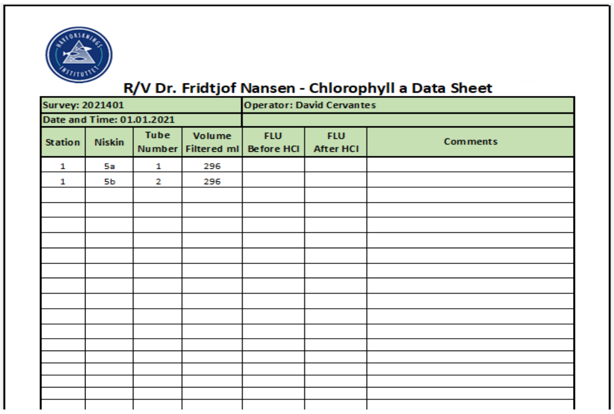
Figure 3. Sample bottle for a pH/total alkalinity sample being filled. Notice that the silicone tube is at the bottom of the bottle and the tube is in the process of being pinched (repeatedly).

1. If this is the first sample being collected from this rosette bottle, Checking for Leaks and remember the steps for Opening a Rosette Bottle.
2. Hold the bottle in your dominant hand (right hand for example).
3. Hold the cap in your nondominant hand (left hand for example).
4. With the tube inserted to the bottom of the bottle, open the spigot.
5. As the flow begins, pinch the tube repeatedly and shake the tube to remove air trapped in and on the tube.
6. Once the bottle is ~30% filled, pinch or bend the tube to stop the flow.
7. Remove the bottle from the tube, swirl the seawater inside vigorously and pour the rinse water on the underside of the cap and sampling tube.
8. Repeat Steps 5 – 7 two more times.
9. On the fourth fill, overflow the bottle 100%.
    - For example: if it takes 9 seconds to fill the bottle, you should have an overflow of 9 seconds.

10. While filling/overflowing and with the tube still at the bottom of the bottle, shake the tube and move the bottle around to release any bubbles that may be trapped inside.
    1. Bubbles like to stick to the outside of the tube and on the insides of the bottle wall.
11. After 100% overflow and with the tube still at the bottom of the bottle, close the spigot by pulling the ring towards you.
12. Once the flow has completely stopped, slowly lower and remove the bottle from the tube.
    - A headspace will now be created from the sampling tube.
13. Screw on the cap **tightly** to prevent any atmospheric gas exchange.
14. As you collect the first few samples (deepest and coldest), put them into the water bath (set to 25°C) and make sure they are covered.
15. Check all screw caps for tightness once sample collection is finished.
16. The bottles in the water bath will be ready for analysis once sample collection is finished.
17. Store the rest of the samples in the dark in their corresponding OA boxes and put them into the water bath as pH samples are analysed.

Chlorophyll a

Samples for the analysis of chlorophyll a are collected in duplicate in 296 ml high-density polyethylene bottles from depths ranging from 5 to 200 m. Immediately after sample collection, the samples will be filtered with 25 mm Munktell MG F filters with a 0.7 µm particle retention on a 200 mm Hg vacuum pumped filtration system. After filtering, samples will either be stored at -20°C or added directly to centrifuge tubes so they can begin extraction with 90% acetone.



R/V Dr. Fridtjof Nansen - Chlorophyll a Data Sheet

Survey: 2021401				Operator: David Cervantes		
Date and Time: 01.01.2021						
Station	Niskin	Tube Number	Volume Filtered ml	FLU Before HCl	FLU After HCl	Comments
1	5a	1	296			
1	5b	2	296			

Figure 4. Centrifuge tubes containing chlorophyll a filters and 90% acetone; duplicate 296 ml chlorophyll a bottles; Chlorophyll a Data Sheet example.

1. With the tube inserted to the bottom of the bottle, open the spigot.
2. As the flow begins, pinch the tube repeatedly and shake the tube to remove air trapped in and on the tube.
3. Once the bottle is ~30% filled, pinch or bend the tube to stop the flow.
4. Remove the bottle from the tube, swirl the seawater inside vigorously and pour the rinse water on the underside of the cap and sampling tube.
5. Repeat Steps 2 – 4 two more times.
6. On the fourth fill, allow the water to flow continuously while you slowly remove the tube from the bottle so the bottle is filled to the rim so absolutely no head space is left.

7. Screw on the cap **tightly**.
8. Check all screw caps for tightness once sample collection is finished.
9. Proceed to the filtration rack in the lab to begin filtering.

Salinity

Salinity samples will be collected in a 250 ml Type III glass bottle with plastic insert and bakelite screw cap (sampling tube not necessary). Four black boxes of 12 salinity bottles each are in the CTD lab. It is incredibly important that the plastic insert is remembered when collecting samples.

Figure 5. Salinity sample bottle with water line below the bottle neck; salinity bottle box with a plastic bag containing the plastic inserts.

1. Acquire the salinity bottle, cap, and plastic insert.
    a. The plastic insert is VERY important for the seal.
2. Hold bottle in your dominant hand (right for example).
3. Hold cap and plastic insert in the other hand (left for example).
4. Fill salinity bottle ~1/4 of its total volume.
5. Put cap on bottle.
6. Shake.
7. Pour rinse water over the cap and the plastic insert.

8. Repeat steps 4-7 two more times.
9. Now fill the salinity bottle just below its shoulder.
10. Put the plastic insert onto the bottle.
11. Screw on the cap **tightly**.
12. When salinity sampling is finished, samples must be stored in the same room as the Portasal for at least 12 hours to equilibrate to the same room temperature as the instrument.

Dissolved nutrients

Nutrient samples will be collected in 20 ml plastic scintillation vials (sampling tube not necessary). Samples will be stored in the freezer until they are ready to be thawed for analysis (Becker et al., 2019). For this reason, it is very important that enough head space is left in the vial to allow for the expansion of water upon freezing. Use the label printer in the CTD lab to label all bottles before sampling.

Figure 6. Nutrient scintillation vial with tightened cap. Arrow indicates appropriate fill level.

1. Place all scintillation vials in the gray vial holder (not pictured).
2. Rinse the vial and cap three times.
3. On the fourth fill, fill the vial just below the shoulder.
4. Screw on the cap **tightly**.
5. When finished, place the vials in a cardboard nutrient box in the CTD lab freezer.

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