Benthic Monitoring of the LOOP Offshore Brine Diffuser

Study Plan for a 3-Year Monitoring Project

Prepared for:

LOOP LLC Covington, LA

Prepared by:

Barry A. Vittor & Associates, Inc. 8060 Cottage Hill Rd. Mobile, AL 36695

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Project Objectives

The objective of this 3-year monitoring program is to assess potential impacts of brine release on biological resources in the vicinity of the LOOP offshore brine diffuser. Hydrography and macroinfaunal assemblages, which represent the integrated effects of habitat conditions, will be assessed at a spatial array of sampling stations after known periods of brine release. Various macroinfaunal metrics (abundance, taxa richness, abundance of key macroinfaunal groups/specific taxa) will be utilized to assess potential short- and long-term spatial impacts on the benthos.

Site Description

The brine diffuser facility is located 4.8 km south of the Louisiana coastline. The offshore diffuser, 161.5 m in length with 26 equally spaced exit ports, was installed perpendicular to the coastline in 9.1 m of water. The 10.2 cm diameter exit ports are spaced at 6.1 m intervals and designed for an exit velocity of 3.05 m/sec, at a maximum discharge rate of 600,000 barrels per day (bpd). The diffuser is located in an area dominated by the discharge of the Mississippi and Atchafalaya Rivers. Nine sampling stations will be arrayed at various distances from the diffuser (Table 1, Figure 1). Three stations (473, 474, 475) are be located 150 meters east, south and west of the diffuser – these stations have been extensively monitored (1979 – 2002) for macroinfaunal assemblages as part of the LOOP Environmental Monitoring Program (Barry A. Vittor & Associates, Inc. 1995, 2002). Four stations (467, 468, 469, 470) will be located 500 meters north, west, south and east of the diffuser. Two stations (471, 472) will be located 1000 meters south and east of the diffuser and serve as controls.

Project Description

Benthic macroinfauna samples will be collected from nine (9) stations arrayed at distance around the LOOP brine diffuser (Figure 1, Table 2). At each station, five (5) grab samples will be collected with a 0.04 square meter (m2) Young grab (modified Van Veen) sampler. Additionally, a vertical (1 m depth intervals, subsurface to bottom) hydrographic profile will be made at each station to characterize water column salinity and dissolved oxygen (DO).

Monitoring macroinfaunal assemblages at the diffuser will take place up to six times over a three-year period beginning in 2018. Benthic sampling will take place twice annually during periods of brine release if LOOP has more than one brine release during the year – if LOOP only releases brine a single time in a calendar year, there will only be one sampling event.

Benthic grab samples will be analyzed by BVA in Mobile, Alabama. Staff from BVA will sort and identify

all macroinvertebrates to the lowest possible identification level, which in most cases will be to a species level. Wet-weight biomass of major taxonomic groups (e.g., Polychaeta, Mollusca, Crustacea) for each macroinfaunal sample will be measured and reported.

Macroinfaunal data will be analyzed by BVA using univariate and multivariate approaches to identify any temporal and spatial differences in community structure between stations (e.g., stations within the influence of the brine diffuser and control stations located at distance from the diffuser).

Data interpretation will consist of habitat characterization (water depth, salinity, dissolved oxygen) and benthic community characterization including faunal composition, abundance, and community structure, numerical classification analysis, and taxa assemblages. The discussion will also include a comparison of samples collected as part of previous LOOP brine diffuser surveys. Data for other parameters (e.g. sediment texture and total organic carbon) may be available from on-going routine monitoring by LOOP; such data may be used in statistical treatments of the benthic macroinfauna data.

Benthic Analytical Methods

Benthic Macroinfauna – Species Composition and Diversity

Upon receipt and inventory by BVA's laboratory, the samples will be rinsed through a 0.5millimeter (mm) mesh sieve that is partially submerged in a tray of water and gently agitated. The purpose of this procedure is to minimize damage to delicate organisms and to remove sediments. Upon completion of sieving, the residue and water in the bottom of the tray will be poured through the 0.5-mm mesh screen again to ensure that all borderline-sized 0.5-mm organisms are retained. The sieve will be carefully examined after sieving to ensure that all organisms have been removed from the screen. The sieved samples will be stained with Rose Bengal and stored in 70% isopropanol solution until processing.

Sample material retained on the 0.5-mm sieve will be transferred to a white enamel pan and sorted under a dissecting microscope. All macroinvertebrates will be removed and placed in labeled glass vials containing 70% isopropanol, with each vial representing a major taxonomic group (e.g., Polychaeta, Mollusca, Arthropoda). All sample labels will be written in pencil (not pen) to ensure preservation in the isopropanol. Organisms will be identified to the lowest practical taxon (typically species) using dissecting and compound microscopes. The identified animals will be placed in vials containing 70% isopropanol according to biomass category (i.e., major taxon). To identify certain taxa of macroinvertebrates, it is often necessary to make slide mounts of the organisms for examination under a compound microscope. The mounting medium used for clearing is CMC10. The slide will then be labeled appropriately and allowed to dry for approximately one week before identification. The number of individuals of each taxon, excluding fragments, will be recorded. A voucher collection will be

prepared, comprised of representative individuals of each species not previously encountered in samples from the region and previous LOOP monitoring surveys. The voucher specimens will be stored in stoppered and labeled glass vials containing 70% isopropanol.

Taxonomists will use a wide variety of resources for the accurate identification of benthic organisms including an in-house museum of specimen vouchers that have been assembled over the last 30+ years, taxa specific consistency cards for all unique taxa BVA have identified, a library of taxonomic keys and journal reprints of relevant scientific literature, and past experience working with benthic samples from the LOOP site. BVA also maintains a network of taxa specific experts from private industry, state and federal agencies, universities, and museums for the identification of problematic specimens.

Benthic Macroinfauna – Wet-Weight Biomass

Each sample will be analyzed for wet weight biomass of each major taxon (e.g., Annelida, Arthropoda, Mollusca, Echinodermata). Each of these groups of organisms will be in a separate vial, preserved in 70% isopropanol solution. The biomass technician will remove the organisms from the vial, place them on a filter paper pad, gently blot them with a paper towel, then immediately place them in a tared dish and measure their weight in a Mettler Model AG 100 or equivalent balance, to the nearest 0.1 milligram (mg). Results will be reported for each major taxon as well as a summation for each station. Specimens that are unique or have not been encountered before in this location will be included in BVA's project reference (voucher) collection. All specimens required for the voucher collection will be returned to the appropriate vial/jar in that collection. The voucher collection is kept internally at BVA and used to verify identifications as needed for QC. It is not a client deliverable.

Data Reduction and Reporting

Standard Data Reporting

The following basic numerical indices will be calculated for each macroinfaunal sample:

- Infaunal abundance (total number of individuals per station);
- Infaunal density (total number of individuals per square meter);
- Taxa richness (total number of taxa represented in a given station);
- Taxa diversity (Pielou's Index H´);
- Taxa evenness (Pielou's Index J´); and
- Wet-Weight biomass of dominant macroinfaunal groups.

Electronic Deliverables for Taxonomic and Weight-Wet Data

BVA will provide the raw taxonomy and wet-weight biomass data in electronic (digital) format as Microsoft Excel files. The following files will be provided to LOOP:

- Abundance Data: Station, Phylum, Class, Family, Genus, Species, Abundance
- Biomass Data: Station, Major Taxonomic Group, Biomass

Statistical Analyses

BVA will apply the appropriate test of significance on the univariate indices to determine if significant spatial differences exist between stations. Multivariate analysis will consist of ordination of station species abundance data by multi-dimensional scaling using the Bray-Curtis similarity measure and hierarchical clustering of similarity values using the group-average sorting strategy. If statistically appropriate, a test of the significance of dissimilarities determined by the ordination will be conducted using a non-parametric permutation procedure on the ordination similarity matrix. This randomization test is the Analysis of Similarities (ANOSIM) module in the Primer statistics program. A species analysis will be done to determine the contribution of taxa to the average dissimilarity between groups using the SIMPER (similarity percentage) module of the Primer statistical package.

In summary, BVA will develop descriptive statistics, univariate comparisons of stations arrayed around the brine diffuser, seasonal effects, multivariate analyses (ANOSIM, SIMPER, multidimensional scaling [MDS]) on the assemblages, and comparisons to past brine diffuser studies.

Electronic Deliverables for Statistical Analyses

BVA will provide the following for all samples:

Abundance Statistical Worksheet (2-way table): Stations as column headers, taxa as row headings with abundance data in the matrix.

Data Interpretation Reports

BVA will provide a summary field sampling report after each sampling event. On an annual basis, BVA will develop a data interpretation report consisting of habitat characterization and benthic community characterization including faunal composition, abundance, and community structure, numerical

classification analysis and taxa assemblages. The focus of each annual report will be on the extent to which benthic communities differed among the station array, and whether any such differences could be attributed to the discharge of brine at the diffuser. This report will include a comparison of samples collected as part of previous LOOP surveys. The comparison will determine the relative dominance of major taxonomic groups and overall assemblage composition on a spatial and temporal scale and a comparison to data collected in past surveys. The report will also include as an appendix the phylogenetic list of macroinvertebrate taxa for the survey. Field summary reports and annual reports will be provided to U. S. Environmental Protection Agency, Louisiana Department of Environmental Quality, U. S. Coast Guard Captain of the Port, Secretary of Louisiana Department of Transportation and Development, Louisiana Offshore Terminal Authority and LOOP LLC.

The Louisiana Offshore Terminal Authority will distribute the field summary reports and annual report to the Program Review Committee that has technical oversite of the LOOP