Louisiana Department of Environmental Quality
Wetland System Monitoring Requirements
Implemented in Permitted Wetland Assimilation Projects

Wetland System Monitoring

The five (5) year LPDES permits contain technology-based effluent limitations for BOD_5, TSS, and pH reflecting the best controls available. Where these technology-based permit limits do not protect water quality or the designated uses, additional water quality-based effluent limitations and/or conditions are included in the LPDES permits. State narrative and numerical water quality standards are used in conjunction with EPA criteria and other available toxicity information to determine the adequacy of technology-based permit limits and the need for additional water quality-based controls.

The state has established a narrative water quality criterion, which states that:

"No substances shall be present in the waters of the state or the sediments underlying said waters in quantities that alone or in combination will be toxic to human, plant, or animal life or significantly increase health risks due to exposure to the substances or consumption of contaminated fish or other aquatic life." (*Louisiana Surface Water Quality Standards*, LAC Title 33, Part IX, Chapter 11, Section 1113.B.5.)

However, the State of Louisiana has set the following specific criteria for protection of the receiving Natural Wetlands:

- **NO MORE THAN A 20% DECREASE IN NATURALLY OCCURRING LITTER FALL OR STEM GROWTH;**
- **NO SIGNIFICANT DECREASE IN THE DOMINANCE INDEX OR STEM DENSITY OF BALD CYPRESS;**
- **NO SIGNIFICANT DECREASE IN FAUNAL SPECIES DIVERSITY AND NO MORE THAN A 20% DECREASE IN BIOMASS**

The EPA document *Biological Criteria: National Program Guidance for Surface Waters*, discusses the Clean Water Act and states that "the general authority for biological criteria comes from Section 101(a) of the Act which establishes as the objective of the Act the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters, including natural wetlands. To meet this objective, water quality criteria must include criteria to protect biological integrity. Section 101(a)(2) includes the interim water quality goal for the protection and propagation of fish, shellfish, and wildlife." Biological integrity is functionally defined in this EPA manual as "the condition of the aquatic community inhabiting the unimpaired waterbodies of a specified habitat as measured by community structure and function." The importance and function of wetlands include, but are not limited to the following: erosion and flood control, saltwater intrusion control, water quality enhancement, habitat for threatened and endangered species, wildlife habitat, nutrient material cycling, recreation and aesthetics.

Natural wetland loss is a problem in Louisiana. This problem is caused by insufficient sedimentation and relative sea level rise each year. The introduction of nutrient rich wastewater to natural wetlands is beneficial in that it stimulates productivity in the wetland. This productivity promotes vertical accretion through increased organic matter deposition and the formation of soil through increased root growth. This vertical accretion helps maintain the wetlands, despite the rising water levels. Additionally, the total suspended solids, provided by the wastewater, also increase the sediment level in the wetland.

Although the introduction of wastewater into natural wetlands renders benefits to the wetland system, changes to the system will occur. Therefore, it is important to address issues, which will indicate the extent of these changes and to determine if the changes are acceptable.

While standard biomonitoring indicates affects on organisms found in free flowing streams and rivers, the biological monitoring schedule proposed below is broader in scope, and more specific to the wetland ecosystem, than standard biomonitoring. It will provide a more direct indication of change in functions of the wetland system as a whole. The proposed biological monitoring schedule for the Wastewater Assimilation Project is based on Best Professional Judgment, taking into account the size and characteristics of the wetland system.

REMOVAL OF FAUNAL MEASUREMENTS

The analysis of the benthic and nekton communities at all studied wetland assimilation sites have shown no clear patterns with respect to treated effluent. The organisms present at the different wetland assimilation sites are typical of healthy systems in Louisiana and are similar to those of other wetland sites in Louisiana not affected by treated effluent (Sklar 1983, Conner et. al. 1989, Day et. al. 1994, 1997 a, b, 1998, 1999, Pratt 1998), despite the fact that several of the treatment wetlands had received effluent from 10 to 50 years. In a detailed study of the benthic community of a system of mixed marsh and swamp vegetation at Amelia that had received wastewater discharge for 25 years, Pratt (1999) reported very little differences in areas receiving effluent as compared to control areas. This is consistent with findings from other areas. Differences do exist in benthic communities between swamp and marsh sites. For example, multidimensional scaling of benthic community data from Mandeville exhibits a separation of marsh and swamp stations, reflecting the differences in these two communities (Day et al. 1999). There were no significant differences, however, in the groupings of the different swamp stations with respect to treated effluent. The Bayou Castine control site and the Bayou Chinchuba reference site did not separate out from the sites influenced by the treated effluent discharge. This indicates that the effluent was not having a significant impact on the benthic community.

Benthic communities similar to those in the sediments of southern Louisiana have been used to evaluate the impacts created by organic enrichment (Hellawell 1986). Most studies have related from nutrient enrichment to secondary effects, largely oxygen reduction and increased primary productivity (Hellawell 1986). Results at Amelia, Louisiana (Pratt 1998) indicate that the benthic assemblage abundance and taxa composition had a predictable response to secondary effects of wastewater. It is speculated that the invertebrate community structure in Louisiana wetlands result from interaction of the moderate to low nutrient concentration near effluent discharge and the effects of floating aquatic vegetation community on surface discharge (Pratt 1998), but it is difficult to attribute variations in the benthic community to wastewater impacts (Pratt 1998, Day et. al. 1998, Sklar 1983). Taxa that are characteristic of those near highly enriched effluent discharges are not characteristic of wetland assimilation sites in Louisiana (Lenat 1983). The organic nature of deltaic swamps and marshes can also influence the community composition toward taxa that are tolerant of low oxygen levels (Pratt 1998). Major factors that most commonly influence invertebrate structure are water flow, oxygen levels in the water column, and an invertebrate community that is tolerant to organic enrichment (Pratt 1998, Day et. al. 1998, Kadlec and Avord 1989, and Lenat 1983).

From the results from a number of wetland assimilation sites in Louisiana, we conclude that the benthic and nekton community sampling is not likely to provide relevant data for the monitoring program. Therefore, benthic monitoring will no longer be included as part of wetland assimilation permits.

The following parameters are proposed to be sampled and monitored for the specified wetland component at all three (3) wastewater management areas and all three (3) control areas:

- **Sampling and classifying the flora** present and determining percentage of total cover for each vegetative species. The sampling will provide information on whether dominance and species diversity of the community is being altered.
- **Growth studies of vegetative productivity**, which will provide an indication of health and vigor of the plant community.

- **Water stage** is a gaged measurement of the water depth, which will assist in determining stress in the wetlands from hydrologic loadings and will determine the existence of a zone of influence resulting from wastewater applications. The zone around the discharge serves to assimilate the wastewater most effectively. This zone grows larger as wastewater continues to be discharged and the assimilative capacity of the immediate area becomes saturated.
- **Metals and nutrient data from plant tissue samples**, which will identify excesses or deficiencies that could become problematic.
- **Sediment analysis for metals, and nutrients**, which will indicate whether or not metals are bound and buried in the sediments, and nutrients assimilated.
- **Corresponding analysis of surface water** must be made to provide a comparison of water quality in the vicinity of the discharge and at increasing distance from it.

Compared to data from the baseline study, the effects of the discharge on the biological integrity (as defined above) may be accurately assessed.

BASIS FOR ESTIMATE OF WETLAND PLANT PRODUCTIVITY

To measure tree production, two 16 x 16 meter plots were established at each site. Within each plot, all trees with a diameter at breast height (dbh) greater than 10 cm were marked with an aluminum identification tag and the species recorded. Tree productivity (total above ground) will be determined from measurements of litter fall and dbh measurements. Litter fall will be collected from established litter boxes, separated into leaf and woody material, dried at 60^{B} C and weighted. Monthly litter fall will be summed for each box to obtain annual leaf litter fall. Tree biomass will be estimated using dbh vs. tree biomass allometric equations calculated for each species in similar forests in the southeastern U.S. Changes in biomass from year to year represents annual wood production. These values for annual litter fall and stem growth can be summed to give annual above ground productivity.

To calculate marsh grass above ground productivity, five clip plots of peak biomass will be collected from each established location at the end of the growing season for each year and will be estimated as equivalent to the harvested live material (Peak Standing-Crop Method).

The wetland monitoring procedures stipulated as a condition of this permit are as follows:

PARAMETER	WETLAND COMPONENT		
	FLORA	SEDIMENT	SURFACE WATER
Species Classification	**P**		
Percentage of Whole Cover (for each species)	**P**		
Growth Studies	**A_1**		
Water Stage			**M**
Metals Analysis: Mg, Pb, Cd, Cr, Cu, Zn, Fe, Ni, Ag, Se	**P_1**	**P_1**	**S**
Nutrient Analysis I: TKN, TP	**P_1**	**P_1**	**S**
Nutrient Analysis II: NH_3N, NO_2N, NO_3N, PO_4		**P_1**	**S**
Others: BOD_5, TSS, pH, Dissolved Oxygen			**S**

Water quality will be monitored by taking water samples along the path of flow of the effluent in the treatment site and from one or more control sites.

Sampling in the **WASTEWATER MANAGEMENT AREA** must be conducted as follows:

Collection of a minimum of three samples per site in each of three sites: 1) approximately 100' from the discharge point, 2) midway, and 3) at the point of entrance into the Terrebonne/Lafourche Drainage Canal.

Sampling for the **CONTROL AREA** must be conducted as follows:

Collection of a minimum of three samples per site in each of three sites. All three samples will be taken from a site or sites similar to the wastewater management area in the receiving stream.

A: **ANNUALLY.** Sample once per year at all three (3) WASTEWATER MANAGEMENT AREAS and all three (3) CONTROL AREAS and included in the yearly report.

A_1 – Stem growth and litter fall

M: **MONTHLY.** Samples should be taken at all three (3) WASTEWATER MANAGEMENT AREAS and all three (3) CONTROL AREAS each month and included in the yearly report.

P: **PERIODICALLY.** Sampling must be made once during March through May, and once during September through November in the fourth year of the permit period for all three (3) WASTEWATER MANAGEMENT AREAS and all three (3) CONTROL AREAS.

P_1 – Sample preservation, handling, and analysis must meet the specifications of the Test Methods for Evaluating Solid Waste Physical/Chemical Methods, third edition (EPA Publication Number SW-846, 1986, or most recent revision) or an equivalent substitute as approved by the administrative authority.

S: **SEMI-ANNUAL.** Sample twice per year: once during September through February, and once during March through August (sampling events must be a minimum of 4 months apart) for all three (3) WASTEWATER MANAGEMENT AREAS and all three (3) CONTROL AREAS and included in the yearly report.

Sampling procedures to be used during the wetland monitoring phase. (*The Use of Louisiana Swamp Forests for Application of Treated Municipal Wastewater: Standard Operating Procedures for Monitoring the Effects of Effluent Discharge*. John W. Day, Jr., Joel Lindsey, Jason N. Day, and Robert R. Lane, Comite Resources, Inc. Used with the permission of Dr. John W. Day, Jr., March 14, 2003)

WATER QUALITY

1. **Dissolved oxygen and water temperature:** is measured using a Yellow Springs Instrument Co. meter or an ORION Model 820 Dissolved Oxygen meter or equivalent. The probe will be calibrated within four hours of use with a known standard (100% air saturation).

2. **pH & TDS:** Measurements of pH and TDS (Total Dissolved Solids) are made in the field using a Corning Checkmate M90 Field System or equivalent. Water samples will be collected in 500 ml polyethylene bottles and returned to the laboratory where pH will again be measured in the lab using a Jenco Markson pH meter, Model 6100 or equivalent.

3. **Nutrients:** Discrete water samples will be taken 5 to 10 cm below the water surface with effort taken not to stir bottom sediments or include any film that may be present on water surface. Samples are collected in 500 ml acid washed polyethylene bottles. The samples will be immediately stored at 4^{B}C, on ice, for preservation. The samples will be transported to an analytical laboratory, and within 24 hours filtered and sub-sampled. Samples analyzed for NO_2 + NO_3, NH_4 and PO_4 will be filtered in the laboratory using 0.45 um Whatman GF/F glass fiber filters or equivalent, and unfiltered samples will be sub-sampled into 125 mL bottles. Both filtered and unfiltered samples will be frozen until analysis. The samples will be analyzed for nitrite + nitrate ($NO2+NO_3$-N), ammonium (NH_4-N), total nitrogen (TN), total phosphorus (TP), and phosphate

(PO_4-P) by an EPA and DEQ approved analytical laboratory using Standard Methods.

4. **Total Suspended Solids:** TSS will be determined by filtering 100-200 mL of sample water through re-rinsed, dried and weighed 47 mm 0.45 um Whatman GF/F glass fiber filters. Filters will then be dried for 1 hr at 105^{B} C, weighted, dried for another 15 minutes, and reweighed for quality assurance (Standard Methods 1992).

5. **Biological Oxygen Demand:** BOD samples will be collected in standard 300 ml glass BOD bottles. BOD_5 analysis will be from water samples collected in 500ml polyethylene bottles, stored on ice and taken to the laboratory for analysis. Initial D.O. will be measured within 24 hours. Final D.O. will be measured after 5 days of incubation at 20^{B}C. Measurement of BOD is the responsibility of the facility.

6. **ICAP Analysis:** Water samples will be collected from the effluent pipe and surface water in the treatment and control area for ICAP and IC analysis. The following will be measured: Mg, K, S, Na, Ca, B, P, Pb, Zn, Cr, Si, Co, Fe, Mn, Ni, Al, Cd, Cu, F, Cl, Br, NO_3, NO_2, PO_4, SO_4.

7. **Coliform Analysis:** Fecal coliform (i.e. *Escherichia coli)* will be tested using membrane filtration as a field preparation, and then sent to an EPA certified laboratory for analysis. Ten ml of sample water will be passed through a 0.45 micron filter. The filter will be stored in a sterile petri dish and brought within 8 hrs to a certified laboratory for analysis.

8. **Statistical Analysis:** One-way analysis of variance analysis will be carried out to compare treatment and control area parameters using statistical software. An alpha probability level of <0.05 will be used to define a significant difference. Comparisons of means with significant ANOVA tests will be made using Tukey-Kramer Honestly Significant Difference (HSD) test (Sall and Lehman 1996). Other statistical tests may be used as appropriate.

SOILS

Sediment Cores: At least one sediment core will be taken from each study site (Treatment & Control) with a 7.5 cm stainless steel corer. Following the removal of large litter debris, the top 10 to 20 cm of the samples will be separated by horizon, dried, ground and analyzed. Parameters measured will include: pH, electrical conductivity (EC), and Mg. Pb, Cd, Cr, Cu, Zn, Fe, Ni, Ag, Se, NH_3-N, NO_2+NO_3-N, PO_4-P, TKN, and TP. All elemental analyses will be done using an inductively coupled argon plasma quantometer (ICP). Results will be reported as the average of duplicate analyses that are within a 10% confidence interval. The results will be based on oven dry weight.

VEGETATION

To sample forest vegetation, three or more 20 m x 20 m (or equivalent size) subplot should be established at each main plot. Normally, main plots will be established at a near, mid, and outlet locations in the Treatment site, and another main plot established at a Control site. The plots will be orientated perpendicular to the hydrological gradient. All trees >10 cm in diameter at breast height (dbh) within each plot will be tagged with an identification number.

1. **Tree Species Composition:** The relative importance of each major tree species in both the treatment and control areas will be based on the density (total number), dominance (basal area), and frequency of occurrence in each of the plots using equations 1-4 (Barbour et al. 1987).

Relative density = (individuals of a species)/(total individuals of all species) (1)
Relative dominance = (total basal area of a species)/(total basal area of all species) (2)
Relative frequency = (frequency of species)/(total frequency of all species in area) (3)
Importance Value = Relative density + Relative dominance + Relative Frequency (4)

2. **Above Ground Biomass:** Biomass production of a forested wetland is defined as the sum of the leaf and fruit fall (ephemeral productivity) and aboveground wood production (perennial productivity, Newbould 1967).

   A. **Ephemeral or litter fall Productivity:** To estimate ephemeral productivity, litter fall should be collected using 0.25 m^2 boxes with 1 mm mesh bottoms. At least 2 leaf litter boxes should be installed in each subplot (a minimum of 6 boxes at each main plot). The boxes will be placed randomly in each plot. The baskets will be elevated to prevent inundation during high water periods. Litter fall should be collected bimonthly or monthly, depending on the season (litter fall is highest during Fall and Winter). We use the term 'leaf litter' in reference to all non-woody litter including flowers, fruits, and seeds that typically account for <10% of the non-woody litter fall total (Megonigal and Day 1988). Leaf litter will be separated from woody litter, dried to constant mass at 65^{B}C, and weighed. Leaf litter weights throughout any given year will be summed and extrapolated to g $m^{-2}yr^{-1}$ units.

   B. **Perennial Productivity:** Stem biomass will be estimated from annual changes in wood biomass calculated using allometric equations based on stem diameter at breast height (dbh ~ 0.3m) as the independent variable (Table 1). The diameter at breast height (dbh) of all tagged trees will be measured above and below (~5 cm) the identification tag during the winter dormant period. This method allows measurement a safe distance from the tag's nail, which often caused the trunk to swell. Diameter will be measured above the butt swell on large cypress trees. Woody production will be calculated using regression equations (Scott et al. 1985; megonigal et al. 1997, Table 1) based on the diameter for each species as the independent variable. We assume that the contribution of wood and stems <10 cm dbh and herbs will be a relatively small fraction of above-ground net primary production (megonigal et al. 1997). The change in biomass from one winter's measurement to the next represents woody production for the year and will be extrapolated to g $m^{-2}yr^{-1}$ units.

   C. **Net Primary Production:** Aboveground net primary production (NPP) will be calculated as the sum of leaf litter and wood protection, and will be given in g $m^{-2}yr^{-1}$ units.

Table 1. Regression equations used to convert diameter at breast height (DBH) measurements to overall perennial biomass. All equations are in the form: Biomass = f (DBH), where biomass is in kg, DBH is in cm and f is the parameterized function.

Species	**Biomass Reference**	**f(D)**	**DBH Range**
Fraxinus	Biomass (kg) = ((2.669*((DBHcm*0.394)^1.16332))*0.454		>10 cm
spp.	Megonigal et al. '97 Biomass (kg) = 10^(-.97+2.34*LOG10(DBHcm))		
Taxodium	>10 cm Megonigal et al. '97		
distichum	Biomass (kg) = 10^(-919+2.291*LOG10(DBHcm))		>10 cm
Nyssa	Megonigal et al. '97		
aquatica	Biomass (kg) = ((2.39959*((DBHcm*0.394)^2)^1.2003))*0.454		10-28 cm
Acer	Megonigal et al. '97		
rubrum	Biomass (kg) = ((3.15067*((DBHcm*0.394)^2)^1.21955))*0.45		10-28 cm
Quercus	Megonigal et al. '97		
nigra	Biomass (kg) = ((5.99898*((DBHcm*0.394)^2)^1.08527))*0.45		>28 cm
	Megonigal et al. '97		
Salix spp.	Biomass (kg) = 10^(-1.5+2.78*LOG10(DBHcm))		n.a.
Other	Scott et al. 1985		
Species	Biomass (kg) = ((2.54671*((DBHcm*0.394)^2)^1.20138))*0.45		10-28 cm
	Megonigal et al. '97		
	Biomass (kg) = ((1.80526*((DBHcm*0.394)^2)^1.27313))*0.45		>28 cm
	Megonigal et al. '97		

3. **Understory Vegetation:** Shrubs, saplings (individuals <10cm dbh but >2.5 cm dbh), and seedlings (individuals <2.5 cm dbh) will be tabulated by species in a 5m X 5m subplot established in each 20m X 20m plot. From the data, density and basal area will be calculated for trees and density will be calculated for sapling and seedling species.

   The present cover for herbaceous vegetation will be determined by a modified line-intercept technique patterned after that proposed by DS&N, Inc. (1988). The method consists of observations made of plant species occurring along a 1m X 10m transect located at the eastern edge of each 20m X 20m plot. East 10m section is divided into 1m X 1m intervals. Species cover will be determined on the basis of the percent cover occupied within each 1m X 10m unit. Herbaceous plots will be measured at least once during the study.

4. **Nutrient and Metals Analysis of Green Leaves:** Green leaf samples should be collected during the last year of the monitoring from the major species in the treatment and control areas, once during March through May and once during September through November. Samples will be oven-dried at 70^{B}C for at least 48 hours, ground in a Wiley mill to pass a 40 mesh screen, and stored in whirl-pak bags. Samples will be analyzed in the laboratory for Mg, Pb, Cd, Cr, Cu, Zn, Fe, Ni, Ag, Se, TKN and TP. The tissue analyses should be done by a wet digestion method.

5. **Marsh Vegetation Production:** Net production in areas dominated by non-woody herbaceous vegetation will be determined by end of season live (EOSL) biomass analysis. Sampling should be conducted during the last week of September or the first week of October. At least five 0.06 m^2 clip plots will be taken at each location using randomly placed quadrants. Vegetation within the quadrant will be cut as close to the surface as possible, stored in labeled paper bags, brought back to the laboratory, and refrigerated until processing. Live material will be separated from dead, and dried at 60^{B} C to a constant weight. All data will be presented on a live dry weight per square meter basis (g dry wt m^{-2}).