

## Procedure: GOMA Round Robin 2

### Preparatory procedures:

Clean carboys for each analyte group/preparation method (= analyte group) as follows:

1. Rinse 3 times with DI water
2. Rinse 3 times with small amount of 10% HCl
3. Rinse 3 times with DI water

Prepare 10% Hydrochloric acid solution for cleaning carboys and tubing as follows:

- Fill cleaned 1-L glass container about half way with distilled water
- Add 10 mL of HCl
- Fill with distilled water to 1L
- Cap and invert bottle to mix.

Set up stations in close proximity to a sink and source of DI water. Each station needs to have the following supplies:

wheeled cart	filter (disposable	Pen
magnetic stir plate	cartridge filter for	labeling tape
stir bar and its magnetic	0.45 $\mu$ m filtering and	10% HCl solution
retriever tubing	reusable cartridge	KimWipes (large and
razor blade	filter with new paper	small)
peristaltic pump	for 105 $\mu$ m filtering)	Notebook
zip ties	filter tighteners (for	procedure notes
goggles	reusable cartridge	absorbent paper
gloves	filter)	Salinometer
large beaker	Sharpie	Narrow-range pH paper

Three sites will be sampled. One will be a coastal or estuarine site that is expected to have high nutrient concentrations. One will be a coastal or estuarine site that is expected to have low nutrient concentrations. The last site will be a location that will represent open-Gulf conditions. For each site, the carboys will be set up for the following analyte groups:

- Filtered nutrients (orthophosphate and dissolved nitrite)
- Total nutrients (TKN, total nitrate+nitrite, ammonia, total phosphate, and total organic carbon)
- Dissolved nutrients (dissolved nitrate+nitrite and dissolve organic carbon)
- Total nitrite
- Chlorophyll a
- BOD
- CBOD

If salinity was not recorded in the field, take a salinity measurement. Record salinity measurement on laboratory sheets that will accompany the samples to the laboratories.

### General sample splitting procedure

#### Day 1:

Samples from the sites will be brought to the lab immediately prior to filtering or acid preservation.

**Basic activities:**

1. Filter carboys for analyte groups as required.
2. Acidify carboys for analyte groups as required.
3. Split and chill analyte groups not requiring filtration or acidification.

**Filtered nutrients preparation (un-preserved, filtered):**

1. Rinse graduated 1-L beaker and stir bar using the same procedure as above for carboys.
2. Place 20-L carboy of ambient water on magnetic stir plate.
3. Add stirring rod.
4. Stir 20-L carboy of ambient water sample for ~10 minutes prior to filtering. Check periodically to make sure that it continues to stir. In the meantime carry out steps 3 to 4. Continue stirring throughout filtering procedure.
5. Set up peristaltic pump with tubing.
6. Attach 0.45- $\mu\text{m}$  inline filter to tubing using small pieces of silicone tubing at either end of the filter and tighten tubing with zip ties to prevent leakage.
7. Rinse tubing and filter assembly by pumping through the system the following solutions in this order:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
8. Filter volume required for samples (see sample volume spreadsheet) plus 20% of sample into another clean carboy (pump should be set around speed 6) and change filter as needed to prevent clogging. Each filter change should be followed by an equipment volume rinse of site water.
9. Label carboy with “Un-preserved, Filtered,” site letter, and analyte group (further distinguish carboys by site with colored tape).
10. Moved filtered carboy into ice or walk-in cooler (4 °C).

**Dissolved nutrients preparation (acid-preserved, filtered):**

1. Continue to use the remaining unfiltered sample from the previous procedure (add water if necessary).
2. Stir carboy for ~10 minutes prior to filtering. Check periodically to make sure that the sample is being stirred.
3. Retain tubing from previous procedure.
4. Attach a new 0.45- $\mu\text{m}$  inline filter to tubing using small pieces of silicone tubing at either end of the filter and tighten tubing with zip-ties to prevent leakage.
5. Rinse tubing and filter assembly by pumping through the system the following solutions in this order:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI

- d. 1 equipment volume (200-400 mL) of site water
6. Filter volume required for samples (see sample volume spreadsheet) plus 20% of sample into another clean carboy (pump should be set around speed 6) and change filter as needed due to clogging. Each filter change should be followed by an equipment volume rinse of site water.
7. Label carboy with “Filtered,” site letter, and analyte group (further distinguish carboys by site with colored tape).
8. Retrieve and clean magnetic stir bar from un-filtered sample in the following manner:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water.
9. Place remaining unfiltered sample on ice.
10. Place filtered sample on the magnetic stirring plate.
11. Add magnetic stirring rod.
12. Stir filtered sample ~10 minutes. Check periodically to verify that the sample is still being stirred.
13. With filtered sample still stirring, add sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for preservation (1-mL per Liter of sample).
14. Stir acidified sample for ~10 minutes.
15. Check to see if pH <2 by running an equipment volume through tubing and then dispense a small amount onto narrow-range pH paper (if pH is > 2, then add acid 1 mL at a time, stir and check pH until pH <2 is reached).
16. Label carboy “acid preserved, filtered,” the number of mL of acid required to get pH<2, site letter, and analyte group (further distinguish carboys by site with colored tape).
17. Move “filtered” and “acid preserved, filtered” carboys into walk-in cooler (4 °C).
18. Retrieve and clean magnetic stir bar from filtered sample in the following manner:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water.

**Nutrients preparation (both sites were prepared simultaneously at two stations):**

1. Continue to use the remaining unfiltered sample and add more un-filtered sample if necessary.
2. Place carboy on magnetic stirring plate.
3. Add magnetic stirring rod.
4. Stir for ~10 minutes. Check periodically to ensure that the sample is being stirred.
5. Retain tubing from previous procedure.
6. Use filter apparatus as a stencil to cut out 105- $\mu$ m filter paper with razor blade.
7. Rinse graduated 1-L beaker and filter apparatus pieces using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water

8. Assemble filter pieces, 105- $\mu\text{m}$  filter paper, and tubing; tighten with tighteners.
9. Attach filter apparatus to tubing assembly.
10. Rinse tubing and filter assembly as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
11. Filter volume required for samples (see sample volume spreadsheet) plus 20% of sample into another clean carboy (pump should be set around speed 7) and check periodically to make sure the filter paper doesn't get clogged and need to be changed out. Each filter paper change should be followed by an equipment volume rinse of site water.
12. Label carboy with "105- $\mu\text{m}$  Filtered," site letter, and analyte group (further distinguish carboys by site with colored tape).
13. Retrieve and clean magnetic stir bar from unfiltered sample in the following manner:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water.
14. Place remaining unfiltered sample on ice.
15. Stir 105- $\mu\text{m}$  filtered sample ~10 minutes. Check periodically to ensure that the sample is still being stirred.
16. With filtered sample still stirring, add sulfuric acid ( $\text{H}_2\text{SO}_4$ ) for preservation (1-mL per Liter of sample).
17. Stir acidified sample for ~10 minutes
18. Check to see if  $\text{pH} < 2$  by running an equipment volume through tubing and then dispense a small amount onto narrow-range pH paper (if  $\text{pH} > 2$ , then add acid 1mL at a time, stir and check pH until  $\text{pH} < 2$  is reached).
19. Label carboy "acid-preserved, un-filtered," the number of mL of acid required to get  $\text{pH} < 2$ , site letter, and analyte group (further distinguish carboys by site with colored tape).
20. Move un-filtered, acid preserved carboy into walk-in cooler (4 °C).

**Total nitrite sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water
2. Fill appropriately sized carboy (see sample volume spreadsheet) with ambient water.
3. Place carboy on magnetic stirring plate.
4. Add magnetic stir bar.
5. Stir carboy for ~ 10 minutes prior to splitting. Check periodically to ensure that the sample is still being stirred. In meantime, carry out steps 6 to 9. Continue stirring throughout splitting procedure.
6. Rinse tubing as follows:

- a. 400-mL DI
- b. 150-mL 10% HCl
- c. 400-mL DI
- d. 1 equipment volume (200-400 mL) of site water
7. Place label with analyte group and preservation information on each bottle.
8. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps face down on large KimWipes).
9. Pump sample into sample containers in thirds, leaving the neck of the bottle as head space.
10. Cap bottles randomly.
11. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
12. Place bottles in boxes that are labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper that placed inside each box.
13. Store boxes in walk-in cooler (4 °C).

**BOD sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water
2. Fill appropriately sized carboy (see sample volume spreadsheet) with ambient water.
3. Place carboy on magnetic stirring plate.
4. Add magnetic stir bar.
5. Stir carboy for ~ 10 minutes prior to splitting. Check periodically to ensure that the sample is still being stirred. In meantime, carry out steps 6 to 9. Continue stirring throughout splitting procedure.
6. Set up peristaltic pump with large diameter tubing.
7. Rinse tubing as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
8. Place label with analyte group and preservation information on each bottle.
9. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps face down on large KimWipes).
10. Pump sample into sample containers in thirds, leaving the neck of the bottle as head space.
11. Cap bottles randomly.
12. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
13. Place bottles in boxes that are labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper that placed inside each box.
14. Store boxes in walk-in cooler (4 °C).

**CBOD sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water
2. Fill appropriately sized carboy (see sample volume spreadsheet) with ambient water.
3. Place carboy on magnetic stirring plate.
4. Add magnetic stir bar.
5. Stir carboy for ~10 minutes prior to splitting. Check periodically to make sure that the sample is being stirred. In meantime, carry out steps 6 to 9. Continue stirring throughout splitting procedure.
6. Retain large diameter tubing from previous analyte group (BOD).
7. Rinse tubing as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
8. Place label with analyte group and preservation information on each bottle.
9. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps on large KimWipes face down).
10. Pump sample into sample containers in thirds, leaving the neck of the bottle as head space.
11. Cap bottles randomly.
12. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
13. Place bottles in boxes labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper placed inside each box.
14. Store boxes in walk-in cooler (4 degrees C).

**Chlorophyll a sample splitting:**

1. Rinsed graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - a. Rinse 3 times with DI water
2. Fill appropriately sized carboy (see sample volume spreadsheet) with ambient water.
3. Place carboy on magnetic stirring plate.
4. Add magnetic stir bar.
5. Stir carboy for ~10 minutes prior to splitting. Check periodically to make sure that the sample is being stirred. In meantime, carry out steps 6 to 10. Continue stirring throughout splitting procedure.
6. Retain large diameter tubing from previous analyte group (CBOD).
7. Rinse tubing (using peristaltic pump) as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl

- c. 400-mL DI
8. Remove tubing from peristaltic pump.
9. Place label with analyte group and preservation information on each bottle.
10. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps on large KimWipes face down).
11. Siphon off 1 equipment volume (200-400 mL) of site water. Start siphoning by feeding some of the tubing inside the carboy, placing a finger over the end of the tubing outside the carboy, pulling enough of the water-filled tubing out of the carboy so that removing the finger on the end will start the siphon.
12. Siphon sample into sample containers in thirds, leaving the neck of the bottle as head space.
13. Cap bottles randomly.
14. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
15. Place bottles in boxes labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper placed inside each box.
16. Store boxes in walk-in cooler (4 °C).

### **Day 2:**

#### **Basic activities:**

1. Split remaining samples.
2. Label bottles with replicate and laboratory information.
3. Pack coolers.
4. Ship coolers.

#### **Orthophosphate/dissolved nitrite sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water
2. Place “un-preserved, filtered” carboy on magnetic stirring plate.
3. Add stirring rod.
4. Stir filtered sample ~10 minutes. Check periodically to ensure that the sample is being stirred. In meantime, carry out steps 5 to 8.
5. Set up peristaltic pump with small-diameter tubing.
6. Place label with analyte group and preservation information on each bottle.
7. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps on large KimWipes face down).
8. Rinse tubing as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
9. Pump sample into sample containers in thirds, leaving the neck of the bottle as head space.
10. Cap bottles randomly.

11. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
12. Place bottles in boxes labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper placed inside each box.
13. Store boxes in walk-in cooler (4 °C).

**Dissolved nutrients sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - a. Rinse 3 times with DI water
  - b. Rinse 3 times with small amount of 10% HCl
  - c. Rinse 3 times with DI water
2. Place “acid-preserved, filtered” carboy on magnetic stirring plate.
3. Add stirring rod.
4. Stir sample ~10 minutes. Check periodically to ensure that the sample is being stirred. In meantime, carry out steps 5 to 8.
5. Retain tubing from previous splitting procedure (Orthophosphate).
6. Place label with analyte group and preservation information on each bottle.
7. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps on large KimWipes face down).
8. Rinse tubing as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
9. Pump prepared sample into sample containers in thirds, leaving the neck of the bottle as head space.
10. Cap bottles randomly.
11. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
12. Place bottles in boxes labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper placed inside each box.
13. Store boxes in walk-in cooler (4 °C).

**Nutrients sample splitting:**

1. Rinse graduated 1-L beaker and stir bar using the following procedure:
  - d. Rinse 3 times with DI water
  - e. Rinse 3 times with small amount of 10% HCl
  - f. Rinse 3 times with DI water
2. Place “acid-preserved, un-filtered” carboy on magnetic stirring plate.
3. Add stirring rod.
4. Stir sample ~10 minutes. Check periodically to ensure that the sample is being stirred. In meantime, carry out steps 5 to 8.
5. Retain tubing from previous splitting procedure (Dissolved N and DOC).
6. Place label with analyte group and preservation information on each bottle.
7. Set out bottles on absorbent paper in a square or rectangle and remove caps (place caps on large KimWipes face down).

8. Rinse tubing as follows:
  - a. 400-mL DI
  - b. 150-mL 10% HCl
  - c. 400-mL DI
  - d. 1 equipment volume (200-400 mL) of site water
9. Pump prepared sample into sample containers in thirds, leaving the neck of the bottle as head space.
10. Cap bottles randomly.
11. Tighten caps, inspect bottles for defects, verify analyte group and preservation information, and dry the outsides.
12. Place bottles in boxes labeled with the site letter, analyte, and preservation on the box sides and on a slip of paper placed inside each box.
13. Store boxes in walk-in cooler (4 °C).

Packaging samples for shipment:

1. Assigned each cooler(s) a lab letter and arrange them in alphabetical order in an area on the floor below the bench space where the sample bottles would be arranged.
2. A bottom layer of ice was placed in each empty cooler to which bottles would be placed on top of in a later step.
3. Using four people, one person opened a box of sample bottles from the walk-in cooler and called out the site label and analyte group from the box. A second person agreed to the site and analyte group called out.
4. This person then dried the condensation off each bottle, checked that the caps were tight and handed it to the labeler.
5. The labeler read out loud the first label for that site and analyte group before placing the label on one of the bottles handed to them.
6. A fourth person checked each label off on the master label spreadsheet as they were called.
7. The labeled bottles were then handed to the first person who placed each bottle on the bench space designated for each lab letter in a line and grouped by analyte.
8. This continued until all the bottles were labeled, sorted by lab and analyte group, and checked off the spreadsheet.
9. All four people then chose a lab's section and arranged the bottles within their letter group in numerical order 1 through n [need to decide # of replicates] checking to make sure they were in the correct letter group and analyte group, there were no defects to the bottles, and the correct number of replicates were present.
10. People then took different letter groups from the ones they arranged and placed them in the appropriate cooler. At the same time they marked on the "list of bottles in each cooler" sheet what samples went into that cooler.
11. One person came around and added ice to surround the bottles once the cooler had all its bottles packed.
12. After all the coolers were filled, the printed copies of paperwork (chain of custody form, instructions sheet, and data entry spreadsheet) for each lab were placed in a plastic zip-lock bag and put on top of the ice inside each cooler and one or two

bottles were briefly lifted to check that the lab letter on the bottles matched the paperwork.

13. Each lab letter was matched up with the corresponding waybill for shipping and the waybills were adhered to the top of each cooler. Senders copies were saved for tracking purposes.
14. Coolers were placed in the loading area to be picked up by DHL (shipped overnight).
15. The extra set of bottles (blank) was stored in a walk-in cooler in case needed.