Solving the Mystery Behind an Iconic Question

What Happens to Terrestrial Organic Matter in the Ocean?

<u>Alterações desde a última versão:</u>

1. Definação o local offshore de alta salinidade que nos é fornecido pelo INPE (Tabela 1a, página 4)

2. Definação quais ferramentas serão utilizadas para multicoring - o ato de amostrar a interface sedimento-água (Tabela 1a, página 4)

3. Lista atualizada da equipe científica (página 2, página 24)

5 June 2023

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United States

Solving the Mystery Behind an Iconic Question

What Happens to Terrestrial Organic Matter in the Ocean?

Expedition Plan

v.8

June 4, 2023

Solving the Mystery Behind an Iconic Question – What Happens to Terrestrial Organic Matter in the Ocean?

June 2023

1. Vessel: R/V F.G. Walton Smith

Port of embarkation: Miami, Florida (ship's crew)

Port of embarkation: Cayenne, France (scientific crew and Brazilian Navy Observing Officer)

Maximum capacity: 12 scientists

2. Dates: June 3-20, 2023

3. **Project Title**: NSF: Collaborative Research: What Happens to Terrestrial Organic Matter in the Ocean? Solving the Mystery behind an Iconic Question

4. Project Groups and Participants:

a. Rosenheim group, University of South Florida

i. Brad Rosenheim, USF

ii. Calyn Crawford, USF

b. Galy group, Woods Hole Oceanographic Institution

i. Brenna Boehman, WHOI

ii. Manuel Colombo, WHOI

c. Hein group, Virginia Institute of Marine Science

i. Christopher Hein, VIMS

ii. Evan Flynn, VIMS

d. West group, University of Southern California

i. Ronmel Rugama-Montenegro

e. University of Western Pará (UFOPA) group

i. José Mauro Sousa Moura - Universidade Federal do Oeste do Pará

ii. Raphael Muniz - Universidade Federal do Oeste do Pará

f. Brazil National Institute for Space Research (INPE) group

i. Andrea de Lima Oliveira - Instituto Nacional de Pesquisas Espaciais – São José dos Campos

g. ETH Zurich (Switzerland)

i. Davide Calvarese - ETH

g. Alternates

i. Milton Kampel, laboratory leader, INPE

ii. Vitor Galazzo de Paiva - Brazil National Institute for Space Research (INPE)

iii. Felipe Stanchak, University of South Florida

5. Project Locations and Itinerary:

a. General Itinerary Outline (*Transit itinerary is included COVID contingency planning in case the scientific party needs to embark and disembark in St. Thomas, USVI, instead of Cayenne, F.G.)*

May 4, 2023: Mobilization – Saint Petersburg, FL. Load equipment onto ship, secure items, set up laboratory work spaces

Transit – Miami, FL to Cayenne, F.G.

Resupply ports:

St. Thomas

Trinidad

Science party boards vessel in Cayenne, F.G.

June 4, 2023: Health and Safety Checks - see section 12.

June 5 - 12, 2023: Science operations (Leg 1) and return to Cayenne, F.G.

June 12-14, 2023: Refuel in Cayenne, personnel exchange (disembark Brazilian Naval Officer)

June 14-20, 2023: Science operations (Leg 2) and return to Cayenne, F.G.

June 20, 2023: **Science party disembarks vessel in Cayenne, F.G.**

July 5, 2023: Likely arrival date and demobilization of ship in Saint Petersburg, FL

*Contingency plan itineraries are described in the appendices.

b. Sampling Locations and Shipboard Tasks

Table 1a. Station locations and tasks outside of 12NM from Brazil

Section	**Station**	**Latitude**	**Longitude**	**Shipboard Tasks**
Marine Endmember	Marine Endmemb er	3.07842	47.47328	CTD/Rosette x2 (240 L), Incubation set up (**INPE Staff will determine a nearby location in real time with satellite imagery**)
Amazon	Outer	1°10'48.67"N	49° 6'37.60"W	Multicore*, Gravity core, CTD/Rosette
Amazon	Mid	1° 1'55.54"N	49°25'1.79"W	Multicore, Gravity core, CTD/Rosette x2
Araguari	Outer	1°42'51.05"N	49°22'43.37"W	Multicore, Gravity core, CTD/Rosette
Araguari	Mid	1°36'20.53"N	49°35'52.98"W	Multicore, Gravity core, CTD/Rosette x2
Guaymas	Outer	3°13'14.51"N	50°27'4.19"W	Multicore, Gravity core, CTD/Rosette
Guaymas	Mid	3° 5'40.57"N	50°40'42.02"W	Multicore, Gravity core, CTD/Rosette x2
Oiapoque	Estuary	4°15'2.04"N	51°37'43.49"W	Bottom Sediment Grab, CTD/Rosette, Bucket Surface Sample
Oiapoque	Inner	4°39'20.11"N	51°46'42.60"W	Multicore, Gravity core, CTD/Rosette
Oiapoque	Mid	4°49'36.72"N	51°43'12.84"W	Multicore, Gravity core, CTD/Rosette x2
Oiapoque	Outer	4°57'32.69"N	51°40'28.53"W	Multicore, Gravity core, CTD/Rosette
Cayenne	**Degrad de Cannes**			**End of Leg 1, disembark the naval officer, embark additional scientist, refuel**
Iracoubo	Outer	5°51'46.38"N	53°17'10.91"W	Multicore, Gravity core, CTD/Rosette
Iracoubo	Mid	5°44'45.48"N	53°18'57.74"W	Multicore, Gravity core, CTD/Rosette x2 Type text here
Iracoubo	Inner	5°36'24.01"N	53°20'33.39"W	Multicore, Gravity core, CTD/Rosette
Iracoubo	Estuary			Bottom Sediment Grab, CTD/Rosette, Bucket Surface Sample
Maroni	Outer	6° 7'2.62"N	54° 4'48.18"W	Multicore, Gravity core, CTD/Rosette
Maroni	Mid	5°59'44.85"N	54° 5'57.96"W	Multicore, Gravity core, CTD/Rosette x2
Maroni	Inner	5°52'54.88"N	54° 6'55.44"W	Multicore, Gravity core, CTD/Rosette
Maroni	Estuary	5°38'36.13"N	54° 1'1.38"W	Bottom Sediment Grab, CTD/Rosette, Bucket Surface Sample

***For this table, we use the term "Multicore" to mean recovery of the sediment-water interface. See section 8.1.a for the sequence of coring techniques that we will use to recover the sediment-water interface.**

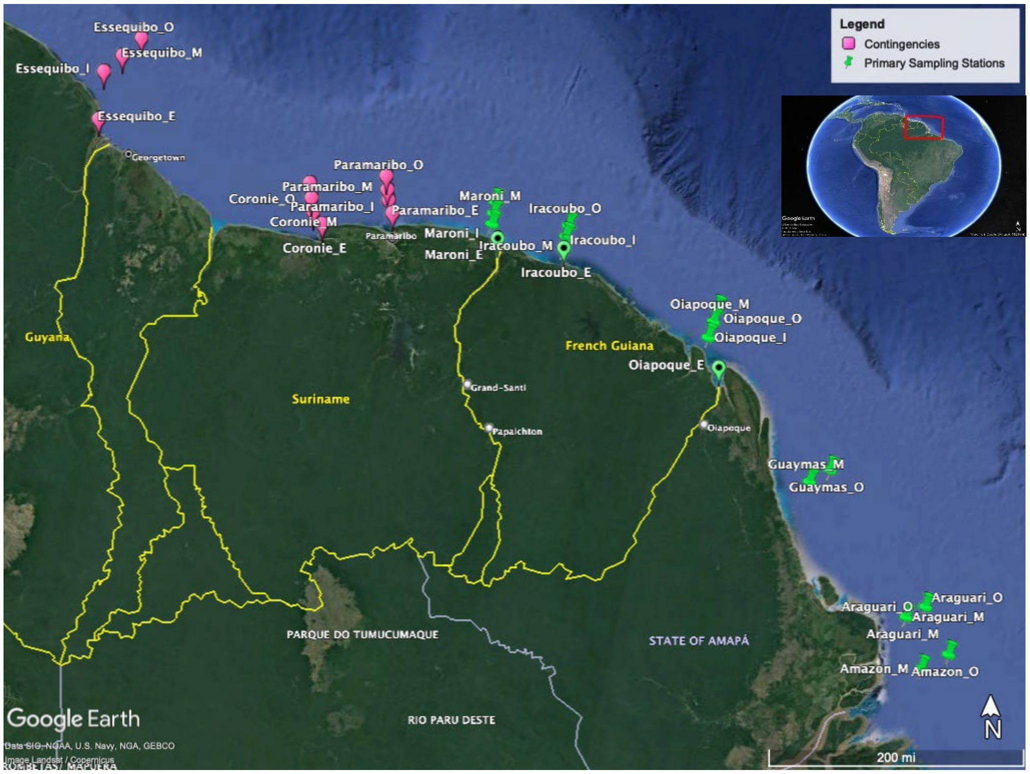

Figure 1: *Our preliminary ocean-going expedition sampling plan includes 6 cross-shelf transects of the Guianas coast drainage system. Each transect consists of 3 stations (yellow pins). The Maroni, Iracoubo, Oipaoque, Araquari, and Amazon transects will have an estuary site (green bubbles) for water sampling and bottom surface grab sampling. The Guaymas transect is not adjacent to a drainage system.*

c. Station Time Calculations

For coastal sampling stations where we will be sampling with the CTD/Rosette, multicore, and gravity core, time on station is approximately 6 hours. For the Amazon River/Estuary sites where we are taking bottom grabs and surface water samples in addition to rosette sampling with CTD, time on station is max. 8 hours. Some estuary sites we will deploy the 16' RHIB for sampling, which could add an hour for launch and retrieval. At least one site in the Amazon main stem, we will deploy the isokinetic depth sampler for 1 hour (depending on observations of how much sediments are sampled with each deployment). For sites with water depth < 50m, we expect high suspended sediment concentration near the bottom, and may need to deploy the isokinetic sampler to collect a near-bottom 20L sample, to ensure particle composition is unbiased.

d.Science Operations Schedule

Leg 1:
Expedition Depart (Cayenne, F.G.): June 5, 2023, 15:00 h
Expedition Arrive (Cayenne, F.G.): June 12, 2023, 18:45 h

Leg 2:
Expedition Depart (Cayenne, F.G.): June 14, 2023, 08:00 h
Expedition Arrive (Cayenne, F.G.): June 20, 2023, 06:30 h

Mobilization will occur in St. Petersburg, Florida prior to the ship's departure. Departure date will be May 14 and the ship will reprovision in transit in St. Thomas and Trinidad. The scientific party will embark the ship prior to 3 June 2023 (see section 12 for health and safety considerations due to COVID-19) and the ship will leave port on 3 June 2023. We will transit to our furthest site (Amazon Mid, Table 1a) prior to commencing work. We will go out to open ocean, outside of the Economic Exclusion Zone, to sample endmember seawater at full strength salinity for incubations. Stations will then be sampled on our way back to Cayenne.

Table 2. Arrival and departure time **estimates** for sampling stations. We assume straight-line distance between stations calculated on Google Earth and a forward speed of 8 knots for transit times. Station times are based on estimates derived from the planned work (below). *These times can, and will likely, change due to weather and/or equipment malfunctions – this table is for estimation purposes only and has not been updated with delays due to Trinidad processing and French Guiana refueling.*

Station/Port	**Arrive**	**Start Sampling**	**Depart**
Degrad des Cannes (Cayenne, F.G.)			June 3 15:00
Marine Endmember	June 5 04:30	June 5 04:30	June 5 10:30
Amazon Outer	June 6 05:15	June 6 05:15	June 6 13:15
Amazon Mid	June 6 16:00	June 6 16:00	June 7 00:00
Araguari Outer	June 7 04:30	June 7 06:00	June 7 14:00
Araguari Mid	June 7 16:15	June 7 16:15	June 8 00:15
Guaymas Outer	June 8 15:15	June 8 15:15	June 8 23:15
Guaymas Mid	June 9 01:07	June 9 08:00	June 9 16:00
Oiapoque TSS and Bottom Grab	June 10 10:00	June 10 10:00	June 10 19:00
Oiapoque Inner	June 10 22:45	June 11 06:00	June 11 14:00
Oiapoque Mid	June 11 15:45	June 11 15:45	June 11 23:45
Oiapoque Outer	June 12 00:52	June 12 06:00	June 12 14:00
End of Leg 1 - Steam to Cayenne for Refuel	June 12 18:45	N/A	June 14 07:00
Disembark:	Lt. Andre Paiva	**Embark:**	Felipe Stanchak
Leg 2:			
Iracoubo Outer	June 14 18:43	June 14 18:43	June 15 02:43

Iracoubo Mid	June 14 03:58	June 14 06:00	June 14 14:00
Iracoubo Inner	June 14 14:52	June 14 14:52	June 15 22:52
Iracoubo TSS and Bottom Grab	June 15 23:00	June 16 06:00	June 16 15:00
Maroni Outer	June 16 22:07	June 16 22:07	June 17 06:07
Maroni Mid	June 17 7:07	June 17 07:07	June 17 15:07
Maroni Inner	June 17 16:00	June 18 06:00	June 18 14:00
Maroni TSS and Bottom Grab	June 19 06:00	June 19 06:00	June 19 14:00
Degrad des Cannes (Cayenne, F.G.)	**June 20 06:30**		

6. Scientific Scope and Objectives:

This cruise will be initiated through funding from the National Science Foundation's Chemical Oceanography program. The main objective of this cruise is to dedicate a sampling campaign to the Guianas Mudbanks in order to test hypotheses set forth in our proposal that are partially based on piecemeal datasets generated by other expeditions to this region but not dedicated to (and usually not able to sample) the Guianas Mudbanks. The hypotheses of this work are:

H_1: *Terrestrial OC from the Amazon River is characterized by a wide continuum of reactivity, such that its most refractory component gets selectively preserved in the coastal ocean while its labile component gets rapidly and efficiently oxidized, ultimately replaced by marine OC.*

H_2: *The refractory component of terrestrial OC that is selectively preserved in the ocean is compositionally distinct from bulk terrestrial OC delivered by the Amazon River such that previous estimates of terrestrial OC burial efficiency based on bulk composition are biased. Specifically, we posit that the overall terrestrial OC burial efficiency offshore the Amazon River mouth has been underestimated by up to 100% (i.e. it should be doubled).*

The tasks of this ocean-going expedition as they relate to the proposal are:

a. Sample a large volume of shelf water to generate marine primary productivity endmember.
b. Sample sediments at the sediment-water interface at the shelf stations of each section by multicoring.
c. Take water samples from CTD casts to measure Total Suspended Solids and water mass properties at all shelf stations of each section.
d. Sample water from CTD casts at all estuary stations of each section.
e. Sample sediment-water interface at all estuary stations of each section using a variety of surface grab technologies.

7. Water Sampling and Budget:

1) Marine endmember (1x 240 L sample at DCM
    a. CTD/Rosette cast x2 (collect at DCM - determined by chl a)

    b. 140 L for incubation, 20 L of that filtered, remaining 100 L to filter to save filter for marine endmember characterization. Pass 50 L onto 5 g PPL.
    c. Expect 600-1000m water depth
2) Shelf/Coastal – (15 sites)
    a. 5 depths with 2x 10 L/depth + 2x10 L collected at surface/2m
    b. one 10 L for
    c. 120 L 50% depth sample at each Mid site e.g: Amazon_M, Araguari_M (6 sites)
3) Estuary – (3 sites)
    a. 3 depths with 4x 10 L/depth

The CTD/Rosette available on the F.G. Walton Smith is a 12-bottle system with 10L Niskin bottles.

Sampled water will be filtered (push with bike pump or N2) with 0.22µ porosity (142mm). Filtration will begin with 10 L, and follow with an additional 10 L collected at the same depth if additional particle is needed (evaluate as filtration is proceeding), and up to an additional 10 L if water is available and deemed necessary (30 L max). Large samples (120 L) will be filtered in their entirety and water will be stored in wine bags as interim storage & refrigerated.

Suspended sediment on filters will be immediately placed in labeled whirlpaks frozen for later analysis, described below. Flow through will be saved and immediately processed. 125 mL of filtered water will be poisoned with mercuric chloride (4 uL saturated $HgCl_2$) for DIC analysis (Subhas Lab, WHOI). 10 mL flowthrough will be required to inject sample into 12 mL exetainers for D14C (carbonate system, MICADAS, NOSAMS). 500 mL flowthrough will be stored for [DOC] analysis (Pohlman/Sylva, WHOI). Remaining flowthrough (20 L) will be passed through PPL SPE columns for DBC analysis. Marine salinity DBC sites Poisoned bottles do not need to be frozen. SPE columns will be placed in labeled whirlpaks and immediately frozen for later analysis.

Marine endmember collected at DCM will be used for incubations, some filtered and some unfiltered

Estimates for water budget based on 15-100 mg/L TSS and 0.5% OC (Nittrouer, 1986; Kineke, 1991; Kineke & Sternberg, 1995). Suspended sediment will be high and variable with depth on the Northern coast of Brazil, and TSS will decrease French Guiana towards Suriname, Guyana.

Table 3. Water budget for each sampling location

Particulate Analysis	Volume (L)	Notes	Storage requirement
Grain size	0.5-1.5		
Inorganic elemental analysis	1-3	at CNRS	
%OC, %ON, $\delta^{13}C$, $\delta^{15}N$	2-6		
Bulk ^{14}C	0.2-0.6	at NOSAMS, WHOI	
Bulk RPO	1-3	Rosenheim Lab, USF	

Compound specific ^{13}C	2-6	Galy Lab, WHOI	
Compound specific ^{14}C	120	*Shelf, Mid 2m cast only*	refrigerator for water storage during processing
TOTAL	**20 L/depth**		Filters in whirlpak/site, freezer

Unfiltered water	**Volume (L)**	**Notes**	**Storage requirement**
CDOM	0.5	keep 100 mL of water	4 C
MP	1-2	keep only the filter	-80 C
Chla/HPLC?	1-2	keep only the filter	-80 C
Vitamins	8	filtering through 3 filters	frozen filtrate in 1 L Nalgene, freeze 3µm/ 0.2 µm filters
Mya Breitbart	1-2 L	keep filter	
0.2um Flowthrough Analysis	**Volume (L)**	**Notes**	**Storage requirement**
[DIC] & 13C	0.125	poisoned with 4 uL saturated HgCl2	room temp
DIC ^{14}C	0.01	injected into pre-acidified exetainer, 6 mL or less	room temp
[DOC], 13C, 14C	1		frozen
SPE column	20	8 sites, Amazon_M/O (2x5g for each), Araguari_M/O (2x5g for each), other Mid sites (2x1g)	SPE columns in whirlpaks, frozen
Levoglucosan?	1		refrigerated

DBC concentration and 14C DBC	1-2L	acidified	would be ideal to be refrigerated, or freeze packs can be rotated out (filtering DOC, no SPE columns)
TOTAL	**19.47/depth**	SPE step last to add all remaining flowthrough	

7h. Nomenclature of water samples: For water samples, it will be important to index samples with time, date, cast number, equipment, platform, L filtered.

7i. Materials

- 182 0.2um 142mm filters (91 samples, assume 3 filters for 3 surface, 3 filters for 9 estuary, 2 filters for 75 shelf, +3 marine endmember)
- 93 12 mL exetainers for DIC D14C (from NOSAMS, WHOI)
- 72 125 mL airtight bottles for [DIC] 13C (from Adam Subhas, WHOI) (4 depths only)
- 4x 5g, 12 1 g PPL SPE cartridges for DOC
- 72-93 1-2 L Nalgenes for DBC
- 91 1 L Nalgenes for vitamins
- peristaltic pump (for loading filtered sample onto SPE, from WHOI)
- 93 500 mL bottles for [DOC]
- 3 mL saturated HgCl2 solution
- 25 mL conc. HCl
- bike pump or compressed N2 for filtration
- 20? 10-20 gallon, translucent & opaque, 'wine bags' or collapsable carboys for interim storage of water (surface samples for filtering; filtered water for loading onto SPE columns)

7j. Vitamin & Retinal water sampling

2 L of sampled water will be filtered (peristaltic pump) through a 3µm and 0.22 µm filter. 1 L of filtered water will be collected in a 1 L Nalgene bottle and frozen. Filters will be rinsed with DI and frozen. All samples will be processed and analyzed in the Sañudo-Wilhelmy lab (USC).

8. Sediment Sampling:

The sediment for each station is shown in Table 4 in grams of dry mud, with the exception of porewaters.

Table 4: Dry sediment mass for sediment core analyses

	Radionuclides	RPO	Fe-OC	General	Porewaters	Grain size	Bulk minera	lipids/ CSIA

				Geoch em			logy	
Shelf GC	15-20	2	3	2		1	5	5, 10+
Shelf MC	15-20	2	3	2		1	5	5, 10+
Estuary grab	15-20	2	3	2		1	5	5, 10+

1) Shelf/Coastal –
    a. Sediment-water interface
       We will deploy either the Ocean Instruments Multicore (MC400, option A), or the box core (option B) to sample the mud-water interface.
    b. Deep stable sediment
       We will use the Gravity Core (20') to sample the underlying "stable" sediment on which the mudbanks are transported.
2) Estuary –
    a. Estuary samples will focus on surface sediment due to currents and shallower water (sampling often from RHIB).
       Sediment Grab (Option A)
       Box Core (Option B)

8.1.a Sediment-Water Interface Recovery, Nomenclature, Partitioning
8.1.a.1 Multicorer

Objective: To obtain sediment from the sediment-water interface with minimal disturbance and to systematically partition samples between PIs and research groups.

Materials:
- Core tube rack
- Collection tray (if material is oozing from cores)
- Core caps
- Black Tape
- Labeling markers
- Measuring tape
- Munsell Color cards for description
- Rags

Protocol

Pre-deployment
1. Review the deployment and retrieval protocol with the winch operator and the A-frame operator who will assist in coring with the MC400.

Deployment

1. 4 normal core tubes, 1 pre-drilled core tube for porewater Rhizon sampling (see 8.3).
    a. See details of porewater analyses in section (8.3)
2. Depending on sea state, assign gaffers and ropers to stabilize the corer through the A-frame (deck operations will be led by marine technician)

Retrieval

1. Once corer is back on deck, lead science team shift member will decide to keep the cores, redeploy, or go to box corer.
    a. Redeployment should be ordered if:
        i. No penetration, or malfunction: No mud in tubes
            1. Determine if the substrate was too sandy or if the equipment malfunctioned
                a. Substrate too sandy: redeploy with only 2 tubes or increased weight (or both)
                b. Equipment malfunction – redeploy if the problem can be solved
        ii. Over penetration: Mud at extreme top of core tubes, mud streaks on outside of the core tubes that are at or near the tops of the tubes.
            1. Reduce weight and redeploy
            2. If reduced weight and still over-penetrating, go to box core
    b. If coring is deemed successful:
        i. Measure the length of sediment in each core tube
        ii. Label from longest core to shortest core A-D
        iii. For cores that will be analyzed and stored at a core repository, core nomenclature would be as follows:

FWS23XX-NNMC-CT-D

...where XX is the cruise number, NN is the sequential number of the multicore (01, 02, …, NN), MC denotes multicore, CT is the label (A-D) of the tubes, and D is the depth in cm (for extruded cores). For mud that is scraped from the sampling tools and saved, the final suffix would be "S" as depth is not determined. Depth would be added to any subsamples, such as porewaters, but not immediately applicable to cores that will be sent to archival. Porewater samples will be labeled as above, but with the suffix "PW" after core depth (D) in cm.

        iv. Core partitioning for subsampling and archival is as follows:
                a. Gamma Geochronology (^{210}Pb, Cs), grain size, bulk organic geochemistry (%TOC, %N, $\delta^{13}C$, $\delta^{15}N$, C:N), geochemistry and bulk mineralogy, bulk radiocarbon, ^{14}C (CSRA, RPO) - with focus on ^{14}C, we will subsample with stainless steel tools into pre-combusted glass vials. **2.5 cm resolution of extrusion, See**
                b. Porewater (Rhizons) - **2.5 cm resolution**
                    i. Core tubes will be pre-drilled. Because it will not be known where the top of the sediment column will be in the tube, each tube may have different intervals sampled.
                    ii. Will fabricate a rack for core tubes while being sampled for porewaters.

c. X-ray and archive
d. Full archive – no X-ray

v. Siphon water and freeze as a sample (FWS20XX-NNMC-SW).

8.1.a.2. Box Core Sediment samples
Objective: To obtain sediment-water interface samples when multicoring is not possible, and to distribute samples to PI's and research groups.

Materials:
- Multi core tubes
- Collection tray (if material is oozing from cores)
- Core caps
- Black Tape
- Labeling markers
- Measuring tape
- Color cards for description
- Trowels and scrapers to free cores from box
- Spades to free cores from box
- Rags and paper towels to clean outsides of the core tubes
- Hose clamps (If needed)
- Plywood (if needed)

Pre-deployment

2. Review the deployment and retrieval protocol with the winch operator and the crane operator who will assist in coring with the Box Core.

Deployment

3. Depending on sea state, assign gaffers and ropers to stabilize the corer through the A-frame (deck operations will be led by marine technician)

Retrieval

2. Once box corer is back on deck, lead science team shift member will decide whether or not redeployment is necessary.
    a. Redeployment should be ordered if:
        i. No penetration, or malfunction: No mud in box
            1. Determine if the substrate was too sandy or if the equipment malfunctioned
                a. Substrate too sandy: redeploy with more weight if possible
                b. Equipment malfunction – Generally this will be a failure to "fire" or close. Was the box core obstructed, or was there an internal equipment malfunction that is evident?
        ii. Over penetration: Mud at extreme top of box, mud streaks or mud above the upper surface of the box or on rope/cable of deployment.
            1. Reduce weight and redeploy
            2. Fit with Aller-type plywood "snowshoes" to increase the surface area. Attach with hose clamps

3. If still over-penetrating with less weight and/or snowshoes, sample nepheloid layer with the hydrostatic water sampler

b. If coring is deemed successful:
    i. Emplace multicore tubes into the box corer at 4 locations
    ii. Push gently so as to not disturb the sediment surface, and use constant force all the way down rather than hammering or similar pulsed forces.
    iii. Cap and tape the tops of the tubes for suction.
    iv. Dig out the areas around the cores with the trowels and/or spades. Measure the length of sediment in each core tube
    v. Cap the bottom of the tubes
    vi. Label from longest core to shortest core A-D (arbitrary if the same length)
    vii. For cores that will be analyzed and stored at a core repository, core nomenclature would be as follows:

FWS23XX-NNBC-CT-D

...where XX is the cruise number, NN is the sequential number of the multicore (01, 02, …, NN), BC denotes box core, CT is the label (A-D) of the tubes, and D is the depth in cm (for extruded cores). For mud that is scraped from the sampling tools and saved, the final suffix would be S as depth is not determined. Depth would be added to any subsamples, such as porewaters, but not immediately applicable to cores that will be sent to archival. Porewater samples would receive a "PW" suffix after the core depth (cm).

    viii. Core partitioning for subsampling and archival is as follows (same as multi-core):
        a. Gamma Geochronology (^{210}Pb, Cs), grain size, bulk organic geochemistry (%TOC, %N, δ^{13}C, δ^{15}N, C:N), geochemistry and bulk mineralogy, bulk radiocarbon and potential RPO/RPO ^{14}C
        b. ^{14}C (CSRA, RPO) - with focus on ^{14}C, we would likely be sampling with stainless steel tools into pre-combusted glass vials.
        c. X-ray and archive
        d. Full archive – no X-ray
    ix. Siphon water and freeze as a sample (FWS20XX-NNBC-SW).

8.1.a.3. Hydrostatic Water Sampler (Nepheloid Layer Samples)

Objective: To obtain sediment-water interface samples when the sediment water interface is deemed too thick (>50 cm) by CTD measurements of transmissivity, fluorescence, and/or salinity. Bob Aller has warned us that a salinity reading of 0 near the sediment-water interface is usually a telltale sign that the "water" has such a high sediment content that conductivity is not well-measured (i.e. sediment causes too much resistivity).

Materials:
- Buckets
- Filters
- Labeling markers
- Color cards for description

- Ziplock or Whirlpack bags for storage of filters

Pre-deployment

3. Review the deployment and retrieval protocol with the winch operator and the crane operator who will assist in sampling with the water sampler.

Deployment

4. This may be deployed off the side of the ship.

Retrieval

5. Water sampler should be full with 10L of muddy water. If not full, investigate for firing malfunction and redeploy..
    a. If sampling is deemed successful:
        i. Empty water into cleaned buckets. It is fine to clean the buckets with surface water.
        ii. Draw the water into filtration units in volumes that will result in a maximum of sediment on the filter but a minimum of time of filtering.
        iii. Change filters when necessary as the flow through the membrane decreases. Remember that the head pressure shouldn't be set higher than a few bars (otherwise the membrane can tear).
        iv. Filters will be numbered as follows:

FWS23XX-NNHS-F

...where XX is the cruise number, NN is the sequential number of the hydrostatic sampler's deployment (01, 02, …, NN), HS denotes hydrostatic sampler, and F is the sequential filter number for that sample. For mud that is scraped from the sampling tools and saved, the final suffix would be S as depth is not determined. No porewater samples would be saved from this sort of sampling, however we could save the filtered water if thought valuable.

        v. Filter partitioning for subsampling and archival is as follows:
            a. Filter 1: Gamma Geochronology (^{210}Pb, Cs),
            b. Filter 2: grain size, bulk organic geochemistry (%TOC, %N, δ^{13}C, δ^{15}N, C:N), bulk radiocarbon and potential RPO/RPO ^{14}C
            c. Filter 3: ^{14}C (CSRA, RPO)
            d. Filter 4: Full archive

8.1.b. Benthos Gravity Core Cutting and Capping Protocol

<u>Objective</u>: To obtain deeper "stable" Holocene mud over which the mobile mud belts are transported.

<u>Materials</u>:

- Core tubes (PVC Schedule 40)
- Core caps (approximately 4 caps per apparent meter of core)
- PVC cutting wheel
- Black Tape

- Duct Tape
- Towels/Rags
- Labeling markers
- Measuring tape
- Sampling tray
- Siphon tubing

Protocol
The operations of core retrieval will be guided by the crane operators and the winch operators. It is imperative to get the corer into a safe position prior to science team access to the core catcher and core liner due to the weight of the corer and the possibility of injury.

1. When the winch operator gives the all clear signal, the science team can commence working on the core and core liner.
2. Upon retrieval, photograph core – make sure to document mudline in notes!
3. Extraction of the core catcher.
    a. Core catcher can be separated by gentle prying. Place core catcher into a labeled Whirlpak bag.
    b. Cap bottom of core with a cap labeled "Bottom."
4. Once the core catcher is extracted, the core liner can be slid out of the core barrel.
5. Once core liner is removed, siphon off top water using a hand pump and Tygon tubing. Siphon into pre-labeled bottles.
6. Cap top with cap labeled "Top." Cut into 5 foot sections; cap and label appropriately. Core sections will be labeled from the bottom up, 1, 2, 3, 4, etc. as they come out of the core barrel:

    FWS23XX-NNGC-S

    …where XX is the cruise number, NN is the sequential core number indexed with site location (name and coordinates) and S is the section of the core.

    All of the gravity core sections will be capped, labeled, stored in cold storage (4 degrees Celsius), and ultimately shipped to the core repository in Corvallis, OR.

8.2.a. Bottom Sediment Grabs

Bottom sediment grabs will be taken in estuary sites, especially when the RHIB is necessary for exploration and sampling. These samples will likely be sandier than offshore samples. Samples will be stored in labeled whirlpaks and frozen once on board the Walton Smith. Nomenclature will be as follows:

FWS23XX-NNBG

…where XX is the cruise number, NN is the sequential number of the bottom grab, BG stands for Bottom Grab. Samples can be split according to scientific needs as follows

a. Gamma Geochronology (^{210}Pb, Cs), grain size, bulk organic geochemistry (%TOC, %N, $\delta^{13}C$, $\delta^{15}N$, C:N), bulk radiocarbon and potential RPO/RPO ^{14}C
b. ^{14}C (CSRA, RPO)
c. Full archive

All samples should be frozen aboard the Walton Smith.

8.3 Porewater Collection & Analysis:

Objectives:

1) Obtain representative surface porewater sample (0-30cm) for assessment of carbon content, source and degree of remineralization (Multicore).
2) Obtain ~30 cm downcore porewater data to examine the dominant biogeochemical processes occuring in the mobile suboxic zone (Multicore).
3) At each Mid site, obtain porewater samples to characterize the biogeochemistry over the transition from mobile suboxic muds to stable mud deposits (Gravity core)

Materials:

Multicore sampling:

- Rhizosphere Macro Rhizons (19.21.35) (1/depth/core; 9 max per core, 18 cores)
- 20x 100 mL syringes (maybe smaller?)
- 18 12 mL exetainer for DIC D14C (from NOSAMS, WHOI)
- 36 4 mL airtight bottles for [DIC] (from Adam Subhas, WHOI)
- 18 SPE cartridges for DOC
- 18 40 mL bottles for [DOC] (VOA glass vials with Teflon lined septa. Bake the vials at 450 for 4 hrs and acid wash (10% HCl v/v) the septa)
- <1 mL HgCl2

Gravity core sampling (only at Mid sites):

- Rhizosphere Macro Rhizons (19.21.35) (1/depth/core; 4 per core, 6 cores)
- 50x 100 mL syringes (maybe smaller?)
- 24 12 mL exetainer for DIC D14C (from NOSAMS, WHOI)
- 24 20 mL airtight bottles for [DIC] (from Adam Subhas, WHOI)
- 24 SPE cartridges for DOC
- 24 40 mL bottles for [DOC] (VOA glass vials with Teflon lined septa. Bake the vials at 450 for 4 hrs and acid wash (10% HCl v/v) the septa)

Protocol:

Multicores will be collected as described in Section 8.1.a.1. One core deployed will be pre-drilled for Rhizon porewater sampling at 3-5 cm intervals.

Gravity cores will be collected as described in Section 8.1.b. One core deployed will be pre-drilled for Rhizon porewater sampling at 25 cm intervals.

Porewater collection will occur quickly with attention to the impacts of time between collection & subsequent fixing/analyses (ie diffusion, O2 exposure, etc).

2 options for porewater extraction from core with Rhizons. Both require 2 sets of hands.

1. Insert rhizons attached to pre-N2 flushed syringe at all depths. Simultaneously, draw out the syringe and hold with a wooden stick (to hold vacuum), start halfway & then full length. Detach from luer lock and divide immediately for ship board analyses, freezing remaining (*in what? something gas tight)*.

Analyses:

In order of priority

[DIC] & d13C - 5 mL

> Method: Transfer porewater volume into 20mL airtight bottle, poison with HgCl, measure in Adam Subhas lab (WHOI)

D14C DIC - 6 mL (or less)

> Method: Inject (syringe) porewater volume into pre-acidified 12 mL exetainers. Measure at NOSAMS.

Ions (anions: Cl-, NO3-, SO42-) - 4-6 mLs, can dilute (IC; Josh West)

[DOC] & d13C - 30 mL

> Method: Transfer porewater volume into 40 mL VOA glass vials with Teflon lined septa (pre-baked and acid washed) +conc. HCl

9. Incubations

9a. Location

Marine endmember site

9b. Objectives

Incubations will examine the mechanisms of terrestrial OC remineralization in the Guianas mudbanks system (water column, surface muds), define rates of OC degradation, and identify the organisms responsible for terrestrial OC degradation.

POC-focused incubations (water column, surface muds)

H1: Terrestrial OC will be remineralized to consume the younger, more depleted terrestrial component, preserving the older, more enriched terrestrial component.

qSIP incubations (water column, surface muds)

H1: Lignin will be degraded by a subset of the microbial community.

<u>9c. Experimental Design</u>

9.c.1 Preparation of marine endmember (marine)

Collected on May 18 by collecting 142 L of surface water, filtering 21 L for incubations, leaving 121 L unfiltered

9.c.2 Preparation of terrestrial endmember (sed)

Collected May 1-15 aboard the Mirage by collecting bottom grabs of muddy sediment and freezing. Need ~ 1 kg sediment.

9.c.3. Surface water Incubations

9.c.3.a Setup

Will require 120 L of marine unfiltered, 20 L filtered marine filtered. 40 g wet terrestrial sediment (Mirage)

Each incubation will have a light and a dark treatment, and a killed (+ * uL $HgCl_2$) and unkilled treatment (x4). Light treatment will be kept on the deck in a seawater flow through bath and exposed to day/night cycle. Dark treatment will be kept inside in the dark. Incubations will have technical replicates for sampling at each time point (x5). Killed treatments will have $HgCl_2$ added. Last time point will be collected when the ship returns to Miami.

1) Marine unamended (2L marine in 2.5 L)
2) Marine unamended filtered (0.8 L marine filtered in 1 L)
3) Marine + terrestrial (2 L marine + 1 g sed in 2.5 L)
4) Marine + d13C lignin (0.8 L marine + 0.5 g sed + ** 13C lignin in 1 L)
5) Marine + unlabeled lignin (0.8 L marine + 0.5 g sed + ** lignin in 1 L)

9.c.3.b Subsampling

2x a day: Measure O_2 w/ Fibox & get water temp

At T0 (immediately), T1 (2 days), T2 (7 days), T3 (14 days), T4 (~28 days)

1)Filter entire time point (1-3 onto 0.22 um (142mm) Millipore PES membrane filters & freeze in whirlpak for POC analysis; 1,4-5 into 0.22um Millipore filter through peristaltic pump cassette, freeze in 15mL falcon tube).

2) 30 mL flowthrough for [DOC], 40mL flowthrough for [DIC] (T0, T4), remaining flowthrough onto SPE (lignin analysis)

9.c.4 Sediment Incubations

9.c.4.a Setup

Will require total of 680 mL wet sediment (from Mirage), 200 mL marine unfiltered, 50 mL filtered.

All incubations will be in 15 mL falcon tubes, in the dark and have a killed treatment (+ * uL $HgCl_2$) (x2). Dark treatment will be kept inside in the dark. Incubations will have technical replicates for sampling at each time point (x5). Last time point will be collected when the ship returns to Miami/St. Petersburg (wherever we demob).

<u>Incubation setup (per site):</u>

1) Terrestrial (+10 mL sed)
2) Marine filtered + terrestrial (+5 mL marine filt, + 8mL sed)
3) Marine + terrestrial (+5 mL marine, + 8mL sed)
4) Marine + terrestrial + d13C lignin (+5 mL marine, + 8mL sed, + ** 13C lignin)
5) Marine + terrestrial + unlabeled lignin (+5 mL marine, + 8mL sed, + ** lignin)

9.c.4.b Subsampling

2x a day: Measure O_2 w/ Fibox (expect suboxic quickly)

At T0 (immediately), T1 (2 days), T2 (7 days), T3 (14 days), T4 (~28 days)

1. Freeze time point specific vials. Processing at WHOI/LMU Munich.

<u>9d. Materials</u>

- 1 L Nalgene x60 (30 + $HgCl_2$)
- 2.5 L Nalgene x40 (20 + $HgCl_2$)
- Water filtration setup (Galy lab)
  - 0.22 um Milipore PES filters (142mm) x100
  - 100 whirlpaks
- Peristaltic pump + tubing + cassette to hold filters (pump from WHOI)
  - 0.22 um Milipore filters x60 (* mm)
  - 1 mL tubes x 60
- SPE columns x 100
- 40 mLs for DOC + $HgCl_2$ x100
- 125 mLs for DIC + $HgCl_2$ x40
- Fibox & 200 O_2 sensor spots
  - Thermometer to measure water temp

- 15 mL falcon tubes x 50 (25+ $HgCl_2$)
- Terrestrial mud from Mirage May 2023 (40 g + 680 mL = ~1 kg max)
- d13C labelled lignin monomers (stock)
- unlabelled lignin monomers (stock)
- bath for continuous flow through sw

10. Equipment and Storage:

10a. R/V F.G. Walton Smith Shipboard Equipment

CTD Systems/Water Sampler

- 12ea. 10L Niskin bottles
- Dissolved Oxygen Sensors
- Fluorometer
- Underwater PAR (2,000 m depth)
- Altimeter
- Transmissometer: 25cm; 660nm
- Conductivity Sensors
- Temperature Sensors

Virtual Integrated Data System (VIDS) – Data Acquisition System

- GPS Position
- Ship Speed
- Water depth
- POSMV320 Inertial Nav
- Sea Surface Temperature
- Sea Surface Salinity
- Fluorometer, Chlorophyll
- Fluorometer, C-DOM

Other Systems/Equipment

- 3.5 kHz CHIRP Depth Sounder
- 600 kHz Broadband Acoustic Doppler Current Profiler
- 16’ RHIB

10b. R/V F.G. Walton Smith Shipboard storage

- Refrigerator for water sample storage: standup fridge/freezer combo with the following dimensions:
  Height: 40 inches   Width: 24 inches   Depth: 21 inches
  The refrigerator has a freezer on top of it that is 15 inches high and has the same width and depth as the fridge.

- 2 chest freezers, one normally stays on the boat and the other can be brought on board as requested. They both have a ledge at the bottom that's 8 X 16.5 inches and only 15 inches deep. The deepest part of the freezers is 22 X 16.5 inches and 25 inches deep.
- Oregon State University MARSSAM Group is supplying a 7'x7' reefer container with shelves for upright multicore storage. This unit will bring cores that are to be analyzed and stored at the Oregon State University Marine Geology Core Repository all the way back to Corvallis, OR, cold and we will have a sampling party post expedition.

10c. Storage Space allocation

10.c.1. Ship storage (room temp)

- Water sampling
  - 93 12 mL bottle for DIC D14C + HgCl2
  - 93 125 mL airtight bottles for [DIC], 13C + HgCl2
- Trace metals sampling
  - 18 surface samples in ** bottles
  - ** porewaters in ** bottles
- Sediment sampling
  - 18 12 mL bottles for DIC D14C + HgCl2 - multicore
  - 18 20 mL airtight bottles for [DIC], 13C + HgCl2 - multicore
  - 20 12 mL bottles for DIC D14C + HgCl2 - gravity core
  - 20 20 mL airtight bottles for [DIC], 13C + HgCl2 - gravity core
- Incubations
  - 100x 40 mLs for DIC + HgCl2

10c.1. Freezer storage

- Water sampling
  - 93 whirlpaks with filters (15x5 shelf +6 Mid surface, 3x3 estuary, 1x3 marine)
  - 93 SPE cartridges in whirlpaks (from each site/depth)
  - 60 1 L Nalgenes (Vitamins)
- Sediment sampling
  - 18 large Whirlpaks with bottom grabs
  - ** Porewater samples (multicores, gravity cores)
- Incubations
  - 1 kg Amazon sediment (from Mirage) from Cayenne-Marine endmember only
  - 100 whirlpaks with 0.22 micron 142 mm filters
  - 60 1 mL tubes with 0.22 micron 45mm filters
  - 50 15 mL falcon tubes (sediment incubations)
  - 100 SPE cartridges (water incubation DOC)

10c.1. Refrigerator storage

- Water sampling
  - 93 250 mL vials (for [DOC], 13C DOC)
- Sediment sampling

- 18 40 mL vials (for porewater [DOC], 13C DOC) - multicores
- 20 40 mL vials (for porewater [DOC], 13C DOC) - gravity cores

- Incubations
    - 100 250 mL vials (for [DOC], 13C DOC)

10d. Scientific equipment for laboratories and deck

a. Rosenheim group:
    i. Calibrated core extruders (2) - Deck
    ii. Deck-mounted multicore tube racks (2) - Deck
    iii. Gravity core tube liners (20 by 20') – Deck/Cargo Hold
b. Galy group:
    i. Filtration unit (Deck/Lab)
    ii. Compressed air bottles or N_2 bottles
    iii. Hydrostatic samplers (2) – Deck
    iv. Field equipment from upstream campaign – Cargo Hold
    v. Fibox (from Bill Orsi) & O2 sensor spots - lab
    vi. Peristaltic pump (from Bill Orsi) - lab
    vii. licor IRGA connected to a headspace equilibration chamber (from Nick Ward) *needs running water/ hook up *
c. Hein group:
    i. Portable X-ray unit – dry lab
    ii. Gamma Prep: petri dishes, balance (?), mortar/pestle, wax, drying oven
    iii. Alpha prep (?)
d. OSU MARSSAM UNOLS equipment pool
    i. MC-400 Multicorer – Deck
    ii. Box core – Deck (6' x 6' x 6' approximate)
    iii. Mutlicore tubes (80) – Deck/Cargo hold
    iv. Extruder (1) - Deck
    v. Core rack (1) - Deck

    vi. 7' x 7' reefer unit for upright multicore storage

e. West

    i. Ronmel Vitamin SPE rig

11. Participants

The following participants will be aboard the R/V F.G. Walton Smith. Emergency contact information for each participant is attached in a spreadsheet.

Current Participants

1. Brad E. Rosenheim, Chief Scientist, University of South Florida

2. Christopher Hein, principal investigator, Virginia Institute of Marine Science
3. Ronmel Rugama-Montenegro, University of Southern California(West group)
4. Brenna Boehman, Ph.D. student, Woods Hole Oceanographic Institute (Galy group)
5. Manual Colombo, postdoctoral researcher, Woods Hole Oceanographic Institution
6. Evan Flynn, graduate student, Virginia Institute of Marine Science (Hein group)
7. Calyn Crawford, M.S. student, University of South Florida (Buck group)
8. Andrea de Lima Oliviera, postdoctoral researcher, Instituto Nacional de Pesquisas Espaciais – São José dos Campos
9. José Mauro Sousa Moura, principal investigator, Universidade Federal do Oeste do Pará
10. Davide Calvarese, graduate student, ETH Zurich

<u>Leg 2</u>

11. Devon Firesinger, laboratory technician, University of South Florida

11b. Brazilian Navy Observing Officer

The Brazilian Navy Observing Officer (BNOO) will embark and disembark the R/V F.G. Walton Smith with the scientific crew from Cayenne, France. One berth will be reserved for the BNOO. All health and safety measures employed by the scientific and ship's crew will be employed also by the BNOO (section 12). Meals will be provided as with other crew, and we ask that the Brazilian Navy inform us of any food allergies or dietary preferences of the BNOO prior to the ship's departure from Miami.

12. Health and Safety

Due to the ongoing global viral SARS CoV-2 (COVID-19) pandemic, limited health and safety measures will be carried out prior to embarking the F.G. Walton Smith in Cayenne, France. Because members of the scientific crew and the BNOO will be arriving with different points of origin, itineraries, and health and exposure histories, participants will have to be up to date on vaccines as per U.S. CDC recommendations 2 weeks prior to the embarkation date. Additionally, scientific crew members and the BNOO will each have to produce 2 consecutive negative RATS COVID tests prior to embarkation. Prior to two tests, mobilization activities aboard the ship will be permitted for scientific crew and the BNOO so long as they properly cover their nose and mouth with a fitted KN-95 mask or equivalent. Cloth masks will be inadequate. Masks and tests will be provided by the ship's crew.

13. Post-expedition sample and data sharing

Upon the disembarkation of the vessel after science operations, all research groups and the Brazilian Navy Observing Officer will have a copy of underway data, station logs, hydrologic data, sub-bottom profile, and other digital data that is able to be reproduced on the ship. Any other data requests will be honored by the chief scientists and/or the marine technical crew within 12 months of the date of disembarkation. Samples that need refrigeration or freezing will be kept on the Walton Smith for transport to the United States unless arrangements can be made in Cayenne to ship samples in required conditions to partner institutions in Brazil and France. Samples will be collected so that they can divided without prejudice to their scientific value within twelve months after the end of the research or scientific investigation. Furthermore, all secondary data, information and results obtained (accompanied by a detailed and complete evaluation) will be shared between all Brazilian and American institutions within one year of the end of data analysis.

Appendices

List of Appendices:

Appendix A…. Amazon River sampling aboard the R/V Mirage

Appendix B….Coastal vs. estuary station time estimates and definition of 24h ops and sprints

Appendix C….Prolonged visit of José for transit to continental shelf and back to Macapa

Appendix D….Only transiting southbound, no sampling at Guaymas southbound

Appendix E….Active expedition plan, but with 24 hour operations

Appendix F….Max time on station with 24h ops and cruise length of 30 days

Appendix G….Excluding sampling sites off the coast of Brazil

Appendix A: Amazon River sampling aboard the R/V Mirage

A1. Objective

To obtain a representative Amazon River endmember for characterization of organic and inorganic geochemical properties.

A2. Personel

A3. Sites & Dates

Table A3. Sites, coordinates and shipboard tasks to be performed aboard the R/V Mirage

Section	Station	Latitude	Longitude	Shipboard Tasks
Amazon	Estuary 1	0°39'1.58"N	50°16'2.60"W	Bottom Sediment Grab, Hydrodynamic Sampler, SPE
Amazon	Estuary 2	0°27'0.81"N	50°29'46.79"W	Bottom Sediment Grab, Hydrodynamic Sampler
Amazon	Estuary 3	0° 1'33.72"S	50°59'7.90"W	Bottom Sediment Grab, Hydrodynamic Sampler, SPE

Amazon	Estuary 4	0° 0'46.42"S	50°21'29.79"W	Bottom Sediment Grab, Hydrodynamic Sampler
Amazon	Estuary 5	0° 7'52.76"S	49°53'47.75"W	Bottom Sediment Grab, Hydrodynamic Sampler
Amazon	Inner	0°53'34.76"N	49°49'11.00"W	Bottom Sediment Grab, Hydrodynamic Sampler, SPE
Araguari	Inner	1°14'9.83"N	49°56'20.45"W	Bottom Sediment Grab, Hydrodynamic Sampler, SPE

A4. Water Sampling

7 sites, 3-4 depths at each site, 21-28 samples, large surface samples E3, E4, E1

Materials

squeeze bottle x2

scale (to weigh water)

200 PES 0.2 micron, 142mm filters

21-28 whirlpaks for filter storage

$HgCl_2$, lil bit

surface only:

4 SPE columns (PPL)

7 1-2L bottles (Vitamins)

7 500 mL bottles for DOC , 13C, 14C

7 125 mL bottles for DIC, 13C, 14C

7 falcon tubes for nutrients (from Kristen, Brad mail to WHOI)

7 GC vials & caps (water isotopes)

7x 60-125mL HDPE bottles - cations/metals + acid? (HCl?)

A5. Sediment Sampling

Bottom grab at each site

Materials

7x large Whirlpaks

A6. Equipment

Hydrodynamic sampler(s)

Filtration device(s)

Peristaltic pump

A7. Storage

Freezer storage

- 8 x Bottom grabs from stations in whirlpaks
- 32 whirlpaks with filters (4 depths x8 stations = 32 bags of filters)
- 8 x Frozen SPE columns
- 8x 1-2L of filtered water

RT storage

- 8+ x 250 mL bottles for DOC, 13C, 14C
- 8+ x 100 mL bottles for DIC, 13C, 14C

Appendix B:

Table B1. Estimated sampling times for coastal and estuary stations.

Station	***Activity***	***Time***
Inner and Outer Shelf stations	Rosette Sampling with CTD	2 hours
	Multicore	2 hours
	Gravity core	2 hours
	TOTAL	**6 hours**

Mid shelf stations	Large surface sample	4 hours
	Rosette Sampling with CTD	2 hours
	Multicore	2 hours
	Gravity core	2 hours
	TOTAL	**10 hours**
Estuary Stations	Rosette Sampling with CTD	2 hours
	Bottom Grab	2 hours
	TOTAL	**4 hours**

The following appendices refer to itineraries based on ***24-hour*** operations or ***sampling sprints***, which are defined as:

- **24-hour operations** would consist of 2 scientific teams alternating 12-hour shifts such that the ship is either transiting between stations or we are actively sampling 24 hours a day.
- **Sampling sprints** roughly follows a 5 am to 11 pm work day, meaning sampling one station in the morning and transiting to the next in the evening or overnight. There would be a few days where we'd sample 2 stations per day rather than one.

Appendix C:

We have run the station time and transit calculator to include José in sampling the Amazon shelf sites before returning to Macapa. For the 24 hour sampling plan shown in Table B1 extending José's time on board only adds a few hours to our expedition. For the sampling sprints itinerary in Table B2, including the Amazon shelf sites in the Macapa loop adds 2 day to our expedition.

Table C1. Including Amazon shelf stations in our Macapa loop with 24 hour sampling

Station/Port	Arrive	Depart
St. Thomas		Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00	Day 8 6:00
Guaymas Inner	Day 9 15:20	Day 9 21:20
Guaymas Mid	Day 10 0:10	Day 10 6:10
Guaymas Outer	Day 10 8:50	Day 10 14:50
Marine Endmember	Day 12 18:30	Day 12 23:18
Macapa	Day 15 17:58	Day 15 23:58
Amazon Station - TSS and Bottom Grab 3	Day 16 0:48	Day 16 8:48
Amazon Station - TSS and Bottom Grab 2	Day 16 15:28	Day 16 23:28
Amazon Station - TSS and Bottom Grab 1	Day 17 2:48	Day 17 10:48
Amazon Inner	Day 17 15:58	Day 17 21:58
Amazon Mid	Day 18 2:08	Day 18 8:08
Amazon Outer	Day 18 12:18	Day 18 18:18
Amazon Station - TSS and Bottom Grab 5	Day 19 9:18	Day 19 17:18
Amazon Station - TSS and Bottom Grab 4	Day 20 4:08	Day 20 12:08
Macapa	Day 20 19:28	Day 21 1:28
Araguari TSS and Bottom Grab	Day 21 13:20	Day 21 22:20
Araguari Inner	Day 22 0:43	Day 22 10:19
Araguari Mid	Day 22 12:11	Day 22 18:11
Araguari Outer	Day 22 19:41	Day 23 1:41
Guaymas Inner	Day 23 15:56	Day 23 21:56
Guaymas Mid	Day 24 0:04	Day 24 6:04
Guaymas Outer	Day 24 8:04	Day 24 14:04
Oiapoque TSS and Bottom Grab	Day 25 6:19	Day 25 15:19
Oiapoque Inner	Day 25 19:04	Day 26 1:04
Oiapoque Mid	Day 26 2:49	Day 26 8:49
Oiapoque Outer	Day 26 9:56	Day 26 15:56
Iracoubo TSS and Bottom Grab	Day 27 6:19	Day 27 15:19
Iracoubo Inner	Day 27 16:04	Day 27 22:04
Iracoubo Mid	Day 27 23:11	Day 28 5:11
Iracoubo Outer	Day 28 6:11	Day 28 12:11
Maroni TSS and Bottom Grab	Day 28 18:41	Day 29 3:41
Maroni Inner	Day 29 6:26	Day 29 12:26
Maroni Mid	Day 29 13:19	Day 29 19:19
Maroni Outer	Day 29 20:19	Day 30 2:19

Degrad des Cannes (Cayenne, F.G.)	Day 30 18:34	Day 31 18:34
St. Thomas	Day 38 0:34	

Table C2. Including Amazon shelf stations in our Macapa loop with sampling sprints (Highlighted = same day sampling)

Station/Port	Arrive	Start Sampling	Depart
St. Thomas			Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00		Day 8 6:00
Guaymas Inner	Day 9 15:20	Day 9 15:20	Day 9 21:20
Guaymas Mid	Day 10 0:10	Day 10 6:00	Day 10 12:00
Guaymas Outer	Day 10 14:40	Day 10 14:40	Day 10 20:40
Marine Endmember	Day 13 0:20	Day 13 0:20	Day 13 5:08
Macapa	Day 15 23:48		Day 16 5:48
Amazon Station - TSS and Bottom Grab 3	Day 16 6:38	Day 16 6:38	Day 16 14:38
Amazon Station - TSS and Bottom Grab 2	Day 16 21:18	Day 17 6:00	Day 17 14:00
Amazon Station - TSS and Bottom Grab 1	Day 17 17:20	Day 18 6:00	Day 18 14:00
Amazon Inner	Day 18 19:10	Day 19 6:00	Day 19 12:00
Amazon Mid	Day 19 16:10	Day 20 6:00	Day 20 12:00
Amazon Outer	Day 20 16:10	Day 21 6:00	Day 21 12:00
Amazon Station - TSS and Bottom Grab 5	Day 22 3:00	Day 22 6:00	Day 22 14:00
Amazon Station - TSS and Bottom Grab 4	Day 23 0:50	Day 23 6:00	Day 23 14:00
Macapa	Day 23 21:20		Day 24 3:20
Araguari TSS and Bottom Grab	Day 24 15:12	Day 25 6:00	Day 25 15:00
Araguari Inner	Day 25 17:22	Day 26 6:00	Day 26 15:36
Araguari Mid	Day 26 17:28	Day 27 6:00	Day 27 12:00
Araguari Outer	Day 27 13:30	Day 27 13:30	Day 27 19:30
Guaymas Inner	Day 28 9:45	Day 28 9:45	Day 28 15:45
Guaymas Mid	Day 28 17:52	Day 29 6:00	Day 29 12:00
Guaymas Outer	Day 29 14:00	Day 29 14:00	Day 29 20:00
Oiapoque TSS and Bottom Grab	Day 30 12:15	Day 30 12:15	Day 30 21:15
Oiapoque Inner	Day 31 1:00	Day 31 6:00	Day 31 12:00
Oiapoque Mid	Day 31 13:45	Day 31 13:45	Day 31 19:45
Oiapoque Outer	Day 31 20:52	Day 32 6:00	Day 32 12:00
Iracoubo TSS and Bottom Grab	Day 33 20 2:22	Day 33 6:00	Day 33 15:00
Iracoubo Inner	Day 33 15:45	Day 33 15:45	Day 33 21:45
Iracoubo Mid	Day 33 22:52	Day 34 6:00	Day 34 12:00
Iracoubo Outer	Day 34 13:00	Day 34 13:00	Day 34 19:00
Maroni TSS and Bottom Grab	Day 35 1:30	Day 35 6:00	Day 35 15:00
Maroni Inner	Day 35 17:45	Day 36 6:00	Day 36 12:00
Maroni Mid	Day 36 12:52	Day 36 12:52	Day 36 18:52
Maroni Outer	Day 36 19:52	Day 37 6:00	Day 37 12:00
Degrad des Cannes (Cayenne, F.G.)	Day 38 4:15		Day 39 4:15

St. Thomas	Day 45 10:15		

Appendix D:

We have run the station time and transit calculator in case we decide not to sample the Guaymas transect on our transit south to Macapa. If we follow the 24 hour sampling plan shown in Table C1, removing the extra Guaymas transect saves 1.5 days, for an expedition totaling 36 days. If we follow the sampling sprints itinerary shown in Table C2 our cruise would still be 43 days.

Table D1. No Guaymas transect on transit south with 24 hour sampling

Station/Port	Arrive	Depart
St. Thomas		Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00	Day 8 6:00
Macapa	Day 11 12:20	Day 11 18:20
Amazon Station - TSS and Bottom Grab 3	Day 11 19:10	Day 12 3:10
Amazon Station - TSS and Bottom Grab 2	Day 12 9:50	Day 12 17:50
Amazon Station - TSS and Bottom Grab 1	Day 12 21:10	Day 13 5:10
Amazon Station - TSS and Bottom Grab 5	Day 13 21:00	Day 14 5:00
Amazon Station - TSS and Bottom Grab 4	Day 14 15:50	Day 14 23:50
Macapa	Day 15 7:10	Day 15 13:10
Amazon Inner	Day 16 1:02	Day 16 7:02
Amazon Mid	Day 16 10:10	Day 16 16:10
Amazon Outer	Day 16 19:17	Day 17 1:17
Marine Endmember	Day 18 8:32	Day 18 13:20
Araguari TSS and Bottom Grab	Day 20 1:35	Day 20 10:35
Araguari Inner	Day 20 12:58	Day 20 22:34
Araguari Mid	Day 21 0:26	Day 21 6:26
Araguari Outer	Day 21 7:56	Day 21 13:56
Guaymas Inner	Day 22 4:11	Day 22 10:11
Guaymas Mid	Day 22 12:19	Day 22 18:19
Guaymas Outer	Day 22 20:19	Day 23 2:19
Oiapoque TSS and Bottom Grab	Day 23 18:34	Day 24 3:34
Oiapoque Inner	Day 24 7:19	Day 24 13:19
Oiapoque Mid	Day 24 15:04	Day 24 21:04
Oiapoque Outer	Day 24 22:11	Day 25 4:11
Iracoubo TSS and Bottom Grab	Day 25 18:34	Day 26 0:34
Iracoubo Inner	Day 26 1:19	Day 26 7:19
Iracoubo Mid	Day 26 8:26	Day 26 17:26
Iracoubo Outer	Day 26 18:26	Day 27 0:26
Maroni TSS and Bottom Grab	Day 27 6:56	Day 27 15:56
Maroni Inner	Day 27 18:41	Day 28 0:41
Maroni Mid	Day 28 1:34	Day 28 7:34
Maroni Outer	Day 28 8:34	Day 28 14:34
Degrad des Cannes (Cayenne, F.G.)	Day 29 6:49	Day 30 6:49

St. Thomas	Day 36 12:49	

Table D2. No Guaymas transect on transit south with sampling sprints (Highlighted = same day sampling)

Station/Port	Arrive	Start Sampling	Depart
St. Thomas			Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00		Day 8 6:00
Macapa	Day 11 12:20		Day 11 18:20
Amazon Station - TSS and Bottom Grab 3	Day 11 19:10	Day 12 6:00	Day 12 14:00
Amazon Station - TSS and Bottom Grab 2	Day 12 20:40	Day 13 6:00	Day 13 14:00
Amazon Station - TSS and Bottom Grab 1	Day 13 17:20	Day 14 6:00	Day 14 14:00
Amazon Station - TSS and Bottom Grab 5	Day 15 5:50	Day 15 6:00	Day 15 14:00
Amazon Station - TSS and Bottom Grab 4	Day 16 0:50	Day 16 6:00	Day 16 14:00
Macapa	Day 16 21:20		Day 17 3:20
Amazon Inner	Day 17 15:12	Day 17 15:12	Day 17 21:12
Amazon Mid	Day 18 0:20	Day 18 6:00	Day 18 12:00
Amazon Outer	Day 18 15:07	Day 18 15:07	Day 18 21:07
Marine Endmember	Day 20 16:52	Day 20 16:52	Day 20 21:40
Araguari TSS and Bottom Grab	Day 22 22:25	Day 23 6:00	Day 23 15:00
Araguari Inner	Day 23 17:22	Day 24 6:00	Day 24 15:36
Araguari Mid	Day 24 17:28	Day 25 6:00	Day 25 12:00
Araguari Outer	Day 25 13:30	Day 25 13:30	Day 25 19:30
Guaymas Inner	Day 26 9:45	Day 26 9:45	Day 26 15:45
Guaymas Mid	Day 26 17:52	Day 27 6:00	Day 27 12:00
Guaymas Outer	Day 27 14:00	Day 27 14:00	Day 27 20:00
Oiapoque TSS and Bottom Grab	Day 28 12:15	Day 28 12:15	Day 28 21:15
Oiapoque Inner	Day 29 1:00	Day 29 6:00	Day 29 12:00
Oiapoque Mid	Day 29 13:45	Day 29 13:45	Day 29 19:45
Oiapoque Outer	Day 29 20:52	Day 30 6:00	Day 30 12:00
Iracoubo TSS and Bottom Grab	Day 31 2:22	Day 31 6:00	Day 31 15:00
Iracoubo Inner	Day 31 15:45	Day 31 15:45	Day 31 21:45
Iracoubo Mid	Day 31 22:52	Day 32 6:00	Day 32 12:00
Iracoubo Outer	Day 32 13:00	Day 32 13:00	Day 32 19:00
Maroni TSS and Bottom Grab	Day 33 1:30	Day 33 6:00	Day 33 15:00
Maroni Inner	Day 33 17:45	Day 34 6:00	Day 34 12:00
Maroni Mid	Day 34 12:52	Day 34 12:52	Day 34 18:52
Maroni Outer	Day 34 19:52	Day 35 6:00	Day 35 12:00
Degrad des Cannes (Cayenne, F.G.)	Day 36 4:15		Day 37 4:15
St. Thomas	Day 43 10:15		

Appendix E:

We have run the station time and transit calculator for our current itinerary (Table 2), but with a 24 hour sampling schedule shown in Table D1. If we follow the 24 hour sampling plan the expedition would last approximately 37 days whereas the itinerary with sampling sprints would be 43 days. Length of expedition in both scenarios depends upon our assumptions for the times of deployment and station occupation.

Table E1. Arrival and departure times for ports and sampling stations with a 24 hour sampling schedule

Station/Port	Arrive	Depart
St. Thomas		Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00	Day 8 6:00
Guaymas Inner	Day 9 15:20	Day 9 21:20
Guaymas Mid	Day 10 0:10	Day 10 6:10
Guaymas Outer	Day 10 8:50	Day 10 14:50
Marine Endmember	Day 12 18:30	Day 12 23:18
Macapa	Day 15 17:58	Day 15 23:58
Amazon Station - TSS and Bottom Grab 3	Day 16 0:48	Day 16 8:48
Amazon Station - TSS and Bottom Grab 2	Day 16 15:28	Day 16 23:28
Amazon Station - TSS and Bottom Grab 1	Day 17 2:48	Day 17 10:48
Amazon Station - TSS and Bottom Grab 5	Day 18 2:38	Day 18 10:38
Amazon Station - TSS and Bottom Grab 4	Day 18 21:28	Day 19 5:28
Macapa	Day 19 12:48	Day 19 18:48
Amazon Inner	Day 20 6:40	Day 20 12:40
Amazon Mid	Day 20 15:48	Day 20 21:48
Amazon Outer	Day 21 0:55	Day 21 6:55
Araguari TSS and Bottom Grab	Day 21 12:10	Day 21 21:10
Araguari Inner	Day 21 23:33	Day 22 9:09
Araguari Mid	Day 22 11:01	Day 22 17:01
Araguari Outer	Day 22 18:31	Day 23 0:31
Guaymas Inner	Day 23 14:46	Day 23 20:46
Guaymas Mid	Day 23 22:54	Day 24 4:54
Guaymas Outer	Day 24 6:54	Day 24 12:54
Oiapoque TSS and Bottom Grab	Day 25 5:09	Day 25 14:09
Oiapoque Inner	Day 25 17:54	Day 25 23:54
Oiapoque Mid	Day 26 1:39	Day 26 7:39
Oiapoque Outer	Day 26 8:46	Day 26 14:46
Iracoubo TSS and Bottom Grab	Day 27 5:09	Day 27 14:09
Iracoubo Inner	Day 27 14:54	Day 27 20:54
Iracoubo Mid	Day 27 22:01	Day 28 4:01
Iracoubo Outer	Day 28 5:01	Day 28 11:01
Maroni TSS and Bottom Grab	Day 28 17:31	Day 29 2:31
Maroni Inner	Day 29 5:16	Day 29 11:16

Maroni Mid	Day 29 12:09	Day 29 18:09
Maroni Outer	Day 29 19:09	Day 30 1:09
Degrad des Cannes (Cayenne, F.G.)	Day 30 17:24	Day 31 17:24
St. Thomas	Day 37 23:24	

Appendix F:

We have run the station time and transit calculator to determine the maximum/average time spent sampling each station if we were to operate on a 24 hour schedule and complete the expedition in 42 days. The itinerary in Table F1 shows that we can spend an average of 10 hours sampling each site and still return to St. Thomas in 42 days.

Table F1. Arrival and departure times for ports and sampling stations with 10 hours of sampling each site and 24 hour operations

Station/Port	Arrive	Depart
St. Thomas		Day 1 0:00
Degrad des Cannes (Cayenne, F.G.)	Day 7 6:00	Day 8 6:00
Guaymas Inner	Day 9 15:20	Day 10 1:20
Guaymas Mid	Day 10 4:10	Day 10 14:10
Guaymas Outer	Day 10 16:50	Day 11 2:50
Marine Endmember	Day 13 6:30	Day 13 16:30
Macapa	Day 16 11:10	Day 16 21:10
Amazon Station - TSS and Bottom Grab 3	Day 16 22:00	Day 17 8:00
Amazon Station - TSS and Bottom Grab 2	Day 17 14:40	Day 18 0:40
Amazon Station - TSS and Bottom Grab 1	Day 18 4:00	Day 18 14:00
Amazon Station - TSS and Bottom Grab 5	Day 19 5:50	Day 19 15:50
Amazon Station - TSS and Bottom Grab 4	Day 20 2:40	Day 20 12:40
Macapa	Day 20 20:00	Day 21 6:00
Amazon Inner	Day 21 17:52	Day 22 3:52
Amazon Mid	Day 22 7:00	Day 22 17:00
Amazon Outer	Day 22 20:07	Day 23 6:07
Araguari TSS and Bottom Grab	Day 23 11:22	Day 23 21:22
Araguari Inner	Day 23 23:45	Day 24 9:45
Araguari Mid	Day 24 11:37	Day 24 21:37
Araguari Outer	Day 24 23:07	Day 25 9:07
Guaymas Inner	Day 25 23:22	Day 26 9:22
Guaymas Mid	Day 26 11:30	Day 26 21:30
Guaymas Outer	Day 26 23:30	Day 27 9:30
Oiapoque TSS and Bottom Grab	Day 28 1:45	Day 28 11:45
Oiapoque Inner	Day 28 15:30	Day 29 1:30
Oiapoque Mid	Day 29 3:15	Day 29 13:15
Oiapoque Outer	Day 29 14:22	Day 30 0:22
Iracoubo TSS and Bottom Grab	Day 30 14:45	Day 31 0:45
Iracoubo Inner	Day 31 1:30	Day 31 11:30
Iracoubo Mid	Day 31 12:37	Day 31 22:37
Iracoubo Outer	Day 31 23:37	Day 32 9:37
Maroni TSS and Bottom Grab	Day 32 16:07	Day 33 2:07
Maroni Inner	Day 33 4:52	Day 33 14:52
Maroni Mid	Day 33 15:45	Day 34 1:45

Maroni Outer	Day 34 2:45	Day 34 12:45
Degrad des Cannes (Cayenne, F.G.)	Day 35 5:00	Day 36 5:00
St. Thomas	Day 42 11:00	

Appendix G:

Below is the station time and transit calculator for excluding sampling sites off the coast of Brazil. In addition to the original Oiapoque, Iracoubo, and Maroni transects, we've added three more at Paramaribo, Coronie, and Essequibo. If we follow the 24 hour sampling plan shown in Table F1 the expedition totals 14 days. If we follow the sampling sprints itinerary shown in Table F2 our cruise would be 20 days.

Table G1. Excluding Brazil from itinerary with 24 hour sampling

Station/Port	Arrive	Depart
Degrad des Cannes (Cayenne, F.G.)		Day 1 6:00
Oiapoque TSS and Bottom Grab	Day 1 14:07	Day 1 23:07
Oiapoque Inner	Day 2 2:52	Day 2 8:52
Oiapoque Mid	Day 2 10:37	Day 2 16:37
Oiapoque Outer	Day 2 17:45	Day 2 23:45
Iracoubo TSS and Bottom Grab	Day 3 14:07	Day 3 20:07
Iracoubo Inner	Day 3 20:52	Day 4 2:52
Iracoubo Mid	Day 4 4:00	Day 4 13:00
Iracoubo Outer	Day 4 14:00	Day 4 20:00
Maroni TSS and Bottom Grab	Day 5 2:30	Day 5 11:30
Maroni Inner	Day 5 14:15	Day 5 20:15
Maroni Mid	Day 5 21:07	Day 6 3:07
Maroni Outer	Day 6 4:07	Day 6 10:07
Paramaribo TSS and Bottom Grab	Day 6 19:30	Day 7 4:30
Paramaribo Inner	Day 7 5:52	Day 7 11:52
Paramaribo Mid	Day 7 12:45	Day 7 18:45
Paramaribo Outer	Day 7 20:00	Day 8 2:00
Coronie TSS and Bottom Grab	Day 8 10:07	Day 8 19:07
Coronie Inner	Day 8 20:30	Day 9 2:30
Coronie Mid	Day 9 3:37	Day 9 9:37
Coronie Outer	Day 9 11:00	Day 9 17:00
Essequibo TSS and Bottom Grab	Day 10 13:00	Day 10 22:00
Essequibo Inner	Day 11 2:07	Day 11 8:07
Essequibo Mid	Day 11 10:15	Day 11 16:15
Essequibo Outer	Day 11 18:30	Day 12 0:30
Degrad des Cannes (Cayenne, F.G.)	Day 14 6:15	

Table G2. Excluding Brazil from itinerary with sampling sprints

Station/Port	Arrive	Start Sampling	Depart
Degrad des Cannes (Cayenne, F.G.)			Day 1 6:00
Oiapoque TSS and Bottom Grab	Day 1 14:07	Day 2 6:00	Day 2 15:00
Oiapoque Inner	Day 2 18:45	Day 3 6:00	Day 3 12:00
Oiapoque Mid	Day 3 13:45	Day 3 13:45	Day 3 19:45
Oiapoque Outer	Day 3 20:52	Day 4 6:00	Day 4 12:00
Iracoubo TSS and Bottom Grab	Day 5 2:22	Day 5 6:00	Day 5 15:00
Iracoubo Inner	Day 5 15:45	Day 5 15:45	Day 5 21:45

Iracoubo Mid	Day 5 22:52	Day 6 6:00	Day 6 12:00
Iracoubo Outer	Day 6 13:00	Day 6 13:00	Day 6 19:00
Maroni TSS and Bottom Grab	Day 7 1:30	Day 7 6:00	Day 7 15:00
Maroni Inner	Day 7 17:45	Day 8 6:00	Day 8 12:00
Maroni Mid	Day 8 12:52	Day 8 12:52	Day 8 18:52
Maroni Outer	Day 8 19:52	Day 9 6:00	Day 9 12:00
Paramaribo TSS and Bottom Grab	Day 9 21:22	Day 10 6:00	Day 10 15:00
Paramaribo Inner	Day 10 16:22	Day 11 6:00	Day 11 12:00
Paramaribo Mid	Day 11 12:52	Day 11 12:52	Day 11 18:52
Paramaribo Outer	Day 11 20:07	Day 12 6:00	Day 12 12:00
Coronie TSS and Bottom Grab	Day 12 20:07	Day 13 6:00	Day 13 15:00
Coronie Inner	Day 13 16:22	Day 14 6:00	Day 14 12:00
Coronie Mid	Day 14 13:07	Day 14 13:07	Day 14 19:07
Coronie Outer	Day 14 20:30	Day 15 6:00	Day 15 12:00
Essequibo TSS and Bottom Grab	Day 16 8:00	Day 16 8:00	Day 16 17:00
Essequibo Inner	Day 16 21:07	Day 17 6:00	Day 17 12:00
Essequibo Mid	Day 17 14:07	Day 17 14:07	Day 17 20:07
Essequibo Outer	Day 17 22:22	Day 18 6:00	Day 18 12:00
Degrad des Cannes (Cayenne, F.G.)	Day 20 17:45		

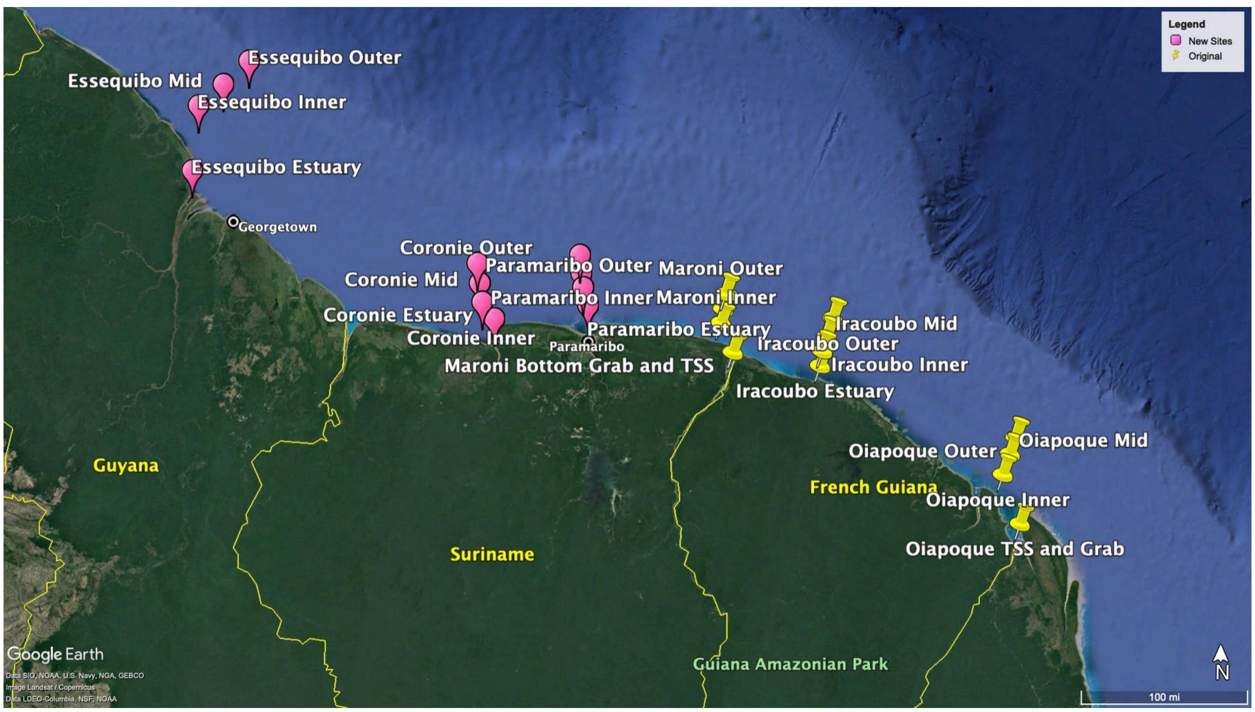

Figure G1. New sampling sites (pink) added if we exclude Brazil from the original sampling plan