Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico: Addendum for Field work to support *Sargassum* mapping

Prepared by:

Chuanmin Hu

College of Marine Science, University of South Florida

For the
Mississippi Canyon 252 Trustees

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Comments and questions should be addressed to:
Ian Zelo (ian.j.zelo@noaa.gov)

Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico: Addendum for Field Work to Support *Sargassum* Mapping

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Each party reserves its rights to produce its own independent interpretations and analyses of any data collected pursuant to this work plan. The trustees have developed a preliminary conceptual model of the DWH release, potential pathways and routes of exposure, and potential receptors. This preliminary model has informed the trustees' decision to pursue the studies outlined in the work plan. By signing this work plan and agreeing to fund the work outlined, BP is not endorsing the model articulated in the work plan.

APPROVED:

Jessica White for Lisa DiPinto 1/5/2012
NOAA Trustee Representative: Date

1/20/2012
Louisiana Trustee Representative: Date

10 - January - 2012
BP Representative: Date

Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico: Addendum for Field Work to Support *Sargassum* Mapping

Objectives of the Assessment Plan for *Sargassum* include the determination of 2011 areal extent and distribution of *Sargassum* in the north-central Gulf of Mexico via aerial surveys, and its spatial relationship to previously observed surface oil and dispersants in 2010 associated with the MC 252 discharge.

The purpose of this addendum is to obtain field data to facilitate the interpretation of satellite imagery of *Sargassum* before and during the MC 252 oil spill, and since the plume has dissipated. The specific goal of this effort is to document two specific conditions – 100% *Sargassum* coverage and 0% *Sargassum* coverage (open water). Any pixel of satellite imagery can have a mixed signal because it contains a range of coverage from 0 to possibly 100%. When analyzing these data it is critical to have clean reflectance measurements for the ends of the spectrum. This plan will help to identify these extremes.

To document these two conditions, *Sargassum* biomass and ecological state (C:N:P ratios) will be quantified in the field. Field surveys will measure the bio-optical (reflectance, biomass) and other properties of pelagic *Sargassum* as well as its surrounding water environment. All field work under this Plan has been completed.

Objectives

1. Measure the spectral reflectance of areas with 100% *Sargassum* coverage and the water environment in different settings (coastal versus offshore, or nutrient-rich versus nutrient-poor);
2. Collect water samples in order to determine environmental parameters (i.e. colored dissolved organic matter, particulate matter, dissolved nutrients, and chlorophyll-a concentration) of the *Sargassum* environment in contrast to nearby *Sargassum*-free environment.
3. Collect *Sargassum* samples to quantify *Sargassum* biomass (wet and dry) per unit area in relation to the measured reflectance;
4. Collect *Sargassum* samples to analyze their C:N:P ratios as well as to determine their chlorophyll-a content per carbon.

Proposed Work

1. Cruise participants

   Two additional NOAA representatives (one person from C. Hu's group, and one from B. Lapointe's group) on *Sargassum* cruise(s)

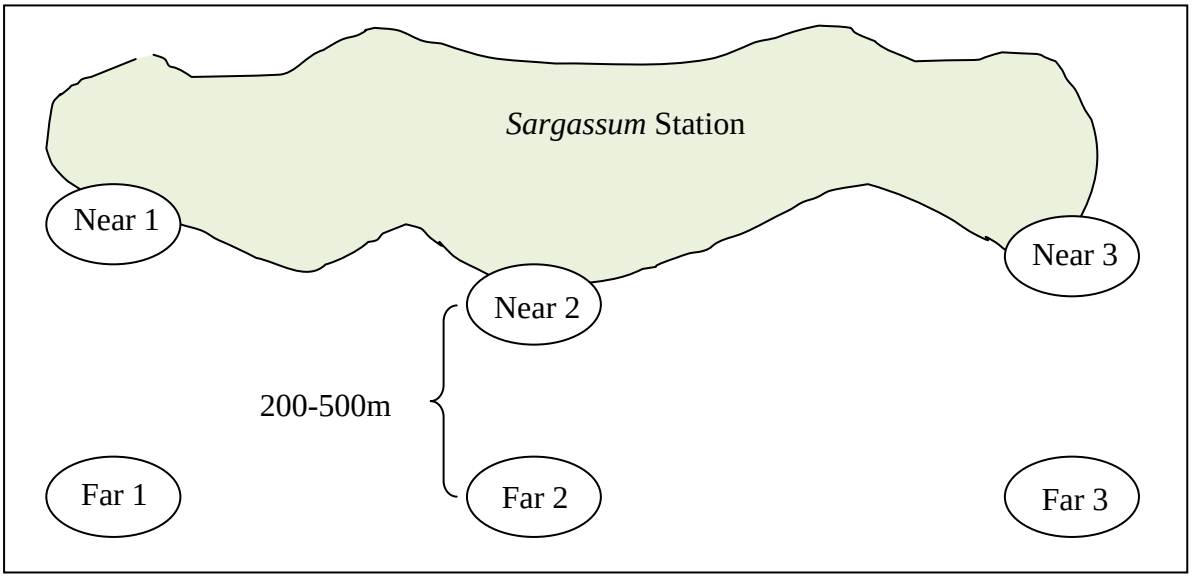
2. Dates of the cruise:

Sampling will occur on the cruise scheduled for 11 – 18 September 2011 (maximum 8 days at sea).

3. Sampling frequency

In the approximate area of two grids pre-defined in the original work plan, *Sargassum* will be sampled: south of Mobile Bay, Alabama (Grid 1) and west of Venice, Louisiana (Grid 2). Within each grid, teams will sample three different stations. At each station teams will sample three sub-stations (Figure 1) and at each sub-station teams may collect samples for two *Sargassum* species (*S. natans* and *S. fluitans*). These stations will be located at the same features that are sampled for the primary plan. Additional stations may be sampled if time and logistics allow.

Figure 1. Substation layout at a *Sargassum* station



At each sub-station a series of measurements and samples will be collected:

- Reflectance measurements – 1 for sargassum, 1 for open water
- Water samples – 2 under the sargassum, 2 in open water
- Wet weight – 1 large sample will be photographed and weighed
- Leaf samples – 3 samples collected from the large wet weight sample. 1 mixed, 1 *S. fluitans*, 1 *S. natans*

Table 1. Sample Summary

					Sub-Sampling	
	Grids	Stations	Substations	Species	Samples / Measurements per sub-station	Total
Reflectance	2	3	3		2	36
Water	2	3	3		4	72
Wet weight	2	3	3		1	18
Leaf samples	2	3	3	2	3	54

This is an estimate and subject to change if adequate *Sargassum* samples cannot be obtained.

4. Measurements, with brief overview of protocols (w/ references)

Corresponding to the four objectives, the following four collections will be conducted: 1) in situ reflectance measurements; 2) water sampling to characterize water column properties; 3) sample collection for *Sargassum* biomass (wet and dry) determination; and 4) sample **collection** for laboratory measurements.

These collections will be made at three locations for each *Sargassum* station sampled to ensure representativeness for each location. These will be collected at the ends and the middle of the*Sargassum* station.

I. In *situ* reflectance measurements.

This will be performed above water using a hand-held spectrometer in overcast or clear sky conditions. Two measurements will be taken per sub-station – 1 for 100% *Sargassum* coverage and 1 for open water.

The Spectrix spectrometer has 512 channels from 350 to 880 nm, but another spectrometer covering longer wavelengths may be used. The Lee et al. (1997) protocol to measure above-water remote sensing reflectance will be followed. This type of measurement has been used since the 1990s and all quality controlled data have been submitted to NASA SeaBASS (Cannizzaro et al., 2006 & 2008). These established protocols will be followed in collecting, processing, and submitting the data.

II. Water sampling to characterize water column properties

Water samples collected from surface waters near and away from *Sargassum* stations. The open water samples will be collected 200-500m away from the *Sargassum*stations. Two of each type of sample will be collected per sub-station. They will be filtered to measure 1) particulate absorption spectra; 2) phytoplankton pigment absorption spectra; 3) detrital absorption spectra; 4) chlorophyll-a concentration (fluorometric method) of seawater and *Sargassum*; 5) colored dissolved organic matter absorption spectra.

One filter pad will be stored in liquid nitrogen onboard the ship and then transferred to a -80°C deep freezer upon return to the lab. The second will be stored at -18°C

For measurement #5, filtered seawater is stored in 4-oz amber bottles at ~-18°C (e.g. standard freezer).

The sample collection and laboratory processing will follow standard protocols established by NASA (Mueller and Austin, 1995, Ocean Optics protocols, NASA technical report).

III. Sample collection for *Sargassum* biomass determination.

A portion of 100% *Sargassum* will be separated from the main algal mass ate each *Sargassum* station using a pole. A plaque/quadrat of know area will be placed next to the sample. The two will be photographed together so that size and percent surface coverage can be measured. Teams will target 10-20 pounds of material.

The *Sargassum* sample will then be collected using a standard or home-made net, and its wet weight determined using a scale on the ship deck. Weight per area is then estimated.

IV. *Sargassum* tissue for lab analysis

Subsamples of 15-30 grams of tissue will be collected from the sample above.

The methods for collecting and processing *Sargassum* for tissue analysis and determination of dissolved seawater nutrients will closely follow the methods of Lapointe (1995). Both *S. natans* and *S. fluitans* will be collected from surface waters using a dip net at each station. The samples will be placed in plastic coolers and then separated by species for processing. The samples will be cleaned of epizoa and epiphytes, and rinsed briefly in deionized water. One sample of *S. natans* and one sample of *S. fluitans* will be dried in a laboratory oven at 70°C for 48 hours. The dried tissue samples will be powdered and analyzed for %C, %N, and % P at the Nutrient Analytical Services (NAS), Chesapeake Biological Laboratory, Solomons, MD. (Keefe et al., 2004).

A third sample will be frozen at -18°C and analyzed in the lab for wet weight and chlorophyll-a.

V. Station and sample selection strategy

The primary *Sargassum* stations sampled under this addendum will be those that are selected for the main plan. Additional *Sargassum* may be sampled but any supplemental work will need to fit into the time and logistical constraints imposed by staying on schedule for the fish/invert sampling.

Upon arriving at a sampling station (determined by needs of the primary plan), 100% *Sargassum* will be collected opportunistically according to the schedule of the primary sampling but it is expected that this sampling will be unbiased and representative of the *Sargassum* community.

VI. Schedule of deliverables

The *in situ* remote sensing reflectance data and the biomass data will be available for delivery one month after the cruise end date. The sample analysis data (both water environment and *Sargassum* samples) will take 3-6 months.

All bio-optical data (reflectance, biomass, absorption spectra) will be recorded in NASA-specified format in ASCII computer files, with naming conventions defined later after consulting with the entire group. A sample file is provided in the appendix.

Sargassum tissue data (C:N:P, chlorophyll-a) and seawater nutrient data (ammonium, nitrate plus nitrite, soluble reactive phosphorus, total dissolved nitrogen and total dissolved phosphorus) will be recorded and managed in Excel spreadsheets.

Instruments and accessories:

Measurement	**Instrument(s)**	**Weight**	**Size**	**Notes**
Reflectance	Spectrix or ASD	4-5 lb	1’x0.5’x2’’	
	Laptop	3 lb	1.5’x1’x1’’	
Water samples	Filtration rig	10 lb	3’x2’x1’	
	Nitrogen dewer	15 lb	1’x1’x1.5’	
	Amber bottles	10 lb	1’x0.5’x0.5’	
	Freezer	20 lb	2’x2’x2’	Approx.
Sargassum biomass	Scale	2 lbs	1’x2’x2”	
	Reference board	1 lb	3’x3’x1’’	
	Oven	50 lbs	3’x3’x2’	Approx.
Sargassum samples	Oven	50 lbs	3’x3’x3’	Approx.

Site Selection Strategy

Refer to plan

Health and Safety

Refer to plan

Data Handling and Sharing

Refer to plan

Durable Equipment

Refer to plan

Budget Estimate

Labor	*Costs*
Principal Investigators:	
Dr. Brian Lapointe	
Dr. Chuanmin Hu	
cruise planning, guidance, coordination, technical consulting, document preparation, etc.	
Field Samplers	
Two technicians: (rate = [redacted])	
[redacted] field days [redacted] x 2 technicians	
[redacted] travel days [redacted] x 2 technicians	
[redacted] days (material/equipment prep etc) [redacted] x 2 technicians	
[redacted] days training for overall field effort x 2 technicians	
Total labor costs	$34,400
Travel	
Air Fare - two round trip flights from Florida to New Orleans @$350	$700
Car rental ($300 per cruise)	$300
Meals during travel ($120 per person per cruise)	$240
Total travel	$1,240
Materials and supplies	
Filters, reagent, boxes, spring scale, bags, liquid nitrogen, etc.	$500
Instrument calibration ($1000 per cruise)	$1,000
Oven for drying samples (provided by BP/CSA) --	
Shipping ($1000 per cruise)	$1,000
Total materials and supplies	$2,500
Total Estimated Costs	***$38,140***

The Parties acknowledge that this budget is an estimate and that actual costs may prove to be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this approved work plan that may arise. The trustees will make a good faith effort to notify BP in advance of any such increased costs

References

Lapointe, B. E. 1995. A comparison of nutrient-limited productivity in Sargassum natans from neritic vs. oceanic waters of the western North Atlantic Ocean. Limnol. Oceanogr. 40(3):625-633.

Lee, Z., Carder, K.L., Steward, R.G., Peacock, T.G., Davis, C.O., Mueller, J.L., 1997. "Remote sensing reflectance and inherent optical properties of oceanic waters derived from above-water measurements", Proc. SPIE 2963, 160; doi:10.1117/12.266436.

Cannizzaro, J.P., Carder, K.L., 2006. Estimating chlorophyll a concentrations from remote-sensing reflectance in optically shallow waters. Remote Sensing of Environment. 101, 13-24.

Cannizzaro, J.P., Carder, K.L., Chen, F.R., Heil, C.A., Vargo, G.A., 2008. A novel technique for detection of the toxic dinoflagellate, Karenia brevis, in the Gulf of Mexico from remotely sensed ocean color data. Cont. Shelf Res. 28, 137–158.

Keefe CW, Blodnikar KL, Boynton WR, Clark CA, Frank JM, Kaumeyer NL, Weir MM, Wood KV, Zimmerman CF (2004) Nutrient Analytical Services Laboratory Standard Operating Procedures, Special Publication Series No. SS-80-04-CBL, Center for Environmental and Estuarine Studies, The University of Maryland System, PO Box 38, Solomons, MD 20688-0038, 65 pp.

Mueller, J.L., and R.W. Austin, 1995: Ocean Optics Protocols for SeaWiFS Validation, Revision 1. NASA Tech. Memo. 104566, Vol. 25, S.B. Hooker, E.R. Firestone, and J.G. Acker, Eds., NASA Goddard Space Flight Center, Greenbelt, Maryland, 67 pp

Appendix A: Sample reflectance file Rrs_PR650_MR05.txt

```
!Rrs_PR650_MR05.txt  Above-Water measurements - Lu, Ls, Ed, Lw, Rrs
/begin_header
/investigators=C. Hu
/affiliations=Univ. South Florida
/contact=hu@marine.usf.edu
/experiment=M_River
/cruise=MR2001
/station=MR05
/data_file_name=MR05.meta
/original_file_name=pr650_MR05.xls
/documents=readme.txt
/calibration_files=calib_pr650.txt
/data_type=above_water
/data_status=final
/parameters=Lu,Ls,Ed,Lw,Rrs
/start_date=20010305
/end_date=20010305
/start_time=13:33:49 [GMT]
/end_time=13:35:22 [GMT]
/north_latitude=28.500 [DEG]
/south_latitude=28.500 [DEG]
/east_longitude=-91.5 [DEG]
/west_longitude=-91.5 [DEG]
/water_depth=250
/measurement_depth=0
/secchi_depth=NA
/cloud_percent=15
/wind_speed=2
/wave_height=0.5
! PR650 Spectrometer
!
!
! Data reduction at Thu Jul 12 15:11:33 2001
! Meta file used:   sample.meta
! Water channels used: 1,2,3
! Sky channels used:  1,2,3
! Plaq channels used: 1
! Fresnel reflectance:   0.0200000
! Normalize to 750 nm (1/Yes, 0/No):    0
! Smooth data (1/Yes, 0/No):        0
/missing=-999
/delimiter=space
/fields=Wavelength,Lu,Ls,Ed,Lw,Rrs
/units=nm,uW/cm^2/nm/sr,uW/cm^2/nm/sr,uW/cm^2/nm,uW/cm^2/nm/sr,1/sr
```

/end_header@

380.000	0.1059547	3.5012798	24.8782692	0.0359291	0.0014442
384.000	0.1137478	3.6982524	25.1287193	0.0397828	0.0015832
388.000	0.1178947	3.7580280	25.9303989	0.0427342	0.0016480
392.000	0.1269038	3.9875152	27.6090794	0.0471535	0.0017079
396.000	0.1348542	4.1712489	29.2250519	0.0514292	0.0017598
400.000	0.1616160	4.9336457	36.2631798	0.0629431	0.0017357
404.000	0.1963803	5.9442563	45.5091400	0.0774952	0.0017028
408.000	0.2068117	6.1768022	48.5017509	0.0832756	0.0017170
412.000	0.2103985	6.1751218	50.2423210	0.0868961	0.0017295
416.000	0.2145428	6.1920066	51.5506401	0.0907027	0.0017595
420.000	0.2140149	6.0601082	52.3899307	0.0928127	0.0017716
424.000	0.2091417	5.8003030	52.2814941	0.0931357	0.0017814
428.000	0.2017531	5.4890213	50.5959206	0.0919727	0.0018178
432.000	0.1951443	5.1955190	49.8385010	0.0912339	0.0018306
436.000	0.2043228	5.3319507	52.8027878	0.0976838	0.0018500
440.000	0.2167073	5.5494881	57.1170616	0.1057176	0.0018509
444.000	0.2263711	5.6772304	60.4184799	0.1128265	0.0018674
448.000	0.2377530	5.8252563	63.8752823	0.1212479	0.0018982
452.000	0.2468566	5.9016299	66.8765640	0.1288240	0.0019263
456.000	0.2490265	5.8155932	67.8550110	0.1327146	0.0019559
460.000	0.2514010	5.7237353	68.8558273	0.1369263	0.0019886
464.000	0.2515748	5.5789056	69.1744995	0.1399967	0.0020238
468.000	0.2509683	5.4102669	69.0474701	0.1427630	0.0020676
472.000	0.2524163	5.2803206	69.3320541	0.1468098	0.0021175
476.000	0.2561271	5.1771922	70.2887421	0.1525833	0.0021708
480.000	0.2587279	5.0716252	71.0171890	0.1572954	0.0022149
484.000	0.2560950	4.8682804	69.9850693	0.1587294	0.0022680
488.000	0.2503849	4.6032934	68.0802002	0.1583191	0.0023255
492.000	0.2536565	4.5128927	68.7551498	0.1633987	0.0023765
496.000	0.2610650	4.5046153	70.5382385	0.1709727	0.0024238
500.000	0.2612876	4.3438640	70.0862808	0.1744103	0.0024885
504.000	0.2617683	4.1891780	69.4683304	0.1779847	0.0025621
508.000	0.2665182	4.1059346	69.7860031	0.1843995	0.0026424
512.000	0.2689425	3.9938536	69.6278992	0.1890654	0.0027154
516.000	0.2644213	3.7764852	67.8499222	0.1888916	0.0027840
520.000	0.2659203	3.6372235	66.8294754	0.1931758	0.0028906
524.000	0.2763198	3.6157303	68.3428421	0.2040052	0.0029850
528.000	0.2843298	3.5747337	69.1828613	0.2128352	0.0030764
532.000	0.2927895	3.5356705	70.1762009	0.2220761	0.0031645
536.000	0.2979322	3.4574974	70.1937485	0.2287823	0.0032593
540.000	0.3006414	3.3489704	69.5889130	0.2336620	0.0033577
544.000	0.3040577	3.2361181	69.0017090	0.2393353	0.0034685
548.000	0.3108826	3.1659567	69.2824097	0.2475635	0.0035733
552.000	0.3182041	3.1063499	69.5845108	0.2560771	0.0036801
556.000	0.3237984	3.0268557	69.2864151	0.2632613	0.0037996

560.000	0.3261175	2.9142714	68.2818451	0.2678321	0.0039224
564.000	0.3307508	2.8288743	67.8976440	0.2741733	0.0040380
568.000	0.3320581	2.7255223	67.1621628	0.2775477	0.0041325
572.000	0.3323567	2.6455760	66.7162781	0.2794452	0.0041886
576.000	0.3322451	2.5897434	66.8164902	0.2804502	0.0041973
580.000	0.3298880	2.5461993	67.2101669	0.2789640	0.0041506
584.000	0.3237195	2.5036495	67.4802780	0.2736465	0.0040552
588.000	0.3015842	2.3553300	64.9198608	0.2544776	0.0039199
592.000	0.2819589	2.2369995	63.0674591	0.2372189	0.0037614
596.000	0.2696213	2.2012167	63.5181885	0.2255969	0.0035517
600.000	0.2578573	2.1940768	64.6522903	0.2139758	0.0033096
604.000	0.2465793	2.1755028	65.4242401	0.2030693	0.0031039
608.000	0.2388639	2.1381528	65.7107010	0.1961008	0.0029843
612.000	0.2322141	2.0756750	65.1242599	0.1907006	0.0029283
616.000	0.2260648	2.0128343	64.5090866	0.1858082	0.0028803
620.000	0.2224402	1.9715849	64.5508194	0.1830085	0.0028351
624.000	0.2166172	1.9060760	63.7512817	0.1784957	0.0027999
628.000	0.2087684	1.8212843	62.2928009	0.1723427	0.0027667
632.000	0.2053030	1.7768540	62.0052567	0.1697659	0.0027379
636.000	0.2047920	1.7580730	62.5185738	0.1696306	0.0027133
640.000	0.2036443	1.7301787	62.6681213	0.1690408	0.0026974
644.000	0.1984368	1.6657571	61.6648979	0.1651216	0.0026777
648.000	0.1911758	1.5960793	60.2681084	0.1592542	0.0026424
652.000	0.1840999	1.5486013	59.5339470	0.1531279	0.0025721
656.000	0.1743990	1.5052186	59.0259323	0.1442947	0.0024446
660.000	0.1683174	1.5041164	60.3322678	0.1382351	0.0022912
664.000	0.1668766	1.5185803	62.2115059	0.1365050	0.0021942
668.000	0.1669748	1.4998857	62.5243187	0.1369771	0.0021908
672.000	0.1698982	1.4647384	62.2376938	0.1406034	0.0022591
676.000	0.1763157	1.4323710	61.9882889	0.1476683	0.0023822
680.000	0.1819746	1.3889664	61.1574097	0.1541953	0.0025213
684.000	0.1772564	1.2948080	57.8839722	0.1513602	0.0026149
688.000	0.1660551	1.1831347	53.7762413	0.1423925	0.0026479
692.000	0.1582734	1.1413254	52.9756775	0.1354469	0.0025568
696.000	0.1508866	1.1454953	54.0463371	0.1279766	0.0023679
700.000	0.1429182	1.1409303	54.6326981	0.1200996	0.0021983
704.000	0.1340082	1.1362888	55.1865234	0.1112825	0.0020165
708.000	0.1259369	1.1372486	56.1438332	0.1031919	0.0018380
712.000	0.1117770	1.0904437	54.5612869	0.0899682	0.0016489
716.000	0.0924480	0.9638652	48.6218224	0.0731707	0.0015049
720.000	0.0773347	0.8643134	44.1342888	0.0600484	0.0013606
724.000	0.0697938	0.8400514	43.3107491	0.0529927	0.0012235
728.000	0.0642953	0.8437596	44.0003891	0.0474201	0.0010777
732.000	0.0638703	0.8971302	47.4718399	0.0459276	0.0009675
736.000	0.0648588	0.9594169	50.9487228	0.0456704	0.0008964
740.000	0.0657470	0.9954613	52.9681511	0.0458378	0.0008654

744.000	0.0672312	1.0309669	54.5547714	0.0466119	0.0008544
748.000	0.0679197	1.0549546	54.9190712	0.0468206	0.0008525
752.000	0.0682419	1.0720114	54.3821793	0.0468017	0.0008606
756.000	0.0619120	0.9974464	47.9910507	0.0419631	0.0008744
760.000	0.0485076	0.8126184	35.7890091	0.0322553	0.0009013
764.000	0.0495014	0.8059340	35.6174126	0.0333828	0.0009373
768.000	0.0626423	0.9900270	45.0568581	0.0428417	0.0009508
772.000	0.0735545	1.1677984	52.3167915	0.0501986	0.0009595
776.000	0.0790391	1.2715000	54.1772499	0.0536091	0.0009895
780.000	0.0828048	1.3509578	54.5403137	0.0557857	0.0010228

Appendix B: Liquid Nitrogen Handing SOP and MSDS

This plan will adopt the approved Liquid Nitrogen Handling and Safety Protocol developed cooperatively for the NRDA Summer 2011 Plankton Imaging Sampling Cruise Plan.

Deepwater Horizon Oil Spill (DWHOS)

NRDA Plankton Sampling Plans: Liquid Nitrogen Handling and Safety Protocol

June 16, 2011

The primary dangers associated with liquid nitrogen are suffocation and freezing. As liquid nitrogen vaporizes to gas, it can build up in enclosed spaces very quickly, displacing oxygen and depriving biological tissues of oxygen. If nitrogen gas concentrations are sufficient to reduce oxygen levels below 19.5%, rapid asphyxiation may occur, leading to unconsciousness or death. Liquid nitrogen will quickly freeze and permanently damage body tissues (skin, hands, eyes, *etc.*) and most other materials (clothing, shoes, floors, ship decks, *etc.*). The safe handling measures and protocols outlined in this document will help prevent injury and damage to the crew, scientists, and equipment on NRDA plankton surveys.

Preventative Measures for the Safe Handling of Liquid Nitrogen:

Personal Protective Equipment (PPE) when filling Dewar and transferring liquid nitrogen:

- Wear dry clothing that covers the arms and legs.
- Wear cryogenic gloves tucked under the sleeves so no liquid can fall into the gloves.
- Wear a face shield or safety glasses.
- Wear non-slip, closed footwear and an apron.

Storage and handling:

- Always make sure that liquid nitrogen is stored and handled in an area where there is sufficient ventilation. Liquid nitrogen is always producing gas. Special care is necessary on ships because nitrogen gas is heavier than air, and can sink into ship holds that are not ventilated.
- Never ride in an elevator with liquid nitrogen.
- Liquid nitrogen should be stored in Dewars (specially designed vacuum flasks used to store cryogenic materials) that are properly maintained and rated for liquid nitrogen.
- All Dewars should have pressure venting lids/stoppers (or a pressure relief valve) as designed by the manufacturer.
- The Dewar should be placed on the floor.
- The Dewar should be securely held to the ship structure in a well ventilated area with metal brackets or positive cargo straps at all times, from before the ship leaves the dock until the Dewar is offloaded.
- Never pour liquid nitrogen from the Dewar.
- Two people are required to move the Dewar on and off the ship.
- All scientists, technicians and ship's crew will be trained on hazards, risks, safe handling and response of emergencies involving liquid nitrogen. Training should cover information presented in the MSDS and this protocol prior to departing any cruise where liquid nitrogen will be on board.

Clean-up Procedures in the event of a liquid nitrogen spill:

- Evacuate the area immediately; move up-wind of the spill site.
- Notify the bridge and crew immediately.
- If it is necessary for anyone to enter the area of the spill (*e.g.* for rescue), they must don all PPE as well as a self-contained breathing apparatus (SCBA). The McArthur II has several SCBAs and all personnel are trained in their use, along with all relevant PPE.
- Wait for all of the liquid nitrogen to evaporate, provide ventilation of the area with fans and blowers.
- Re-enter the area only after oxygen levels have returned to normal. The McArthur II has the appropriate oxygen and CO_2 sensors, and personnel have been trained on their use.
- A thorough assessment of any damage, which may include structural damage to deck plates or bulkheads, should be performed and communicated to the ship's commanding officer.

Protocol for the Use of Liquid Nitrogen During Plankton Sampling:

1. Place sample to be preserved into the proper cryopreservation tube.
2. Put on cryogenic gloves and face shield/goggles.
3. Remove the cover from the Dewar.
4. Using tongs, place the cryopreservation tube into the Dewar.
5. Replace the cover to the Dewar.

References

Airgas Inc. 2011. Material Safety Data Sheet: Nitrogen. Revised January 1, 2011. Airgas Inc., Radnor, PA. Available online at (http://www.airgas.com/documents/pdf/001040.pdf).

University of New South Wales. 2008. Protocol – Liquid Nitrogen. Effective January 9, 2008. Available online at (http://www.med.unsw.edu.au/SOMSWeb.nsf/resources/Policies02/$file/Protocol_Liquid_Nitrogen.pdf).

Material Safety Data Sheet

Nitrogen

Section 1. Chemical product and company identification

Product name : Nitrogen

Supplier : AIRGAS INC., on behalf of its subsidiaries
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

Product use : Synthetic/Analytical chemistry. Liquid – cryogenic coolant.

Synonym : nitrogen (dot); nitrogen gas; Nitrogen NF, LIN, Cryogenic Liquid Nitrogen, Liquid Nitrogen

MSDS # : 001040

Date of Preparation/Revision : **1/14/2011.**

In case of emergency : 1-866-734-3438

Section 2. Hazards identification

Physical state : Gas. [NORMALLY A COLORLESS GAS: MAY BE A CLEAR COLORLESS LIQUID AT LOW TEMPERATURES. SOLD AS A COMPRESSED GAS OR LIQUID IN STEEL CYLINDERS.]

Emergency overview : WARNING!

GAS:
CONTENTS UNDER PRESURE.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Do not puncture or incinerate container.

Contact with rapidly expanding gases or liquids can cause frostbite.

Routes of entry : Inhalation

Potential acute health effects

Eyes : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Skin : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Inhalation : Acts as a simple asphyxiant.

Ingestion : Ingestion is not a normal route of exposure for gases. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Medical conditions aggravated by over-exposure : Acute or chronic respiratory conditions may be aggravated by overexposure to this gas.

See toxicological information (Section 11)

Section 3. Composition, Information on Ingredients

Name	CAS number	% Volume	Exposure limits
Nitrogen	7727-37-9	100	Oxygen Depletion [Asphyxiant]

Section 4. First aid measures

No action shall be taken involving any personal risk or without suitable training.If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus.It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Eye contact : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.

Skin contact : None expected.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Inhalation : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Ingestion : As this product is a gas, refer to the inhalation section.

Section 5. Fire-fighting measures

Flammability of the product : Non-flammable.

Products of combustion : Decomposition products may include the following materials:
nitrogen oxides

Fire-fighting media and instructions : Use an extinguishing agent suitable for the surrounding fire.

Apply water from a safe distance to cool container and protect surrounding area. If involved in fire, shut off flow immediately if it can be done without risk.

Contains gas under pressure. In a fire or if heated, a pressure increase will occur and the container may burst or explode.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (section 8). Shut off gas supply if this can be done safely. Isolate area until gas has dispersed.

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Methods for cleaning up : Immediately contact emergency personnel. Stop leak if without risk. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Handling : High pressure gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Never allow any unprotected part of the body to touch uninsulated pipes or vessels that contain cryogenic liquids. Prevent entrapment of liquid in closed systems or piping without pressure relief devices. Some materials may become brittle at low temperatures and will easily fracture.

Storage : Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F).
For additional information concerning storage and handling refer to Compressed Gas Association pamphlets P-1 Safe Handling of Compressed Gases in Containers and P-12 Safe Handling of Cryogenic Liquids available from the Compressed Gas Association, Inc.

Section 8. Exposure controls/personal protection

Engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.

When working with cryogenic liquids, wear a full face shield.

Skin : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

The applicable standards are (US) 29 CFR 1910.134 and (Canada) Z94.4-93

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Insulated gloves suitable for low temperatures

Personal protection in case of a large spill : Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product.

Product name

Nitrogen	Oxygen Depletion [Asphyxiant]

Consult local authorities for acceptable exposure limits.

Section 9. Physical and chemical properties

Molecular weight : 28.02 g/mole

Molecular formula : N2

Boiling/condensation point : -195.8°C (-320.4°F)

Melting/freezing point : -210°C (-346°F)

Critical temperature : -146.9°C (-232.4°F)

Vapor density : 0.967 (Air = 1) Liquid Density@BP: 50.46 lb/ft3 (808.3 kg/m3)

Specific Volume (ft ^{3}/lb) : 13.8889

Gas Density (lb/ft ^{3}) : 0.072

Section 10. Stability and reactivity

Stability and reactivity : The product is stable.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Toxicity data

Other toxic effects on humans : No specific information is available in our database regarding the other toxic effects of this material to humans.

Specific effects

Carcinogenic effects : No known significant effects or critical hazards.

Mutagenic effects : No known significant effects or critical hazards.

Reproduction toxicity : No known significant effects or critical hazards.

Section 12. Ecological information

Aquatic ecotoxicity

Not available.

Environmental fate : Not available.

Environmental hazards : No known significant effects or critical hazards.

Toxicity to the environment : Not available.

Section 13. Disposal considerations

Product removed from the cylinder must be disposed of in accordance with appropriate Federal, State, local regulation.Return cylinders with residual product to Airgas, Inc.Do not dispose of locally.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Class	Packing group	Label	Additional information
DOT Classification	UN1066 UN1977	NITROGEN, COMPRESSED Nitrogen, refrigerated liquid	2.2	Not applicable (gas).		**Limited quantity** Yes. **Packaging instruction** **Passenger aircraft** Quantity limitation: 75 kg **Cargo aircraft** Quantity limitation: 150 kg
TDG Classification	UN1066 UN1977	NITROGEN, COMPRESSED Nitrogen, refrigerated liquid	2.2	Not applicable (gas).		**Explosive Limit and Limited Quantity Index** 0.125 **Passenger Carrying Road or Rail Index** 75
Mexico Classification	UN1066 UN1977	NITROGEN, COMPRESSED Nitrogen, refrigerated liquid	2.2	Not applicable (gas).		-

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Section 15. Regulatory information

United States

U.S. Federal regulations :

TSCA 8(a) IUR: Partial exemption
United States inventory (TSCA 8b): This material is listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: Nitrogen
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: Nitrogen: Sudden release of pressure

State regulations :

Connecticut Carcinogen Reporting: This material is not listed.
Connecticut Hazardous Material Survey: This material is not listed.
Florida substances: This material is not listed.
Illinois Chemical Safety Act: This material is not listed.
Illinois Toxic Substances Disclosure to Employee Act: This material is not listed.
Louisiana Reporting: This material is not listed.
Louisiana Spill: This material is not listed.
Massachusetts Spill: This material is not listed.
Massachusetts Substances: This material is listed.
Michigan Critical Material: This material is not listed.
Minnesota Hazardous Substances: This material is not listed.
New Jersey Hazardous Substances: This material is listed.
New Jersey Spill: This material is not listed.
New Jersey Toxic Catastrophe Prevention Act: This material is not listed.
New York Acutely Hazardous Substances: This material is not listed.
New York Toxic Chemical Release Reporting: This material is not listed.
Pennsylvania RTK Hazardous Substances: This material is listed.
Rhode Island Hazardous Substances: This material is not listed.

Canada

WHMIS (Canada) :

Class A: Compressed gas.

CEPA Toxic substances: This material is not listed.
Canadian ARET: This material is not listed.
Canadian NPRI: This material is not listed.
Alberta Designated Substances: This material is not listed.
Ontario Designated Substances: This material is not listed.
Quebec Designated Substances: This material is not listed.

Section 16. Other information

United States

Label requirements :

GAS:
CONTENTS UNDER PRESURE.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Canada

Label requirements :

Class A: Compressed gas.

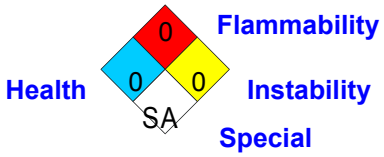
Hazardous Material Information System (U.S.A.) :

Health	0
Flammability	0
Physical hazards	0

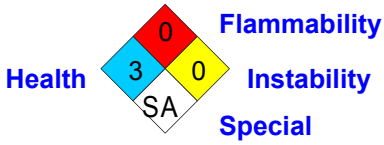
liquid:

Health	3
Fire hazard	0
Reactivity	0
Personal protection	

National Fire Protection Association (U.S.A.) :



liquid:



Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.