Second Addendum to Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico: *Sargassum* Sample Processing Plan for Remotely Operated Underwater Vehicle (ROV) Data, Bongo Net Samples and Neuston Net Samples

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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 — 5/02/2012

NOAA Trustee Representative: — Date

FOR ROLAND GUIDRY — 5/18/2012

Louisiana Trustee Representative: — Date

May 2, 2012

BP Representative: — Date

Background/Justification

This document outlines the protocols for processing biological samples collected under the Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico (Assessment Plan). These procedures are based on 1) well-established methods documented in scientific literature; 2) methods currently approved by NOAA/NMFS for fisheries research (e.g., SEAMAP protocols); or 3) tailored methods developed and recommended by the authors to address the unique nature of *Sargassum* communities. Wherever practical, these methods will complement the approved guidelines for plankton processing and data management adopted by the Water Column Technical Working Group (TWG).

Plan Objectives

- Process neuston net samples for type, abundance and size frequency of fishes and postlarval/juvenile decapods, and bongo net samples for type, abundance and size frequency of ichthyoplankton.
- Create larval/juvenile fish and postlarval/juvenile decapod reference collections based on neuston and bongo net samples.
- Identify encrusting invertebrates (e.g., hydroids, bryozoans, tunicates) on *Sargassum* blades, stems, and vesicles collected via neuston nets.
- Determine species composition, abundance and length frequency of fish observed during ROV video surveys of *Sargassum* patches.

Sargassum Cruises

Sargassum cruises were accomplished using three different vessels under the direction of personnel from Dauphin Island Sea Lab (DISL) and Gulf Coast Research Laboratory (GCRL), including Frank Hernandez, Jim Franks, Bruce Comyns, and Marcus Drymon (Table 1). Sample efforts focused on two pre-defined grids, as outlined in the Assessment Plan: a north-south Mobile grid (off the coast of Mississippi and Alabama) and an east-west Venice grid (off the coast of Louisiana). The Assessment Plan called for sampling three types of *Sargassum* patches within each grid during each cruise: convergent lines, mats and clumps. Sample operations at each targeted patch included 1) a Conductivity, Temperature and Depth (CTD) profile; 2) an ROV video survey; 3) a pelagic longline set; 4) a bongo plankton net tow under or alongside the *Sargassum* canopy; 5) replicate neuston net tows; and 6) collections of biological material for contaminant analysis (from the neuston net and longline). This document addresses protocols for the processing of ROV data, and the bongo and neuston plankton samples (Table 2).

Table 1. Summary of the 2011 NRDA *Sargassum* cruises

Sargassum Cruise	Vessel	Dates	Lead Institution	Chief Scientist
1	Wes Bordelon	May 11-18	DISL	F. Hernandez
2	Wes Bordelon	June 7 - 14	DISL	M. Drymon
3	Tommy Munro	July 18 – 26	GCRL	J. Franks
4	Tommy Munro	Aug 3 – 11	GCRL	J. Franks
5	Dry Tortugas	Aug 16-23	DISL	B. Comyns
6	Dry Tortugas	Aug 28 - Sep 4	DISL	B. Comyns
7	Dry Tortugas	Sep 11-18	DISL	F. Hernandez

Table 2. 2011 NRDA *Sargassum* cruise samples to be processed under this Plan.

Cruise Number	***Cruise Name***	***Neuston Net Samples***	***Bongo Net Samples***	***ROV Videos***
1	Wes Bordelon Cruise 8 – Leg 1 (5/11 – 5/18)	**12**	**12**	**18**
2	Wes Bordelon Cruise 8 – Leg 2 (6/7 – 6/14)	**12**	**12**	**18**
3	Tommy Munroe Cruise 1 – Leg 1 (7/18 – 7/26)	**10**	**10**	**22**
4	Tommy Munroe Cruise 1 – Leg 2 (8/3 – 8/11)	**12**	**12**	**15**
5	Dry Tortugas Cruise 1 – Leg 1 (8/16– 8/23)	**12**	**12**	**18**
6	Dry Tortugas Cruise 1 – Leg 2 (8/28– 8/31)	**6**	**6**	**9**
7	Dry Tortugas Cruise 1 – Leg 3 (9/11– 9/18)	**10**	**8**	**15**

Project Personnel and Management

Samples collected during the cruises 1, 2, and 5 will be processed by DISL under the direction of Frank Hernandez. Samples collected during cruise 3, 4, 6 and 7 will be processed by GCRL under the direction of Jim Franks. The principal investigators have submitted estimated budgets based on the sample processing as described in the methods that follow. Each principal investigator will also have the authority for hiring and training project personnel.

The data products resulting from processing of samples from the NRDA *Sargassum* cruises will include data reports with summaries and all electronic and paper forms of data generated as part of the sample processing plan. Products will not include any analysis, evaluation, or interpretation of project data. Data products include biomass measurements, taxon identification, counts, and length measurements for each component of the samples.

Laboratory Processing

Bongo net and neuston net samples - ichthyoplankton

Bongo and neuston net samples will be processed (sorting and identification) for ichthyoplankton based on protocols used by the Plankton Sorting and Identification Center in Szczecin, Poland (Appendix I). These procedures have been modified to reflect specific gear and methods pertinent to the *Sargassum* Assessment study; for example, provisions in the SEAMAP protocols dealing with gears, such as the MOCNESS, have been removed since those gears were not used in this Assessment Plan.

All specimens from the sample collections sorted and identified will be preserved (long-term) in 85% ethanol. This keeps the specimens in a condition suitable for further study using other analytical methods (e.g., otolith-ageing, molecular identification). If time allows, clearing and staining (C&S) techniques may be used to assist with identifications. Notes on morphology, meristics, pigmentation and other larval characteristics will be taken, and/or silhouette image(s) (e.g., TIFF or JPG file formats) of the specimen will be made to document original characteristics prior to initiation of the C&S process.

Each laboratory (GCRL and DISL) will build and maintain larval/juvenile fish reference collections using specimens from the project samples that are processed. Representative specimens from each taxon will be imaged using a digital camera mounted onto a microscope or the ZooScan Plankton Identifier. Voucher specimens and their associated digital image(s) will be linked through a Reference Collection Number (to be determined by the PIs).

Ichthyoplankton identification information, including scientific name, sample counts, and length measurements, will be recorded on a standardized sample identification sheet (Attachment I, part I through VII), which is based on the sheet currently used by the NOAA Southeast Fisheries Science Center (SEFSC). Number of specimens for each taxon will be recorded, but only those identifiable to family-level or below will be measured, not those in the Unidentified fish category. Field collection and laboratory identification information will subsequently be entered into an electronic database that will facilitate calculation of densities, length measurements, depth strata, location, and related information for each taxon, as requested.

Bongo net and neuston net samples - decapods

Neuston net samples will be processed (sorting and identification) for postlarval and juvenile decapods based on the SEAMAP protocols used by the Polish Sorting and Identification Center (Szczecin, Poland). Decapods from bongo net samples will be sorted during the ichthyoplankton

processing (no identification beyond Order level) and then archived for further analysis, if needed. The decapod protocols (Appendix II) have been revised to reflect the specific gear and methods of the *Sargassum* Assessment study; for example, protocols for gears such as the MOCNESS have been removed. All specimens are to be preserved (long-term) in 85% ethanol. This keeps the specimens in a condition suitable for further study.

Each laboratory (GCRL and DISL) will build and maintain a decapod reference collection based on the samples they are processing. Representative specimens for each taxon will be imaged using a digital camera mounted onto a microscope or a ZooScan. Voucher specimens and their associated digital image(s) will be linked through a Reference Collection Number (to be determined by the PIs).

Decapod identification information, including scientific name and sample counts, will be recorded on two standardized sample identification sheets (Attachment I, part VIII), which are based on the sheets currently used by the SEFSC. Field collection and laboratory identification information will subsequently be entered into an electronic database that will facilitate calculation of densities, length measurements, depth strata, location, and related information for each taxon, as requested.

Neuston net samples - encrusting invertebrates

Sargassum provides attachment substrate for a suite of encrusting invertebrates. These sessile organisms in turn provide food resources and additional refuge for associated decapods and fish (larvae and juveniles). Because they are sessile, the fate of the encrusting invertebrate community is shared with that of the *Sargassum* to which it is attached. The assessment of the encrusting invertebrate community is straightforward and at a relatively coarse taxonomic level, and therefore will not slow down the overall processing of samples.

Sargassum removed from neuston samples will be examined for diversity and abundance of encrusting invertebrates (e.g., hydroids, bryozoans, tunicates). *Sargassum* is composed of three structural components: stems, blades and vesicles (bladders). Since the properties of each of these components differ (e.g., surface area), up to 20 replicate samples from each component (stems, blades, and vesicle) from each neuston net will be examined for encrusting invertebrates.

Encrusting invertebrates will be identified to the lowest possible taxonomic level, which for these organisms will likely be class (e.g., Hydrozoa) (Appendix III). A data sheet will be filled out for each sample with the appropriate header data (as in Attachment I, part IX, for example), and spaces to record counts for the different taxa encountered. Once examined for organisms, individual bladders, stems and blades will be kept separately in small vials with 85% ethanol.

ROV video

Each 10-minute ROV video survey/transect will be "read" for species composition, abundance and length frequency (Appendix IV). For each sample, the viewer will make note of the screen time stamp when a species is most abundant. The count at the screen time where the highest

number of a particular fish is observed is noted as the MIN count, following Gledhill et al. 2004. This is the minimum number of fish present in the sample, and is the most conservative estimate possible because it ensures that fish are not counted twice. The mean MIN count (SE) of the three replicate videos will be calculated for each sampled *Sargassum* patch (mat, clump, or line). A pair of Digi-Key lasers mounted in parallel (3 cm distance) will be used to measure the total length of fish that are illuminated, following Caimi and Tusting 1987. The species composition, fish length frequency, and species abundance will be recorded (Attachment 1, parts X through XII).

Quality Assurance and Control Procedures (QA/QC)

Each bongo net sample will be halved twice, using a plankton splitter, to yield four equal subsamples (25%) by volume, each of which will be processed for larval fishes (sorting and identification) and decapods (sorting only). One of the 25% subsamples will be randomly selected and reprocessed. If there is greater than a 5% error in the number of larval fishes OR in the number of decapods in the reprocessed subsample, the three remaining subsamples will be reprocessed for both fishes and decapods as described in detail in the SOPs attached to this work plan.

Each neuston net sample will be sorted in its entirety for fishes and postlarval/juvenile decapods. It is not practical to quantitatively split these samples due to their large volumes (often multiple gallon-size jars) and high concentrations of non-plankton material (e.g., *Sargassum*, other flotsam and debris). The numbers of fish and decapods will be noted for each jar that comprises a sample, and then summed to determine an estimate for the total sample. After the initial sorting, one of the jars will be randomly selected and reprocessed. If there is greater than a 5% error in the number of fishes OR decapods, the remaining jars that comprise the sample will be reprocessed for both fishes and decapods as described in detail in the SOPs attached to this work plan.

The taxonomic identifications will also be reviewed by a senior taxonomist as part of this quality control process. If the identity of a taxon needs to be corrected during quality control, the corrected scientific name and specimen length will be recorded on an 'ID correction' sheet along with justification(s) for the correction (meristics, spination, pigmentation, etc.). If a consensus cannot be reached among taxonomists, the specimen will be assigned to the lowest agreed upon taxonomic level. Specimens re-assigned to a different taxon during quality control will be placed in a properly labeled vial with some identifier (i.e., 'dot label' or 'X' made with wax pencil) on the vial cap to facilitate locating the vial for specimen re-examination, if necessary. A reference collection of images (and/or specimens) of rare and poorly known taxa will be maintained along with collection information, and shared among researchers to help ensure uniformity in recognizing identification criteria, and to facilitate communication among taxonomists.

For ROV video processing, fish of questionable identity will be reviewed by a separate technician. If agreement cannot be reached on a given taxon, the specimen will be assigned to the lowest agreed upon taxonomic level.

Sample Handling and Chain of Custody (COC) Procedures

Samples are examined upon arriving at the DISL or GCRL. The label on the lid and the internal label are checked against the sample ID on the COC form and any differences will be noted and initialed by the person making the change on the COC form. Once custody has been transferred and the transference documented on appropriate COC forms, all plankton (neuston and bongo net) samples will be stored in locked cabinets or rooms at the destination labs. Key access to the room with the samples will be limited to Frank Hernandez at DISL and Jim Franks at GCRL, or a designated individual. While not being processed or prepped for processing, samples will remained secured in the locked cabinets or rooms, at room temperature. All sample tracking and COC forms will be maintained at DISL and GCRL.

At DISL and GCRL, as samples are removed from the storage bins for processing, the individual removing the samples signs a sample tracking form indicating when the sample(s) were removed, for what purpose, and where they will be located in the lab. While samples are being processed in the labs, access to the work areas will be restricted to authorized personnel. The samples will then be returned to the original storage bin in the lockable cabinet or storage room to which access is limited to Frank Hernandez, Jim Franks, or a designated individual.

Data Handling and Sharing

All field and laboratory data will be collected, managed and stored in accordance with the written SOPs attached to this work plan and following strict chain of custody procedures. The appropriate training on particular equipment or in the conduct of specific field studies for all personnel involved with the project shall be documented and those records shall be kept on file for the duration of this project.

Each individual laboratory (DISL and GCRL) will coordinate with the NOAA NRDA Data Management Team to be registered for the NOAA NRDA Content Management System [redacted] The NOAA NRDA Content Management System will serve as a repository of information required by NOAA from each lab that is receiving and processing samples. Upon registration, labs will receive training on the NOAA NRDA Content Management System and more detailed instructions on the requirements for each of the categories of documentation, including “Confirmation”, “Metadata”, and “Results.”

The NOAA NRDA Data Management Team will coordinate with each lab to determine the appropriate format and required content for each type of data/results. As sample analyses, processing, and lab QA/QC processes are completed, labs will upload results, additional metadata and quality control information, and other information determined to be appropriate to

support these data (i.e., sample tracking forms, lab data sheets, COC forms, and laboratory logs). Once registered, a designated lab contact will log into the system at periodic intervals (determined by the lab and NOAA NRDA Data Management Team) to upload the required information to the system.

Upon receipt and inventory of samples, each laboratory shall deliver an inventory and status review of all samples, including all necessary metadata and splitting or compositing information, generated as part of this sample processing plan to the NOAA NRDA Data Management Team via the NOAA NRDA Content Management System [redacted] There will be limited accessibility (limited to only lab and NOAA NRDA Data Management Team members) to the data until the full internal data review process has been completed in stepwise intervals, as described in this plan. Data will be identified as to its level of review in the noaanrda.org system and updated (monthly) as the review process proceeds.

The laboratory QA/QC processes detailed in the attached SOPs will be completed prior to data release to all parties via noaanrda.org. Throughout the process, any changes made to taxonomic or other information will be documented on bench sheets or forms for recording this information. Imagery will be retained, along with any changes in processing software or results. All of this information will be maintained during all review steps in the process and stored in secure locations under Trustee control and will be provided to all parties as part of the data release process.

In addition to the QA/QC reviews described in the SOPs, the labs will perform a 100% transcription verification of the data. A final level of review will be the marrying of the data provided by the labs with the corresponding field information after the transcription verification by the lab providing the data.. The NOAA NRDA Data Management Team will perform a completeness check to ascertain that the laboratory information matches up properly with field sample information and all field information has associated laboratory information.

Once processing and QA/QC of samples is completed, the data and supporting information referenced in this section will be made available to the parties to this agreement either on noaanrda.org or via other means [e.g. portable hard drives, etc.] as determined by the NOAA NRDA Data Management Team. NOAA and the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and BP (or Cardno ENTRIX on behalf of BP) will be alerted when these data become available for download on noaanrda.org.

In the interest of maintaining one consistent data set for use by all parties, only the QA/QC'd data set made available on noaanrda.org by the NOAA NRDA Data Management Team shall be considered the consensus data set. In order to ensure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 14 days after such data have been made available to the parties. The parties agree to review this requirement under the Adaptive Management provisions below and consider adjustment to this restriction period as implementation proceeds. Any questions raised on the consensus data set as it was made

available to the parties on noaanrda.org shall be handled generally consistent with the procedures in Section 7.2 of the Deepwater Horizon NRDA Analytical Quality Assurance Plan.

- The trustees and BP shall each designate an individual responsible for raising questions, if any, on the consensus data set.
- If questions are raised, the two designated individuals will meet to determine the source of the difference and resolve.
- The questions raised and their resolution shall be distributed to all parties.
- No changes to the consensus data set will be made if the differences are considered immaterial by both designated individuals, acting on behalf of the parties.
- If the parties agree that changes to the dataset should be made, the dataset will be updated in accordance with the resolution and reposted with a notation that the dataset has been revised.
- If the designated individuals do not agree on how to resolve the difference concerning the consensus data set, the designated individuals shall request assistance from the Assessment Managers for the trustees and BP.

Adaptive Management

The parties agree to initiate sample processing as described in this work plan. To address any uncertainty in how the processing will proceed until the labs begin to implement the work plan and to learn in more detail what is involved for each of the various elements, the parties further agree that after the first subgroup of data are available to all parties, the process may be reviewed, discussed, and modified as appropriate with the agreement of all parties.

Work will continue according to this sample processing plan unless and until modified as agreed in writing by the parties.

Progress Reporting Schedule

Progress reports will be submitted quarterly to the co-Trustees and BP by each lab and will include two major sections, one describing the status of sample processing in the laboratory and one describing the data uploading progress for the previous three-month reporting period. To the extent practicable, a standardized format will be used for all lab progress reports (Attachment II). At a minimum, the laboratory operations section of the progress report should include the number of samples analyzed, general location of samples analyzed by station ID, gear and cruise information associated with the samples analyzed, operational/logistical issues, and planned activities for next three months. The data uploading section of the progress report should include, at a minimum, when and what data were uploaded to [redacted] when confirmation, metadata, and results were completed, any operational/logistical issues, and planned activities for next three months. The actual results of the processing effort will not be summarized in the quarterly progress reports, but rather the status of the processing and data uploading effort. In addition to the quarterly progress reports compiled by each lab, the NOAA NRDA Data

Management Team will prepare and submit status reports after each batch of samples has been identified for release and report the status of data uploading to noaanrda.org.

The estimated completion date for the analysis of all samples is one year from the receipt of samples.

Sample Retention

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, including any remains of samples and remains of extracts created during or remaining after analytical testing, must be preserved and disposed of in accordance with the preservation and disposal requirements set forth in Pretrial Orders ("PTOs") # 1, # 30, #35, # 37, #39 and #43 and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Destructive analytical testing of oil, dispersant or sediment samples may only be conducted in accordance with PTO # 37, paragraph 11, and PTO # 39, paragraph 11. Circumstances and procedures governing preservation and disposal of sample materials by the trustees must be set forth in a written protocol that is approved by the state or federal agency whose employees or contractors are in possession or control of such materials and must comply with the provisions of PTOs # 1, # 30, # 35, 37, #39 and #43.

Estimated Budget for *Sargassum* Sample Processing Plan

Labor Costs	**Cost**
GCRL Labor (Jim Franks, Read Hendon, Technicians)	
DISL Labor (Postdoctoral Researcher, Lab Manager / Senior Taxonomist, Research Assistant and Research Technologist)	
Consultant Labor (Frank Hernandez, Bruce Comyns)	
Subtotal Labor	$685,792
Gulf Coast Research Laboratory (GRCL) Direct Costs	
Dissecting microscopes (Nikon SMZ-1000) 3 for 12 months @ $250/mo each – Rental	$9,000
Ethanol, carboys, sample jars, vials, ROV video processing materials, sorting trays, forceps, scales, measuring boards, identification/reference materials, miscellaneous laboratory supplies	$10,500
Subtotal Direct Costs	$19,500
Indirect charge ( )	[redacted]

Total Direct and Indirect for GCRL	[redacted]
Dauphin Island Sea Lab (DISL) Direct Costs	
Glass jars and vials for archival storage of sorted samples and identified fishes and inverts, sorting supplies:	$3,500
Microscope rental (4 dissecting microscopes @ $250/mo. * 6 mo.)	$6,000
Subtotal DISL Direct	$9,500
NON-HOURLY INDIRECT COST [redacted] on Other Direct Costs)	[redacted]
Total Direct and Indirect for DISL	[redacted]
Total Plan Budget	$727,906

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.

Durable Equipment **-** All durable equipment purchased by BP for this study will be returned to BP or their designated representatives at the conclusion of its use for this study.

References

Caimi, F.M. and RF. Tusting. 1987. Laser aided quantitative sampling of the sea bed. Proceedings of Oceans 87'. Mar. Tech. Soc. Washington, D.C.

Gledhill, C.T., G. Ingram, Jr., K.R. Rademacher, P. Felts and B. Trigg. 2004. NOAA Fisheries Reef Fish Video Surveys: Yearly Indices of Abundance for gag (*Mycteroperca microlepis*). SEDAR10-DW-12.

Appendix I: SOP for Ichthyoplankton Sampling Sorting and Identification

The protocols below are based on the SEAMAP ichthyoplankton protocols, Southeast Fisheries Science Center (J. Lyczkowski-Shultz, April 2010). They have been modified as needed to be compatible with the gear used during implementation of the Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico.

Ichthyoplankton Sample Sorting and Identification Protocols

I. Procedures for processing *Sargassum* plankton samples.

A. BONGO SAMPLES

1. Displacement Volume

a) Pour the entire plankton sample into a large sorting tray and remove all nonplankton and other extraneous material (e.g., *Sargassum*, debris) from the sample. Place all nonplankton and other extraneous material removed from the sample into a separate vial or jar with 85% ethanol and label with the sample number.

b) Also remove all large fish and invertebrates >2.5 cm. Set these organisms aside in a temporary container in 85% ethanol.

c) Sieve the plankton sample (0.202 mm mesh) to remove excess liquid and pour the sample into a 500-ml graduated cylinder, using 85% ethanol for rinsing the sieve.

d) Add or subtract enough ethanol (85%) to bring the level of the liquid to an even millimeter increment. Record this Initial Volume on the Bongo Net Sample Sorting Log.

e) Place a funnel in another clean 500-ml graduated cylinder. A draining cone of 0.202 mm mesh is placed in the funnel. Pour the plankton and ethanol into the draining cone. If plankton remains in the original cylinder, it can be rinsed with a known volume of ethanol (record this Rinse Volume on the Bongo Net Sample Sorting Log).

f) The plankton is retained in the cone while the liquid drains into the cylinder. The plankton is considered drained when the interval between drops from the bottom of the cone increases to 15 seconds. Draining times vary with the size and composition of the sample. Record Drained Volume on the Bongo Net Sample Sorting Log.

g) Calculate the Plankton Volume using the following formula:

Plankton Volume = (Initial Volume + Rinse Volume) -Drained Volume

Record the Plankton Volume on the Bongo Net Sample Sorting Log.

h) Return the plankton (along with the >2.5cm organisms) to the sample jar with preservative in preparation for sorting.

2. Sample Splitting

a) Place a Folsom Plankton Splitter on a level surface and level the base of the splitter using the adjusting screws and level indicator.

b) Pour the plankton from the sample jar into the splitter console. Rinse the sample jar with preservative to remove all plankton.

c) Homogenize the sample by rocking the splitter drum 10 times. Then rotate the drum towards the two compartments divided by the cutting edge. Pour the two subsamples into the separate holding trays by rotating the drum. If any plankton remains in the splitter, rinse with preservative and repeat the process (rocking the splitter 10 times each time), until all plankton is split into the two holding trays.

d) Repeat this process (steps a-c above) for each of these two subsamples to yield four equal subsamples (25%) by volume. Place each subsample in a separate jar and label each with the sample number, the aliquot (25%) and a subsample number (e.g., 1 of 4, 2 of 4, etc.)

e) Check the performance of the plankton splitter by allowing the four sample splits to settle undisturbed for twenty minutes on a level surface then compare the amount of sample in each jar to ascertain if the amount of sample in each jar is approximately equal. If the amount of sample in each jar is not equal, reposition and/or adjust the splitter, recombine the splits and aliquot the sample again.

3. Ichthyoplankton and Decapod Sorting

a) Pour a small amount of a subsample into a gridded sorting dish.

b) Using fine (insect) forceps and delicate probes, sort through the plankton in the dish systematically (i.e., along the gridded sections) under a dissecting microscope and remove all fish larvae and decapods from the

dish, enumerating them as they are placed in separate vials. Count, but do not remove, fish eggs during the sample sorting. Continue until the entire dish is sorted.

c) Pour the remaining material from the dish into a temporary container (e.g., another jar) and repeat the steps above until the entire subsample is sorted.

d) When done, pour the remaining subsample (minus removed fishes and decapods) from the temporary holding container back into the original subsample jar. Place a mark on the subsample jar lid indicating that it has been sorted.

e) On the Bongo Net Sample Sorting Log, record the number of eggs counted and the number of fish and decapods removed from the subsample. Also record the date the subsample was sorted and the name of the sorter.

f) Repeat the steps above (a-e) for each of the four subsamples unless the subsamples contain excessively large numbers of fish larvae.

g) If excessively large numbers of fish larvae are present, sort enough subsamples sufficient to yield 1,000 fish larvae. Remember, all fish larvae and decapods must be removed from each subsample that is sorted.

4. Sorting QA/QC

a) Once an entire sample is sorted (i.e., all four subsamples), randomly select one of the subsamples to be sorted again by a different technician using the protocols detailed in the previous section.

b) Any fish larvae or decapods found in the second sort are to be counted, removed and added to their respective vials for the same subsample. Record the number of fish and decapods found in the QA/QC resort on the Bongo Net Sample QA/QC Log (located on the back of the Bongo Net Sample Sorting Log). Also record the date the subsample was sorted for the second time and the name of the second sorter.

c) If there is greater than a 5% addition to the number of larval fishes OR the number of decapods found during the second sorting of the random subsample, then each of the three remaining subsamples will be reprocessed for both fish and decapods using the protocols detailed in the previous section. If there is less than 5% increase in both the larval fish AND decapod counts found during the second sort, the remaining

subsamples are not to be sorted again (note this on the Bongo Net Sample Sorting Log).

d) Record a Revised Fish Subtotal, i.e., the combined number from the Fish Subtotal and QA/QC Fish Count for the subsample(s). Record a Revised Decapod Subtotal, i.e., the combined number from the Decapod Subtotal and QA/QC Decapod Count for the subsample(s). At the bottom of the Bongo Net Sample Sorting Log, record the Total Egg Count, Total Fish Count and Total Decapod Count for the entire sample (sum of each value for all four subsamples).

B. NEUSTON SAMPLES

1. Displacement Volume

a) Because the neuston nets directly targeted *Sargassum*, these samples (which are often preserved in multiple containers) will likely contain high concentrations of nonplankton material. Therefore it is not practical to remove all of the *Sargassum* (and other extraneous material) from the samples. As such, no displacement volume measures will be made for neuston samples (which is in line with SEAMAP protocols).

2. Sample Splitting

a) Because of the anticipated high concentrations of nonplankton material, it is not practical to split the neuston net samples using traditional means (e.g., Folsom plankton splitter). A single neuston sample will likely be separated into multiple jars, and the number of jars per sample will be recorded during processing, but these samples will not be split quantitatively by volume.

3. *Sargassum*, Decapod and Fish Sorting

a) Record the number of jars that comprise a single sample on the Neuston Net Sample Sorting Log. Assign each of the jars a number, e.g., 1 of 2, 2 of 2, etc.

b) Pour contents from one of the sample jars into a large sorting tray. If there are multiple jars per sample, the jars may be sorted one at a time (note this on the Neuston Net Sample Sorting Log).

c) Remove and rinse (with ethanol) a representative number of *Sargassum* algae, making sure to include all components (blades, stems, bladders). At least 20 of each component should be removed for later processing of

encrusting invertebrates, but collecting as much as possible within a reasonable amount of time is preferred. Place the *Sargassum* into a separate jar (85% ethanol) labeled with the sample number for later processing.

d) Sort the remaining portion of the sample (with fine forceps or probes) for fishes and decapods, removing and placing each in separate vials or jars. Record the number of fishes and decapods removed from the sample, as well as the name of the sorter and the date(s) the sample was sorted on the Neuston Net Sample Sorting Log.

e) When done, pour the remaining sample (minus removed *Sargassum* fishes, and decapods) back into the original sample jar. Place a mark on the sample jar lid indicating that it has been sorted.

f) Repeat steps b-e for the remaining jars that comprise this sample.

4. Sorting QA/QC

a) Once an entire sample is sorted (i.e., all jars from the same sample), randomly select one of the jars to be sorted again by a different technician using the protocols detailed in the previous section.

b) Any fish and decapods found in the second sort are to be counted, removed and added to the existing fish vial for the same sample jar. Record the number of fish and decapods found in the QA/QC sort on the Neuston Net Sample QA/QC Log (located on the back of the Neuston Net Sample Sorting Log). Also record the date the subsample was sorted for the second time and the name of the second sorter.

c) If there is greater than a 5% addition to the number of fish OR the number of decapods found during the second sorting of the random jar, then each of the remaining jars that comprise that sample will be reprocessed for both fish and decapods using the protocols detailed in the previous section. If there is less than 5% increase in both the fish AND decapod counts found during the second sort, the remaining sample jars are not to be sorted again (note this on the Neuston Net Sample Sorting Log).

d) Record a Revised Fish Subtotal, i.e., the combined number from the Fish Subtotal and QA/QC Fish Count for the jar(s). Record a Revised Decapod Subtotal, i.e., the combined number from the Decapod Subtotal and QA/QC Decapod Count for the jar(s). At the bottom of the Neuston Net Sample Sorting Log, record the Total Fish Count and

Total Decapod Count for the entire sample (sum of each value for all jars that comprise this sample).

II. Identification and measurement of ichthyoplankton and juvenile fishes.

A. Identification

1. Identification protocols are for both bongo (fish only) and neuston (fish and decapod) samples.

2. Please follow the classification and higher level fish names listed in Table 1 of this SOP.

3. Identify specimens of the following families commonly associated with *Sargassum* to the lowest possible taxon (i.e. genus and species if possible): ANTENNARIIDAE, MUGILIDAE, SYNGNATHIDAE, CORYPHAENIDAE, CARANGIDAE, LOBOTIDAE, MULLIDAE, KYPHOSIDAE, POMACENTRIDAE, SPHYRAENIDAE, SCOMBRIDAE, ISTIOPHORIDAE, BALISTIDAE, MONACANTHIDAE, BELONIDAE, EXOCOETIDAE, HEMIRAMPHIDAE.

4. Identify fish of all other families to the genus or species level only when such identification can be made easily with little additional time (in particular, those of fisheries importance, such as LUTJANIDAE, SERRANIDAE). Otherwise identify to the family level only.

5. Those specimens which cannot be taken to at least the Order level, but which are in identifiable condition, will be classified as 'Unidentified'. It is permissible to use multiple categories of unidentified larvae such as 'Unidentified I' or 'Unidentified II' to denote larvae that are morphologically similar to each other. Head and tail sections that cannot be identified by the taxonomist but are in good condition are placed in vials labeled 'Unidentified'.

6. Larvae in such a poor state that they are impossible to identify are to be labeled 'Disintegrated'. Head and tail sections that are too damaged to be identified are also to be placed in vials labeled 'Disintegrated'.

7. All fish regardless of size or stage of development are to be removed from samples, identified, counted, and placed in labeled specimen vials or jars that match the size of the specimens. However, when only a few specimens of the taxon would require a larger vial or jar, place all the specimens in the larger jar. Do not break spines off specimens or force large specimens into small vials. Use larger jars for these specimens (especially the BALISTIDAE).

8. Record all identifications on the Bongo Net Larval Fish Identification Sheet or the Neuston Net Larval Fish Identification Sheet, along with the Sample Number, the taxonomist's name and the date the fishes were identified.

9. The total number of vials and jars in which specimens of each taxon has been placed must be recorded in the "Number of Vials" column on the Bongo Net Larval Fish Identification Sheet or the Neuston Net Larval Fish Identification Sheet. The label inside a vial or jar containing specimens should specify which vial or jar of the total number of vials or jars it is, for example: 1 of 2, or 2 of 2, etc. This information can be placed on the back of the label.

B. Specimen Counts

1. Specimen count protocols are for both bongo and neuston samples.

2. COUNT the number of specimens of ALL taxa including 'Unidentified', 'Disintegrated', Clupeiformes, Perciformes, Percoidei etc. and record the count for the subsample or sample sorted on the data sheet and inside vial label.

3. When samples contain many parts and pieces of larvae count only the 'heads'. Head and tail sections are sometimes present and may possibly be identified. Head sections that can be identified should be included as a number in the total count for whole specimens; if tail sections are recognizable to species, include them in the appropriate species vial, but counting tails is not necessary.

4. Record all counts on the Bongo Net Larval Fish Identification Sheet or the Neuston Net Larval Fish Identification Sheet for this sample.

C. Measurement

1. Specimen measurement protocols are for both bongo and neuston samples.

2. Measure up to 10 larvae identified to species for <u>all taxa</u> except Order level taxa, Unidentified, and Disintegrated. Randomly choose the larvae from the available pool, but include the largest and smallest specimen.

3. Measure up to 30 specimens of all taxa of carangid, scombrid, coryphaenid, lobotid, kyphosid, balistid and istiophorid larvae (taxa of fisheries importance and common association with *Sargassum*). Randomly choose the larvae from the available pool, but include the largest and smallest specimen.

4. Use the appropriate measurement of body length, i.e. notochord (NL) or standard (SL) length, depending on the stage of development of the specimen.[1]

5. Record all measurements on the Bongo Net Larval Fish Identification Sheet or the Neuston Net Larval Fish Identification Sheet for this sample.

III Ichthyoplankton Identification QA/QC

A. Identification QA/QC

1. Identification QA/QC protocols are for both bongo and neuston samples.

2. Bongo and neuston samples were collected in replicate (n=2). One of the replicates from each pair will be randomly selected for identification QA/QC by a different (and senior) taxonomist (e.g., principle investigator). If any of the fishes are mis-identified, or if there is a discrepancy in identification, specimens from both replicates will be re-examined by the senior taxonomist.

3. During the QA/QC, if the identity of a specimen is in doubt, the senior taxonomist will confer with the taxonomist who made the original identification. If a consensus cannot be reached between taxonomists, the specimen will be assigned to the lowest agreed upon taxonomic level.

4. If any of the fishes are mis-identified, or if there is a discrepancy in identification during the QA/QC of the first replicate, then specimens from both replicates will be re-examined by the senior taxonomist.

5. If the identity of a taxon needs to be corrected during quality control, the corrected scientific name and specimen length will be recorded on an 'ID correction' sheet along with justification(s) for the correction (meristics, spination, pigmentation, etc.). Specimens re-assigned to a different taxon during quality control will be placed in a properly labeled vial with some identifier (i.e., 'dot label' or 'X' made with wax pencil) on the vial cap to facilitate locating the vial for specimen re-examination, if necessary.

6. Corrections in identification will be recorded on the Identification Data Sheet, as well as the internal labels in the specimen vials.

B. Digital Archive and Voucher Specimens

[1] Matarese, A. C., A. W. Kendall, D. M. Blood, and B. M. Vinter. (Oct 1989). *Laboratory Guide to Early Life History Stages of Northeast Pacific Fishes*. U. S. Department of Commerce. NOAA Technical Report NMFS 80. *Available at* http://spo.nwr.noaa.gov/tr80.pdf.

1. Archive and voucher protocols are for both bongo and neuston samples.

2. Representive specimens for each taxon encountered will be removed from samples and placed in vials to serve as a reference or voucher collection. The removal of specimens is to be noted on the Identification Data Sheets under the "Comments" section.

3. Each of these specimens will be photographed using a dissecting microscope and imaging system. Taxonomists at each laboratory should freely and frequently exchange images and questions regarding identifications to ensure consistency between lab groups.

4. File names for images will be assigned sequentially at each institution and include the laboratory name and taxon name, e.g., "DISL_001_C_crysos". The filenames of the images will be recorded on the "Ichthyoplankton Voucher Log", along with the specimen's sample number and size (in mm).

IV Vial or Jar Labeling

A. Internal Label

1. Record the following information on inside vial and jar labels: sample number, taxon, number of specimens, and vial number of total vials used for that taxon (to be placed on back of label).

2. Write identifying sample information on an internal label using an indelible ink, such as India ink, on archival paper that will maintain its stability when wet. Allow ink to dry completely before inserting into the vial.

B. External Label

1. Record the following information on outside vial and jar labels: sample number.

Table 1: This is the classification scheme and higher level names of fishes to be used when identifying larvae.

Elopiformes
- Elopidae
- Megalopidae

Albuliformes
- Albulidae

Anguilliformes
- Synaphobranchidae
- Moringuidae
- Nettastomatidae
- Congridae
- Ophichthidae
- Anguillidae
- Muraenidae
- Derichthyidae
- Serrivomeridae
- Nemichthyidae
- Chlopsidae
- Heterenchelyidae
- Cyematidae

Saccopharyngiformes
- Saccopharyngidae
- Eurypharyngidae
- Monognathidae

Notacanthiformes
- Notacanthidae

Clupeiformes
- Clupeidae
- Engraulidae

Argentiniformes
- Argentinoidei
  - Argentinidae
  - Microstomatidae
    - Bathylaginae
    - Microstomatinae
  - Opisthoproctidae
- Alepocephaloidei
  - Leptochilichthyidae
  - Alepocephalidae
  - Platytroctidae

Stomiiformes
- Gonostomatidae
- Sternoptychidae
- Phosichthyidae
- Stomioidei
  - Chauliodontidae
  - Stomiidae
  - Astronesthidae
  - Melanostomiidae
  - Malacosteidae
  - Idiacanthidae

Ateleopodiformes
- Ateleopodidae

Aulopiformes
- Aulopoidei
  - Aulopidae
  - Synodontidae
- Chlorophthlamoidei
  - Chlorophthalmidae
  - Ipnopidae
  - Notosudidae
- Alepisauroidei
  - Alepisauridae
  - Evermannellidae
  - Paralepididae
  - Scopelarchidae
- Giganturoidei
  - Bathysauridae
  - Giganturidae

Myctophiformes
- Neoscopelidae
- Myctophidae

Gadiformes
- Bregmacerotidae
- Bathygadidae
- Macrouridae
- Moridae
- Melanonidae
- Phycidae
- Merlucciidae
- Steindachneriidae

Ophidiiformes
- Bythitoidei
  - Aphyonidae
  - Bythitidae
- Ophidioidei
  - Ophidiidae
    - Brotulinae
    - Brotulotaeniinae
    - Ophidiinae
    - Neobythitinae
  - Carapidae

Batrachoidiformes
- Batrachoididae

Lophiiformes
- Lophioidei
  - Lophiidae
- Antennarioidei
  - Antennariidae
- Chaunacoidei
  - Chaunacidae
- Ogcocephaloidei
  - Ogcocephalidae
- Ceratioidei
  - Caulophrynidae
  - Centrophrynidae
  - Ceratiidae
  - Diceratiidae
  - Gigantactinidae
  - Himantolophidae
  - Linophrynidae
  - Melanocetidae
  - Neoceratiidae
  - Oneirodidae
  - Thaumatichthyidae

Atheriniformes
- Atherinidae
- Atherinopsidae

Mugiliformes
- Mugilidae

Cyprinodontiformes

Beloniformes
- Scomberesocidae
- Belonidae
- Hemiramphidae
- Exocoetidae

Lampridiformes
- Lamprididae
- Lophotidae
- Radiicephalidae
- Trachipteridae
- Regalecidae
- Stylephoridae

Beryciformes
- Anomalopidae
- Anoplogasteridae
- Berycidae
- Diretmidae
- Holocentridae
  - Holocentrinae
  - Myripristinae
- Trachichthyidae

Stephanoberyciformes
- Stephanoberycidae
- Melamphaidae
- Gibberichthyidae
- Rondeletiidae
- Barbourisidae
- Cetomimidae
- Mirapinnidae
- Megalomycteridae

Polymixiiformes
- Polymixiidae

Zeiformes
- Parazenidae
- Zeidae
- Grammicolepidae
- Oreosomatidae
- Zeniontidae

Syngnathiformes
- Aulostomidae
- Centriscidae
- Fistulariidae
- Syngnathidae
  - Hippocampinae
  - Syngnathinae

Scorpaeniformes
- Scorpaenidae
- Triglidae
- Peristediidae
- Psychrolutidae

Perciformes
- Percoidei
  - Centropomidae
  - Acropomatidae
  - Polyprionidae
  - Howellidae
  - Serranidae
    - Anthiinae
    - Epinephelinae
    - Grammistinae
    - Liopropomatinae
    - Serraninae
  - Symphysanodontidae
  - Grammatidae
  - Priacanthidae
  - Caproidae
  - Apogonidae
  - Epigonidae
  - Malacanthidae
  - Dactylopteridae
  - Scombropidae
  - Pomatomidae
  - Rachycentridae
  - Echeneidae
  - Carangidae
  - Coryphaenidae
  - Bramidae
  - Caristiidae
  - Emmelichthyidae
  - Lutjanidae
    - Apsilinae
    - Etelinae
    - Lutjaninae
  - Lobotidae
  - Gerreidae
  - Haemulidae
  - Inermiidae
  - Sparidae
  - Sciaenidae
  - Polynemidae
  - Mullidae
  - Pempheridae
  - Bathyclupeidae
  - Kyphosidae
  - Ephippidae
  - Chaetodontidae
  - Pomacanthidae
  - Pomacentridae
  - Cichlidae
  - Cirrhitidae
  - Opistognathidae
- Labroidei
  - Labridae
  - Scaridae
- Zoarcoidei
  - Zoarcidae
- Trachinoidei
  - Chiasmodontidae
  - Ammodytidae
  - Uranoscopidae
  - Percophidae
- Blennioidei
  - Tripterygiidae

- Chaenopsidae
- Dactyloscopidae
- Labrisomidae
- Blenniidae
- Gobiesocoidei
  - Gobiesocidae
- Callionymoidei
  - Callionymidae
  - Draconettidae
- Gobioidei
  - Eleotridae
  - Gobiidae
  - Microdesmidae
  - Ptereleotridae
- Acanthuroidei
  - Luvaridae
  - Acanthuridae
- Sphyraenoidei
  - Sphyraenidae
- Scombroidei
  - Scombrolabracidae
  - Gempylidae
  - Trichiuridae
  - Scombridae
- Xiphioidei
  - Istiophoridae
  - Xiphiidae
- Stromateoidei
  - Centrolophidae
  - Nomeidae
  - Ariommidae
  - Tetragonuridae
  - Stromateidae

- Pleuronectiformes
  - Scophthalmidae
  - Paralichthyidae
  - Bothidae
  - Pleuronectidae
  - Poecilopsettidae
  - Achiridae
  - Cynoglossidae

- Tetraodontiformes
  - Triacanthodidae
  - Balistidae
  - Monacanthidae
  - Ostraciidae
  - Tetraodontidae
  - Diodontidae
  - Molidae

Appendix II: SOP for Decapod Sample Processing

The protocols below are based on the SEAMAP zooplankton protocols, Southeast Fisheries Science Center (J. Lyczkowski-Shultz, April 2009). They have been modified as needed to be compatible with the gear used during implementation of the Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico.

Decapod Sample Sorting and Identification Protocols

I. General Instructions:

A. Sorting and Handling

1. Decapods in bongo and neuston samples will be sorted and removed during the sorting of ichthyoplankton (see Appendix I).

2. Decapods are to be sorted from bongo net samples and then archived for future analysis, if warranted. Decapods (list below) from neuston net samples are to be sorted and identified. While sorting decapods from both bongo and neuston samples, the specimens are to be sorted while in ethanol, removed, and placed in individual labeled vials with 85% ethanol from every sample.

II. Decapod Crustacean Protocols

A.. Neuston Net Samples

1. Taxa to be identified from NEUSTON samples:

a) The following decapod taxa are to be sorted from neuston net samples:

1. Lobster phyllosoma (all species)
2. Penaeidae postlarvae and juveniles
3. Portunidae postlarvae and juveniles
4. Sicyoniidae postlarvae
5. *Menippe* postlarvae and juveniles
6. Geryonidae postlarvae and juveniles
7.. Other and Damaged Decapods
8.. *Callinectes similis* (juveniles and adults)
9. *Callinectes sapidus* (juveniles and adults)
10. *Portunus sayi* (juveniles and adults)
11..*Leander tenuicornis* (juveniles and adults)
12. *Latreutes fucorum* (juveniles and adults)

2. Decapod Sorting Procedures for Neuston Net Samples

a) All decapods in the neuston net samples will be enumerated and identified because i) they are generally large and conspicuous; ii), they are found in direct association with *Sargassum*; and iii) there is no accurate way to quantitatively split the neuston samples.

b) The decapods sorted during the ichthyoplankton processing will be identified by an invertebrate taxonomist. Each of these taxa will be placed in a separate vial, individually labeled and sealed. The number of specimens sorted should be recorded on the data sheet together. All specimens are to be placed in labeled vials containing 85% ethanol.

c) The aliquot for neuston samples will be recorded as "1" on the data sheet.

d) The identifications will be verified by a second taxonomist as QA/QC.

Appendix III: SOP for Enumerating and Identifying Encrusting Invertebrates

The processing of encrusting invertebrates is unique to the Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico samples.

Encrusting Invertebrate Sorting and Identification Protocols

I. General Instructions:

A. Source Material for Processing

1. Ample amounts of *Sargassum* components (blades, stems and air bladders) are present in the neuston samples (preserved in ethanol). Once removed from the sample, these components can be examined for encrusting invertebrates to assess their abundance and diversity.

2. Additional *Sargassum* blades, stems and air bladders can be obtained, if needed, from *Sargassum* samples collected (and frozen) for chemical analysis.

II. Encrusting Invertebrate Processing

A. Sample Selection

1. *Sargassum* is composed of three structurally different components (blades, stems and air bladders). Because these components differ in their morphology, there may be differences in the number and types of organisms that attach to each. Therefore, replicates of each component should be examined.

2. *Sargassum* components for this processing are provided by neuston net samples previously sorted for fish processing (see Appendix I).

3. From the available *Sargassum* components pulled from each sample, randomly select 20 of each component (i.e. blades, stems, and air bladders) for examination.

B. Identification of Organisms

1. Taxa to be identified

a) A list of encrusting invertebrates associated with *Sargassum* in the Gulf of Mexico was taken from Turner and Rooker 2006 (Mar. Biol. 149: 1025-1036).

b) Taxa to be identified for encrusting invertebrate processing:

1. *Membraniporum* sp. (bryozoan)

2. *Algaophenia latecarinata* (hydroid cnidarian)
3. *Spirorbis* sp. (serpulid polychaete)
4. Acorn barnacles
5. Gooseneck barnacles
6. Other
7. Unidentified

1. Enumeration and Identification

a) Under a dissecting microscope, examine 20 randomly-selected replicates of each *Sargassum* component. Count and identify the organisms listed in the taxonomic list above. Record all identifications and counts for each replicate of each component on the Encrusting Invertebrate Identification Data Sheet.

b) If identifiable but not listed on the taxon list, count as "Other" and list the taxon on the data sheet. If unknown, count as "Unidentified".

c) After examination, place each replicate of each component in a separate vial with 85% ethanol. Place an internal label in each vial with the sample number and replicate number (e.g., 1 of 20, 2 of 20, etc.).

d) A randomly selected subset (n= 5, or 25%) of each component for each sample will be re-examined by a different taxonomist for QA/QC.

Appendix IV: SOP for Processing Remotely Operated Vehicle (ROV) Video Surveys

The protocols below are based on those used in

> Gledhill, C.T., G. Ingram, Jr., K.R. Rademacher, P. Felts and B. Trigg. 2004. NOAA Fisheries Reef Fish Video Surveys: Yearly Indices of Abundance for gag (*Mycteroperca microlepis*). SEDAR10-DW-12.

They have been modified as needed to be compatible with the gear used during the implementation of the Assessment Plan for *Sargassum* Communities and Associated Fauna in the Northern Gulf of Mexico.

Video Processing Protocols

I. General Background:

A. ROV Video

1. Video surveys were made just beneath the *Sargassum* canopy, between a depth of 0.5 and 3 m.

2. The objectives of the video processing are to identify and quantify fish:

a) Species composition;

b) Length frequency (when possible); and

c) Abundance.

II. Methods for Video Processing

A. Preparation

1. Verify that the ROV video file is labeled with the correct station number. If not, amend as necessary.

2. Open the video file in the video playback software. Options include VLC media player, Sony Vegas Movie Studio HD Platinum (v. 10.0), or compatible player.

3. Watch the entire recorded segment, recording the start and end times. For processing, the start time will begin once the ROV is under the *Sargassum* feature and end no more than 10:00 minutes later. In instances where video was recorded for longer than ten minutes, video after 10:00 will not be processed.

B. Species Composition (first video read)

1. During the initial video viewing, record the time stamp for each new, positively identified species encountered.

2. For example, when the first fish is seen, note the time (**ROV Video Data Sheet A**). This will provide the species composition for the video transect.

C. Fish Lengths (second video read)

1. After the species composition has been recorded, record laser based length measurements during a second viewing.

2. For laser based length measurements to be useful, include only fish that are:

a) Approximately perpendicular to the ROV, and

b) Have two laser points clearly visible on the fish.

3. If the conditions in II.C.2 are met:

a) Record the time of the fish/laser encounter.

b) Measure the distance between the two laser points in millimeters using a pair of dial calipers.

c) Measure the fork length of the fish image using a pair of dial calipers.

d) Multiply the measured fork length of the fish by 30 (the distance in mm between laser points). Divide that quantity by distance between laser measurements. This number is the estimated length of the fish.

e) Repeat for total length.

f) Record all data on **ROV Video Data Sheet B**.

D. Fish Abundance (third video read)

1. Once all fish tagged with lasers have been measured and recorded, estimate relative abundance.

2. To do this, refer to the species composition generated during the first video read (**ROV Video Data Sheet A**). For each species:

a) Forward the video to the time stamp when it was first observed and note the number of individuals present for the species.

b) Continue viewing the video, pausing as needed to make reliable counts, with the goal of obtaining a minimum count (MIN), i.e., the greatest number of individuals for a given taxon that appears on screen at one time.

c) Repeat this method for all three replicate videos and generate a mean MIN count for each species.

d) Record all data on **ROV Video Data Sheet C**.

I. Bongo Net Sample Sorting Log

Bongo Net Sample Sorting Log

Sample Number:	

Subsample #	Displacement Volume (ml)				Egg Subtotal	Fish Subtotal	
	Initial	Rinse	Drained	Plankton			
Sorted By			Date Sorted		QA/QC ? (Y / N)	QA/QC Fish Count	Revised Fish Subtotal

Subsample #	Displacement Volume (ml)				Egg Subtotal	Fish Subtotal	
	Initial	Rinse	Drained	Plankton			
Sorted By			Date Sorted		QA/QC ? (Y / N)	QA/QC Fish Count	Revised Fish Subtotal

Subsample #	Displacement Volume (ml)				Egg Subtotal	Fish Subtotal	
	Initial	Rinse	Drained	Plankton			
Sorted By			Date Sorted		QA/QC ? (Y / N)	QA/QC Fish Count	Revised Fish Subtotal

Subsample #	Displacement Volume (ml)				Egg Subtotal	Fish Subtotal	
	Initial	Rinse	Drained	Plankton			
Sorted By			Date Sorted		QA/QC ? (Y / N)	QA/QC Fish Count	Revised Fish Subtotal

Total Egg Count		Total Fish Count	

II. Bongo Net Sample QA/QC Log

Bongo Net Sample QA/QC Log

QA/QC Subsample #	Resorted By:	Date Resorted:	QA/QC Fish Count	Subsample % Error

If the error rate above is > 5%, the remaining subsamples are resorted. Use fields below. Report all "QA/QC Fish Count" data on this side and opposite side of data sheet, and add to "Fish Subtotal" to get the "Revised Fish Subtotal" for each subsample.

Subsample #	Resorted By:	Date Resorted:	QA/QC Fish Count	Subsample % Error

Subsample #	Resorted By:	Date Resorted:	QA/QC Fish Count	Subsample % Error

Subsample #	Resorted By:	Date Resorted:	QA/QC Fish Count	Subsample % Error

III. Neuston Net Sample Sorting Log

Neuston Net Sample Sorting Log

Sample Number:		No. of Jars	

Jar #	Invertebrate Subtotal	Fish Subtotal	QA/QC ? (Y / N)	QA/QC Invert Count	Revised Invert Subtotal	QA/QC Fish Count	Revised Fish Subtotal
Sorted By				Date Sorted			

Jar #	Invertebrate Subtotal	Fish Subtotal	QA/QC ? (Y / N)	QA/QC Invert Count	Revised Invert Subtotal	QA/QC Fish Count	Revised Fish Subtotal
Sorted By				Date Sorted			

Jar #	Invertebrate Subtotal	Fish Subtotal	QA/QC ? (Y / N)	QA/QC Invert Count	Revised Invert Subtotal	QA/QC Fish Count	Revised Fish Subtotal
Sorted By				Date Sorted			

Jar #	Invertebrate Subtotal	Fish Subtotal	QA/QC ? (Y / N)	QA/QC Invert Count	Revised Invert Subtotal	QA/QC Fish Count	Revised Fish Subtotal
Sorted By				Date Sorted			

Jar #	Invertebrate Subtotal	Fish Subtotal	QA/QC ? (Y / N)	QA/QC Invert Count	Revised Invert Subtotal	QA/QC Fish Count	Revised Fish Subtotal
Sorted By				Date Sorted			

Total Invert Count		Total Fish Count	

Sheet _____ of _____

IV. Neuston Net Sample QA/QC Log

Neuston Net Sample QA/QC Log

QA/QC Jar #	Resorted By:	Date Resorted:	QA/QC Invert Count	% Error	QA/QC Fish Count	% Error

If the error rate above is > 5% for invertebrates or fishes, the remaining jars are resorted. Use fields below. Report all "QA/QC Fish Count" and "QA/QC Invert Count" data on this side and opposite side of data sheet, and add to "Fish Subtotal" and "Invertebrate Subtotal" to get the "Revised Fish Subtotal" and "Revised Invertebrate Subtotal", respectively, for each jar.

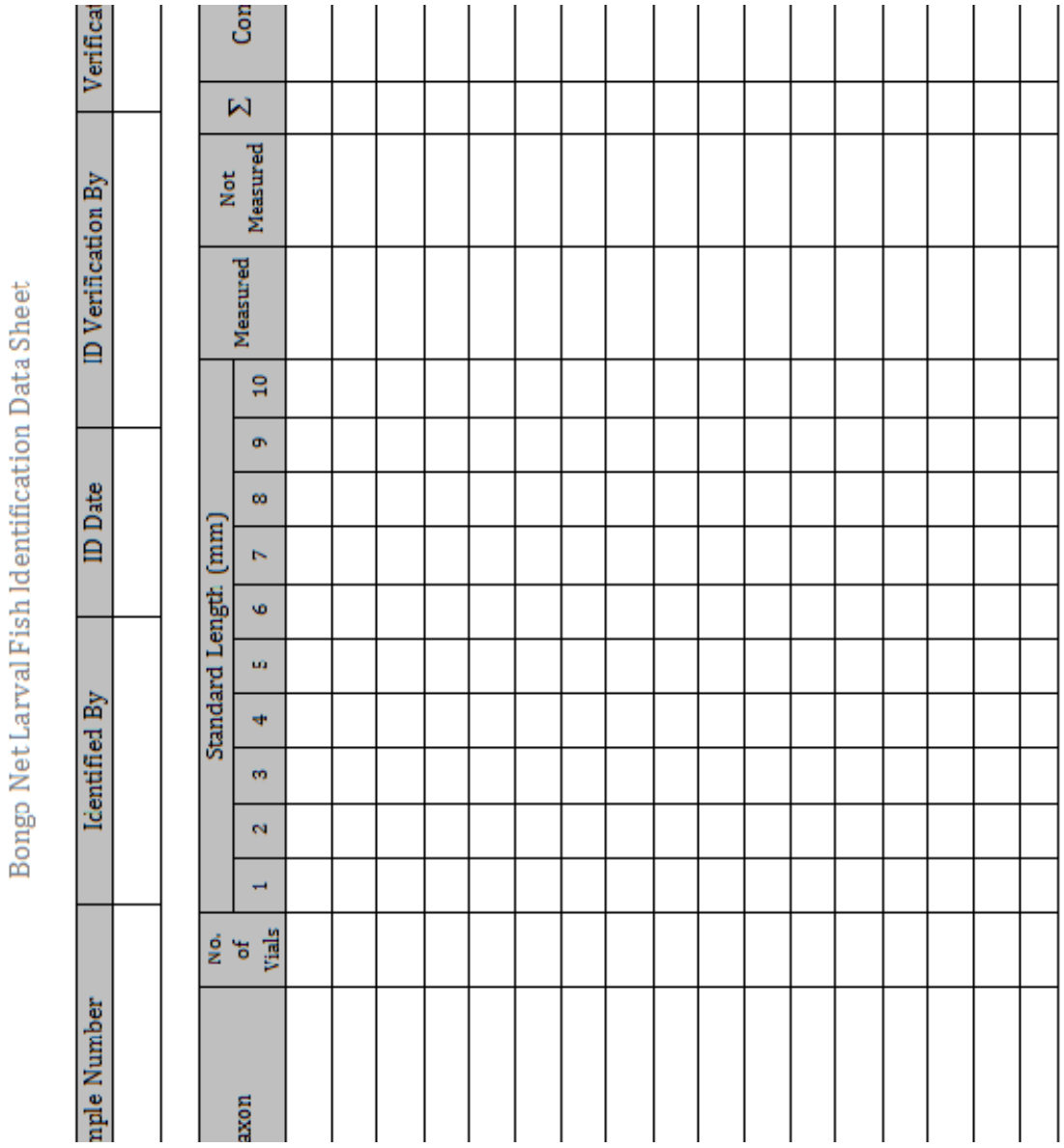
Jar #	Resorted By:	Date Resorted:	QA/QC Invert Count	% Error	QA/QC Fish Count	% Error

Jar #	Resorted By:	Date Resorted:	QA/QC Invert Count	% Error	QA/QC Fish Count	% Error

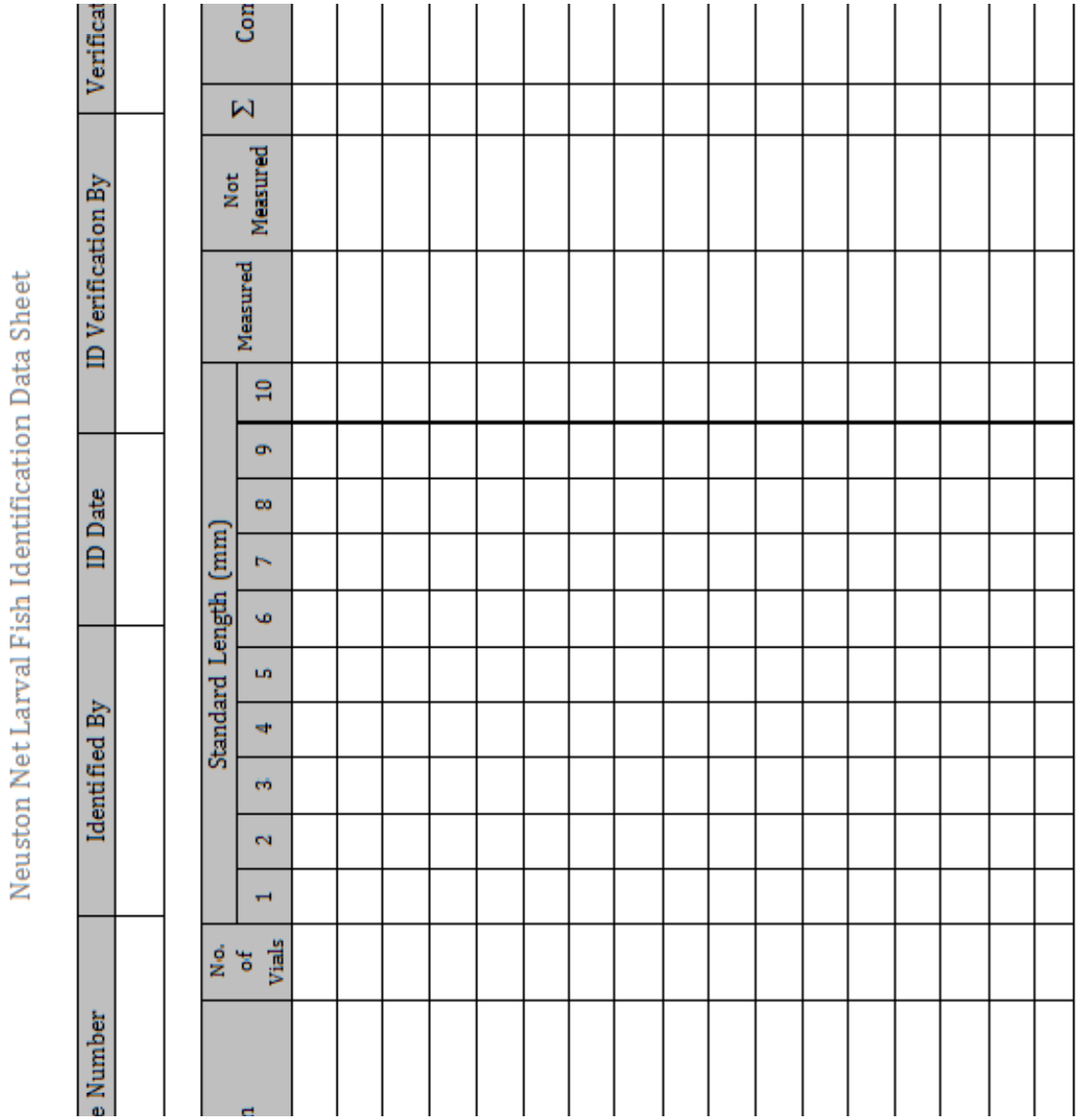
Jar #	Resorted By:	Date Resorted:	QA/QC Invert Count	% Error	QA/QC Fish Count	% Error

Jar #	Resorted By:	Date Resorted:	QA/QC Invert Count	% Error	QA/QC Fish Count	% Error

V. Bongo Net Larval Fish Identification Data Sheet



VI. Neuston Net Larval Fish Identification Data Sheet



VII. Ichthyoplankton Voucher Log

Ichthyoplankton Voucher Log

Sheet ____

Sample #	Size (mm)	Taxon															

VIII. Decapod Crustacean Data Sheet

Sample Number	Gear Type	Identified By	ID Date	ID Verification By	Verification Date

TAXON	COUNT	FINAL ALIQUOT	TOTAL	COMMENTS
1. Lobster phyllosoma (all species)				

2. Penaeidae postlarvae and juveniles				
3. Portunidae postlarvae and juveniles				
4. Sicyoniidae postlarvae and juveniles				
5. *Menippe* postlarvae and juveniles				
6. Geryonidae postlarvae and juveniles				
7. Other and damaged decapods				
8. *Callinectes similis*				
9. *Callinectes sapidis*				
10. *Portunus sayi*				
11. *Leander tenuicornis*				
12. *Latruetes fucorum*				

IX. Encrusting Invertebrate Data Sheet

Encrusting Invertebrate Data Sheet

Sample Number	Identified By	ID Date	ID Verification By	Verification Date

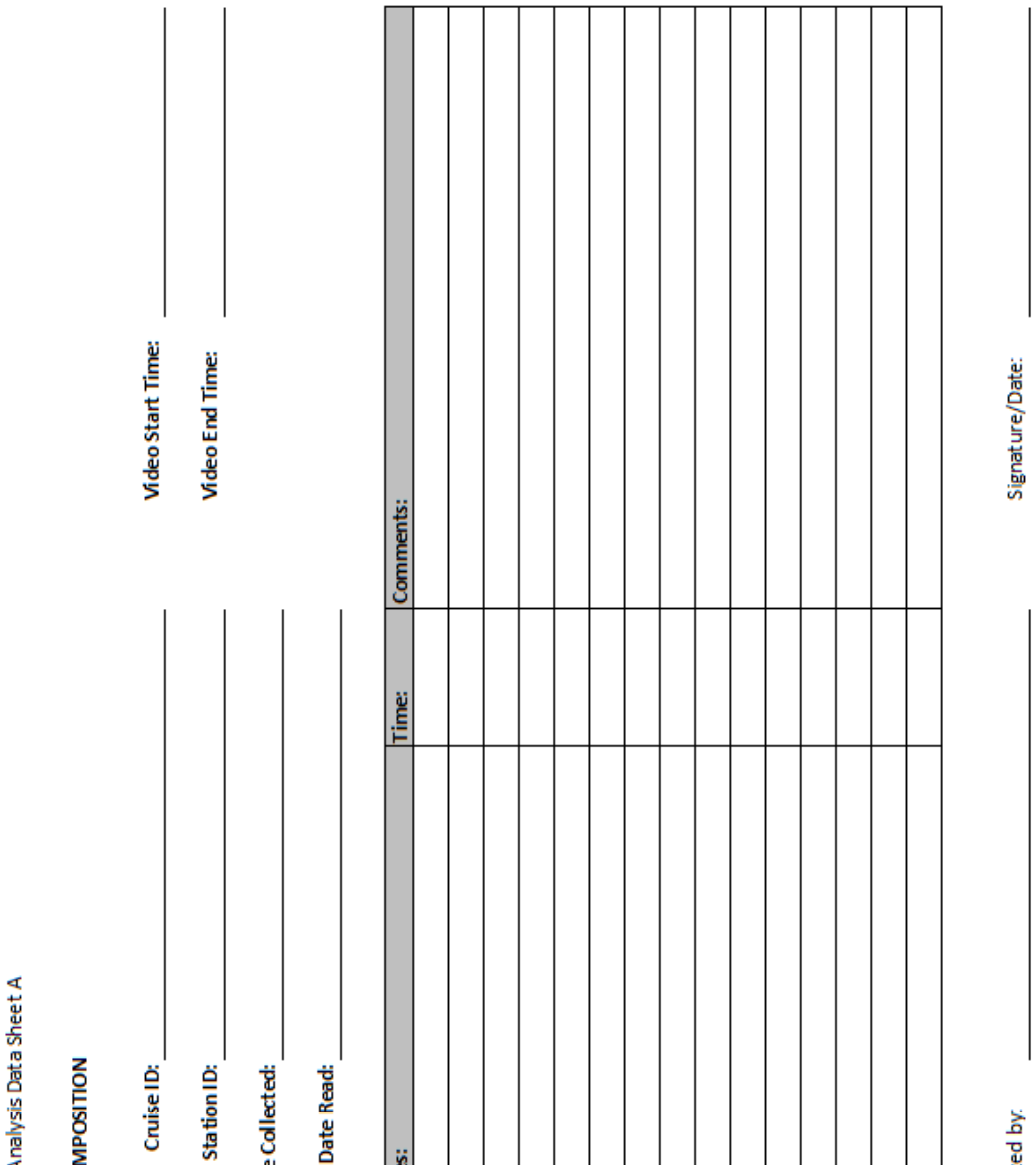
TAXON	BLADES (Replicate Number)																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1. *Membraniporum* sp.																					
2. *Algaophenia latecarinata*																					
3. *Spirorbis* sp.																					
4. Acorn barnacles																					
5. Gooseneck barnacles																					
6. Other																					
6A Other:																					
6B Other:																					
6C Other:																					
7. Unidentified																					
TAXON	**FRONDS (Replicate Number)**																				**Total**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1. *Membraniporum* sp.																					
2. *Algaophenia latecarinata*																					
3. *Spirorbis* sp.																					
4. Acorn barnacles																					
5. Gooseneck barnacles																					
6. Other																					
6A Other:																					

Encrusting Invertebrate Data Sheet

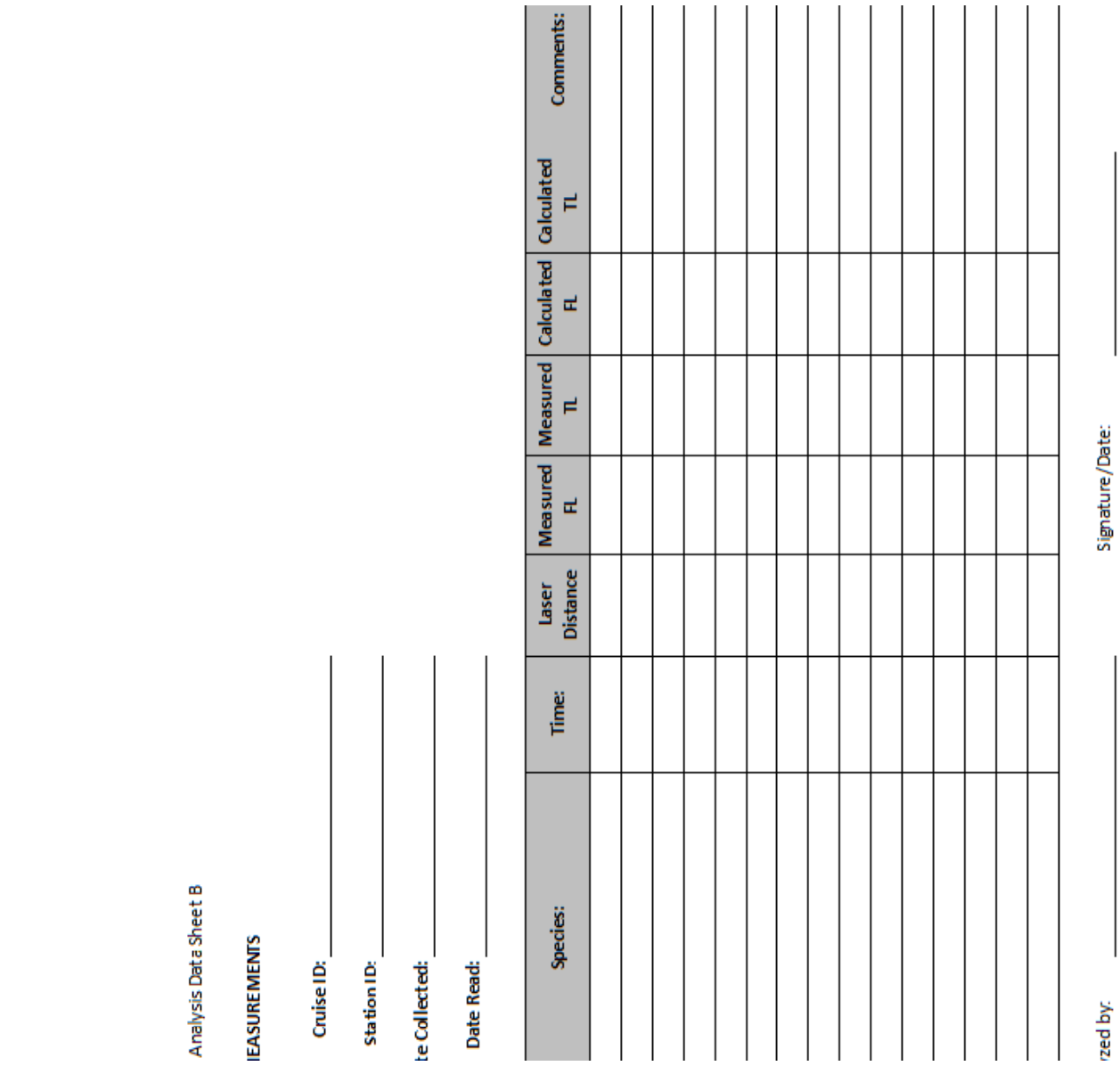
TAXON	FRONDS (Replicate Number) - Continued																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
6B Other:																					
6C Other:																					
7. Unidentified																					
TAXON	**VESSICLES (Replicate Number)**																				**Total**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1. *Membraniporum* sp.																					
2. *Algaophenia latecarinata*																					
3. *Spirorbis* sp.																					
4. Acorn barnacles																					
5. Gooseneck barnacles																					
6. Other																					
6A Other:																					
6B Other:																					
6C Other:																					
7. Unidentified																					

Comments:

X. ROV Video Data Sheet A



XI. ROV Video Data Sheet B



Analysis Data Sheet B

IEASUREMENTS

Cruise ID: _____

Station ID: _____

te Collected: _____

Date Read: _____

Species:	Time:	Laser Distance	Measured FL	Measured TL	Calculated FL	Calculated TL	Comments:

Signature/Date: _____

rzed by: _____

XII. ROV Video Data Sheet C

Analysis Data Sheet C

BUNDANCE

Cruise ID: _____

Station ID: _____

e Collected: _____

Date Read: _____

Species:	Time:	Number of Fish	Mean	Comments:

Signature/Date: _____

ed by: _____

ATTACHMENT II. Quarterly report template.

[Lab Name] Quarterly Report

Period covered: October 1 – December 30, 2012

Report Prepared By:

Author Name	Position	Email

Laboratory Team:

Role	Name	Email
Principal Investigator		
Data Manager		
Scientist		
Scientist		
Scientist		
Scientist		
Scientist		
Scientist		
Scientist		

Summary of Activities for Reporting Period:

Sample Processing Status:

Number of Samples Received:

Number of Samples Identified, Counted, and measured:

Number of Samples QAQC'd internally/externally (%):

Number of Sample Bench Sheets Entered to Database:

Number of Sample Bench Sheet Transcriptions Complete:

Number of Samples Returned to Archive:

Operational / Logistical Issues:

Activities Planned for the Next Reporting Period: