**2022 P02 Leg 2 Bio Cruise Report**

**Total Numbers May Need a Recount**

Sample types collected the same way as Leg 1: DNA/RNA, Large Volume Particulate Organic Matter (POC/N, POP, PCOD), High Performance Lipid Chromatography (HPLC), and Flow Cytometry (FCM). Sample types taken differently or only during Leg 2: Small Volume Particulate Organic Carbon/Nitrogen, Planktoscope, PCOD Volume Trial

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**Underway Sampling**

DNA/RNA, Large Volume Particulate Organic Matter (POC, PON, POP, PCOD), HPLC, and FCM samples were collected at approximately 0600, 1200, and 2000 local time via the underway tap (59 stations). The underway system was pumped with a diaphragm pump instead of the impeller pump so that organic matter would not be shredded up. The timing for collecting samples around 2000 was based on each day’s solar noon + 8 hours. Underway samplings were skipped if the CTD rosette were to collect biological samples within a three to four hour window of an underway sampling time.

**Bio CTD Station Sampling**

At every third GO-SHIP P2 station, the CTD rosette was casted twice (32 stations). The first cast only collected biological samples (called the “bio cast”) and the second cast only collected core GO-SHIP samples (pH, nutrients, etc.). For the bio cast, the CTD rosette was sent to a maximum depth of 1000 m. As the rosette surfaced, it collected seawater only to be used for biological samples (ie. DNA/RNA, Large Volume POM, HPLC, and FCM). Niskin bottles were fired at depths of 1000 m, 500 m, 200 m, 150 m, 100 m, 75 m, 40 m, and 5 m. The CTD rosette was then recovered, the seawater was collected, and then the CTD was cast again to collect the core GO-SHIP samples.

**BGC Argo Float Station Sampling**

At each station where a BGC Argo float was deployed, the sampling scheme for DNA/RNA, Large Volume Particulate Organic Matter (POC, PON, POP, PCOD), HPLC, and FCM was different than the usual bio cast sampling. For every bio cast at a float station, Niskin bottles were fired at depths of 1000 m, 500 m, 200 m, ~30 m below the chlorophyl maximum depth, at the chlorophyl maximum depth, ~ 30 m above the chlorophyl maximum depth, at the bottom of the mixed layer, and 5 m. For Leg 2, four out of the thirty-two bio cast stations were float stations (GO-SHIP P2 stations 122, 146, 170, and 191).

**DNA/RNA**

DNA/RNA samples were collected at each underway sampling, with duplicates collected at 2000. For bio casts, seawater for DNA/RNA was collected at depths of 1000 m, 200 m, 100 m and 5 m. For bio casts at float stations, seawater for DNA/RNA was collected at depths of 1000 m, 200 m, at the chlorophyll maximum depth, and 5 m. 100 DNA/RNA samples were collected from the underway and 120 were collected from the rosette, resulting in a total of 220 DNA/RNA samples collected throughout Leg 2.

Each sample was a Sterivex 0.22μm filter cartridge that had seawater filtered through it. For DNA/RNA, seawater was transported from the underway or rosette to the filtration system using 4L plastic cubitainers. Volumes of 4 - 8L of seawater were filtered through a Sterivex 0.22μm filter cartridge via a peristaltic pump set to a low speed. After the seawater had been filtered, air was pumped out of the filter cartridge using a 5 mL syringe. The cartridge bottom was then sealed with Crito-Seal before pipetting 1620μL of sterile lysis buffer into the cartridge. The cartridge was then capped with a luer-lock. 10 of the 100 underway samples were duplicates that had RNA/DNA Shield buffer added instead of lysis buffer in order to compare these two buffer types. Each filter was placed in a Ziplok bag and stored at -80 °C until time for analysis. Final filtration volume was recorded for all samples. Samplers wore nitrile gloves throughout the entirety of sample collection and filtration.

Before Leg 1, all silicone tubing, Omnifit caps and cubitainers were soaked with soap water, 10% HCl acid, and Milli-Q water. At the end of every week of the cruise, the peristaltic pump tubing and Omnifit caps were cleaned with 10% bleach solution and then rinsed with Milli-Q water. All cubitainers were rinsed three times with sample water before being filled.

**Large Volume Particulate Organic Matter (POM)**

Whatman GF/F filters (pore size 0.7 μm, diameter of 25 mm) were filtered with 2 - 8 L of seawater to serve as particulate organic matter (POM) samples. Filters were collected for post-cruise analyses that determined either particulate organic carbon/nitrogen (POC/N), phosphorous (POP) or particulate chemical oxygen demand (PCOD). POM samples were collected via the underway tap or the CTD rosette at a depth of 5 m (91 stations). A total of 801 samples was collected (297 from the underway and 504 from the rosette).

Nine 8 L carboys were filled with 2 - 8 L of seawater for each POM filtration. The carboys were divided into three groups for desired sample type: POC/N, POP, and PCOD. As a result, triplicates were collected for POC/N, POP, and PCOD at each sampling. Seawater was drawn through the filters using an aspirator pump with a vacuum setting of -0.06 to -0.08 MPa. After all seawater had been filtered, filters for POP were rinsed with 5 mL of 0.017 M Na2SO4 to remove any dissolved organic phosphorous. PCOD filters were rinsed with 5 mL of Milli-Q water to remove excess chloride. Filters were folded and then stored at -80°C in pre-combusted aluminum foil.

Before starting Leg 2, GF/F filters and foil squares were precombusted at 500°C for 5 hours. All silicone tubing, filter cartridges, and carboys were soaked with soap water, 10% HCl acid, and then Milli-Q water. Samplers wore nitrile gloves throughout the entirety of sample filtration. All carboys were rinsed three times with sample water before being filled.

**Small Volume Particulate Organic Carbon/Nitrogen (POC/N)**

Small volume particulate organic carbon/nitrogen (POC/N) samples were only collected at the four float stations. The CTD rosette collected seawater for these samples at a depth of 200 m, ~30 m below the chlorophyl maximum depth, at the chlorophyl maximum depth, ~30 m above the chlorophyl maximum depth, at the bottom of the mixed layer, and at a depth of 5 m. From each of these depths, 2 L of seawater was collected and a single duplicate of 2 L was collected from one of these depths. The depth from which the duplicate was collected was decided at random and varied across float stations. Small volume POC/N were collected to compare the carbon/nitrogen quantified from large volume POC/N samples.

Seawater was transported from the rosette to a filtration manifold using 1 -2 L clear plastic bottles. The filtration manifold was hooked to a vacuum pump set at 100 mmHg. Seawater was filtered onto pre-combusted Whatman GF/F filters (pore size 0.7 μm, diameter of 25 mm). A dry blank and a wet blank were also collected at each float station. A dry blank consisted of taking a GF/F filter out of the pre-combusted foil it was stored in, and then putting it pack into the foil without filtering any seawater. The wet blank consisted of placing another GF/F filter below one of the GF/F filters before filtering seawater through them. The bottom filter was collected and saved as the wet blank. For Leg 2, the wet blank was always placed under the duplicate sample filter, thus the seawater filtered through it also came from a random depth and varied across float stations. All filters were then stored at -80 °C in pre-combusted aluminum foil. Samplers wore nitrile gloves throughout the entirety of seawater collection and filtration. All bottles were rinsed three times with sample water before being filled.

**Particulate Chemical Oxygen Demand (PCOD) Volume Trial**

Volume trials for PCOD samples were performed three times during Leg 2. These trials happened randomly when there was enough time in-between underway or rosette sampling. The volume trial consisted of filling the POM carboys via the underway with volumes ranging from 1 - 8 L. Starting with a carboy filled with 8 L, each consecutive carboy was filled with 1 L less than the carboy filled before it (ie. the second carboy was filled with 7 L, the third was filled with 6 L, etc.). The seawater from each carboy was filtered onto a Whatman GF/F (pore size 0.7 μm, diameter of 25 mm) in the same method for POM filtration. Filters were then rinsed with 5 mL of Milli-Q water to remove excess chloride. The purpose of the volume trials is to test the sensitivity of the PCOD analysis after the cruise.

All carboys were rinsed three times with sample water before being filled. GF/F filters and foil squares were also pre-combusted and then stored at - 80 °C. Nitrile gloves were worn for all steps mentioned above.

**High Performance Liquid Chromatography** (**HPLC)**

HPLC samples were collected with each underway sampling and at each bio cast. For bio casts, seawater for HPLC samples was collected at 100 m, 40 m, and 5 m. For float stations, seawater for HPLC samples was collected at a depth of 200 m, ~30 m below the chlorophyl maximum depth, at the chlorophyl maximum depth, ~30 m above the chlorophyl maximum depth, at bottom of the mixed layer, and at a depth of 5. A total of 181 samples was collected (111 from the rosette and 70 from the underway). The purpose of HPLC samples was to quantify photosynthetic pigment content.

For HPLC samples, 2 L of seawater was transported from the underway or rosette with 1 L amber HPDE bottles. Seawater was then filtered with Whatman GF/F filters (pore size 0.7 μm, diameter of 25 mm) using a vacuum pump set at 100 mmHg. Filters were folded twice, placed into 1 mL cryovials, and then stored at -80 °C. Nitrile gloves were worn throughout seawater collection and filtration. All HPDE bottles were rinsed three times with sample water before being filled.

A duplicate HPLC sample was collected for every other underway sampling. This was to ensure that roughly 10% of total HPLC samples were duplicates. At float stations, a single duplicate of 2 L was also collected from one of the depths chosen by random. The depth chosen for the duplicate varied across float stations.

**Flow Cytometry (FCM)**

FCM samples were collected with each underway sampling and at each bio cast. For bio casts, seawater for FCM samples was collected at depths of 1000 m, 500 m, 200 m, 150 m, 100 m, 75 m, 40 m, and 5 m. For float stations, seawater for FCM samples was collected at depths of 1000 m, 500 m, 200 m, ~30 m below the chlorophyl maximum depth, at the chlorophyl maximum depth, ~30 m above the chlorophyl maximum depth, at bottom of the mixed layer, and 5 m. A total of 305 samples was collected (245 from the rosette and 59 from the underway). FCM samples were to be analysed after the cruise with a flow cytometer.

Seawater for FCM samples was collected with 50 mL tinted falcon tubes. From the tubes, 1.8 mL of seawater was pipetted into a 2 mL cryovial. While under a fume hood, 18 μL of a preservation mixture (half 25% Glutaraldehyde and half 2% Kolliphor) was added to each cryovial. The cryovial was then inverted several times and then placed on a vial stand for about for 10 minutes. The vials were then flash frozen with liquid nitrogen and stored at -80 °C.

**Planktoscope**

At most stations with a bio cast, 40 - 100 L of seawater from the underway tap was filtered by a 10 μm mesh to concentrate >10 μm particles into a volume of ~250 mL (27 stations). The volume used from the underway was determined by filling a 20 L carboy multiple times. For two of the twenty-seven stations, about 4 L of seawater from the chlorophyl maximum depth was concentrated to ~250 mL instead. Chlorophyl maximum depth seawater was collected via the CTD rosette. 100 mL from the ~250 mL was preserved with 0.5 mL of Lugols solution for post-cruise analysis. 1.8 mL was also taken from the ~250 mL and preserved by the same method used for FCM samples. About 5 - 20 mL of the ~250 mL was also fed into a planktoscope. The purpose of the planktoscope was to quantify plankton abundance.

The planktoscope took 100 pictures as 5 mL of seawater flowed in front of a 20 mm tube lens and a 16 mm objective lens. Seawater was set to flow at a rate of 2 mL/min. The planktoscope then segmented parts of the pictures that contained distinct outlines and dark shapes against a white background. The segmenting program was optimized to detect objects in the size range of 40 μm - 200 μm. The lens were focused and a white-balanced was performed before filming each sample