Impacts of Hurricanes Katrina and Rita on the Microbial Landscape of the New Orleans Area

Resubmitted 20 Mar. 2007

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Author contributions: All contributed to writing and editing. CDS, MLG, AH, EL, HMS, LF contributed to sample collection strategy. Specifically the following authors were responsible for the following analyses: AH, EL - *E. coli* and enterococci by MF. CDS, MLG, TS, HMS, LF - *E. coli* by qPCR and enterococci by CS and qPCR. DB - *Bifidobacterium*. LB - algae. LAZ - microbial assemblage. RJG – *Legionella*. GFS, ODN – *Vibrio*. RF, WQB, and GV – coliphage, *C. perfringens, Bacteroidales*. DW, THD, JM – remote sensing.

The authors declare no conflict of interest.

Abstract

Floodwaters in New Orleans from Hurricanes Katrina and Rita were observed to contain high levels of fecal indicator bacteria and microbial pathogens, generating concern about long-term impacts of these floodwaters on the sediment and water quality of the New Orleans area and Lake Pontchartrain. We show here that fecal indicator microbe concentrations in offshore waters from Lake Pontchartrain returned to pre-hurricane concentrations within two months of the flooding induced by these hurricanes. *Vibrio* and *Legionella* species within the lake were more abundant in samples collected shortly after the floodwaters had receded compared to samples taken within the subsequent three months; no evidence of a long-term hurricane-induced algal bloom was observed. *Giardia* and *Cryptosporidium* were detected in canal waters. Elevated levels of fecal indicator bacteria observed in sediment could not be solely attributed to impacts from floodwaters, as both flooded and non-flooded areas exhibited elevated levels of fecal indicator bacteria. Evidence from measurements of *Bifidobacterium* and bacterial diversity analysis suggest that the fecal indicator bacteria observed in the sediment were from human fecal sources. Epidemiologic studies are highly recommended to evaluate the human health effects of the sediments deposited by the floodwaters.

Introduction

Hurricane Katrina made landfall along the US Gulf Coast on 29 Aug. 2005, resulting in extensive flooding in the City of New Orleans (NO), LA, due to storm surge from adjacent Lake Pontchartrain (LP) and several levee failures. These floodwaters had been partially pumped back into LP when the city experienced additional flooding and levee failures from Hurricane Rita on 24 Sep. 2005. Floodwaters completely receded by 11 Oct. 2005. Much of the flooding occurred in urbanized and industrial areas, fueling concerns that a public health crisis could result from exposures to chemically and microbiologically contaminated floodwaters.

Preliminary investigations in mid-Sep. 2005 documented high levels of microbial and toxicant contamination in the NO floodwaters (*1,2,3*). Reports of mean total coliform and total *Escherichia coli* levels as high as 8 x 10^8 CFU per 100 mL and 3 x10^7 CFU per 100 mL, respectively (*1*) indicated the presence of sewage contamination and associated sewage-borne pathogens. Elevated concentrations of fecal coliforms have previously been observed in floodwaters of the NO region, but the 2005 event was characterized by an unusually large volume and long duration of human exposure (*4*). The most contaminated area tested near the Superdome also contained high levels of non-sewage pathogens, with an estimated *Aeromonas* spp. concentration of 1.7 x 10^8 per 100 ml (*1*). Concentrations of *Vibrio* spp. were not measured, but the temperature and salinity of the floodwaters would have been favorable for their growth, and the number of *Vibrio* infections reported in the month following Hurricane Katrina was higher than normal (*5*).

As shown in the satellite floodwater image, approximately 34 billion liters of water covered NO as of 30 Aug. 2005, with floodwater depths in several areas in excess of 3 meters (Fig. 1). As these floodwaters were pumped back into LP, sediments were deposited throughout the flooded regions of the city (including the interior of homes). To a large extent, these sediments, now dried, remain even after one year following their deposition by the floodwaters. Therefore, the human population of the NO region can be exposed to microbial pollution through contact with the waters of LP and the dried sediments, which may be ingested or suspended in the air and inhaled. Long-term environmental impacts of this floodwater discharge on the microbial water quality of the lake, and the potential public health risk associated with exposures to the remaining sediment deposits, are unclear.

The primary objective of this study was to document the microbial water quality of LP after the city was dewatered, and to evaluate canal waters and sediments within residential areas of NO as potential sources of microbial contaminants. This study differs from others in the timing of the measurements, which were conducted shortly after floodwaters receded from the city, and also in that sediments from the urban areas of the city were evaluated. This study is also unique in that a diverse group of microbes were measured, including traditional fecal indicators (enterococci and *E. coli*) using three methods (membrane filtration (MF), chromogenic substrate (CS), and qPCR). A suite of non-traditional fecal indicators (FRNA coliphage, *Clostridium perfringens*, *Bacteroidales*, *Bifidobacterium adolescentis*) was also evaluated. FRNA coliphage, *C. perfringens* and *Bacteroidales* have been recommended as alternative indicators of sewage pollution, and more recently *Bifidobacterium adolescentis* has been shown to grow exclusively in the intestines of humans (*6,7*) and has been proposed for microbial source-tracking of human fecal contamination (*8,9,10*). Efforts were also made to measure pathogenic bacteria of the genus *Vibrio* and *Legionella*, with species detection as

V. vulnificus, V. parahaemolyticus, *V. cholera*, and *L. pneumophila*. Pathogenic protozoa (*Cryptosporidium*, *Giardia*) were measured at the genus level. The study also included an innovative method of evaluating the possible origin of microbial contamination through microbial assemblage analysis as well as a preliminary screening for the presence of algal blooms.

Sample Collection

Sample collection and analysis was completed through a collaborative response of six academic institutions including Louisiana State Univ. (LSU) and three NSF/NIEHS National Centers for Oceans and Human Health at the Univ. of Miami, Univ. of Hawaii, and Woods Hole (Woods Hole Oceanographic Inst., Marine Biological Laboratory, Massachusetts Inst. of Technology), with supporting collaborations from Florida International Univ. and Georgia College and State Univ.

The period of intensive sample collection occurred during the two months immediately following the dewatering of the City of NO. Given the results from this intensive monitoring period, sampling locations within the lake were modified and additional sites were identified within NO. Sampling within NO focused on collecting water samples from interior canals, and collecting sediments from canal banks and from floodwater sediments deposited within residential neighborhoods. After Nov. 2005, the frequency of sample collection was decreased to monthly and ultimate to a quarterly basis.

Lake Transects. During the months of Oct. and Nov. 2005, surface water samples were collected from southern LP along three transects (labeled A-C) radiating outward from the outlet of the 17th St. Canal. These samples were evaluated for traditional and non-traditional fecal indicators plus *Vibrio* and *Legionella* sp. Each transect was comprised of four stations along an approximately 4-km track with stations identified by transect letter and a station number (1-4) assigned consecutively beginning with the station nearest shore. Transect A ran to the W-NW (Stations A1-A4). Transect B was located to the north, perpendicular to shore (Stations B1-B4), and Transect C ran NE (Stations C1-C4) (Fig. 1). In Jan. 2006, transects were redesigned to sample for gradients extending from the outlets of three canals along the south shore (the 17th St., London Avenue, and Industrial Canals). Two near-shore stations were sampled for each new transect, one to the east and one directly in front of the outlet of the respective canal. Two additional stations were sampled along each transect at increasing distances from the outlets of the canals running perpendicular to the shoreline. The transect from the 17th St. Canal was comprised of previously sampled stations (A1, B1, B2, B3), while those from the London Ave. (Stations D1-D4) and Industrial Canals (E1-E4), were new (Fig. 1).

Canal and Shoreline Water Sampling. Water samples from interior canals and from the canal shorelines were collected during Oct. and Nov. 2005. Particular efforts were placed on collecting water samples from the 17th St. (S1) and Industrial Canals (S3). A distinguishing feature of the 17th St. Canal is the presence of a large pump station at its interior end, as this canal was designed to discharge waters from the interior portions of NO. These waters originate from rainfall runoff and from groundwater seepage as the city lies below sea level. The Industrial Canal is a navigational canal that receives flows from the Gulf of Mexico during incoming tide through the Mississippi River Gulf Outlet. Canal and shoreline water samples were analyzed for fecal indicators (traditional and non-traditional) and for *Vibrio* and *Legionella* sp. Microbial assemblage analysis was also evaluated for 3 samples (2 from site S1 and 1 from Site S3). Water samples (sites S1 and A1) were also collected during Dec. 2006 for subsequent analysis of *Cryptosporidium* and *Giardia*.

Sediment Sampling. Canal shoreline sediments from 6 locations and deposited floodwater sediments from 3 homes were collected during Nov. 2005 and Mar. 2006. The 6 shoreline sediment sites corresponded to the outfalls of major canals (17th St. Canal (R1), Bayou St. Johns (R2), London Ave. Canal (R3), and the Industrial Canal (R4)) and from the inner city canal sites (R5 and R6) corresponding to pre-hurricane sampling sites established by the Louisiana Dept. of Environmental Quality. The sites corresponding to deposited floodwater sediments were randomly chosen from within a targeted area. Targeted areas were based upon depth of flooding and the specific canal breach that was the cause of flooding. The first residential site (Y1) corresponded to a home that received moderate flood depth (1.5 to 2 m) from multiple canal breaches. The second site (Y2) received deep flooding (3+ meters) directly from the London Ave. Canal breach; the third site (Y3) received deep flooding (3+ meters) from a breach on the Industrial Canal. In June 2006, additional sediment sampling sites were included to evaluate the impacts of hurricane-induced flooding on sediment sites. These additional sites were geographically distributed within residential neighorhoods from flooded (Y4 through Y11) and non-flooded areas (Z1 through Z10). Sediment samples were analyzed for enterococci, *E. coli*, and *Bifidobacterium*. Microbial assemblages were established for a sediment sample (Y3) showing elevated fecal indicator concentrations.

Regional and Local Surveys of Chlorophyll-a. Water samples were collected during Sep. 2005 along the Mississippi coast near the river delta and in Chandeleur Sound (which receives discharges from LP) to provide data concerning the relative abundance of algae (Fig. 2). Additional samples were collected during Oct. and Nov. 2005 along the initial, radial transects in LP.

Results

In the LP transects (characterized by salinities from 7 to 11 ppt), maximum recommended single sample exposure levels of enterococci (104 CFU/100ml for marine water) and *E. coli* (235 CFU/100 ml for freshwater) (*11*) were only exceeded on occasion (as measured by qPCR and CS) at the stations closest to shore, and the levels dropped with distance away from shore (Fig. 3). None of the lake transect samples exceeded the single sample exposure levels as measured by the MF method. On occasion, the fecal indicator measurements at the lake sites located closest to shore exceeded the single sample exposure level. For the canal sites, single sample exposure levels were frequently exceeded regardless of method utilized. The counts observed within the canals were consistent with the concentrations observed in the near-shore sampling sites within LP suggesting that the interior portions of the city may be the source of the fecal indicator bacteria.

During the sampling period FRNA coliphages and *Bacteroidales* by *Taq* nuclease assay (TNA) were not detected in near-shore and offshore water samples within the lake. *Bacteroidales* and *Bifidobacterium* were detected consistently by standard PCR in the near-shore samples, at sites 1 and 2 within transects A, B, and C, at the locations where fecal indicator bacteria counts were highest. Conversely, *Bacteroidales* and *Bifidobacterium* were consistently not detected in the offshore samples, at sites 3 and 4, in areas where fecal indicator counts were low. *Bifidobacterium* were also detected in shoreline waters adjacent to the canal outfalls (Fig. 3). During the sampling period, the highest *C. perfringens* levels were observed at near-shore sites 1 and 2 within transect A. Low to undetectable levels of *C. perfringens* were observed in offshore sites of transect A and in all sites within transects B and C (Supplemental Table S-2).

For the Industrial Canal (Site S3), fecal indicator bacteria levels (enterococci: 9 CFU/100 ml, *E. coli*: 140 CFU/100 ml, means for all methods used collectively) were lower compared to the interior portions of the 17th St. Canal (site S1, enterococci: 380 CFU/100 ml, *E. coli*: 4,600 CFU/100 ml, means for all methods collectively). Concentrations were much lower, on average, at the outlet of the 17th St. Canal (sites A1, B1, and C1, mean enterococci: 70 CFU/100 ml, *E. coli*: 120 CFU/100 ml) relative to the interior site. During 12 Nov. 2005, samples were taken at the effluent side of the 17th St. Canal pumping station (site S1), both before and after pumping. The relative abundance of enterococci in the canal water at this site increased by over an order of magnitude after pumping (mean of 93 CFU/100 ml before pumping and 1,400 CFU/100 ml after pumping), thereby suggesting that the source of fecal indicators to this canal is water from the interior portions of the city which is then transported by the 17th St. Canal to LP. On the day that the pumps were active, lake shore-line water samples exceeded the US EPA single sample exposure levels at the mouths of Bayou St. John, the London Ave. Canal, the Industrial Canal, and at the interior canal sites within the London Ave. Canal and Bayou St. John (Fig. 3). Samples for *Cryptosporidium* and *Giardia*, also collected during a pumping event on the 17th St. Canal, showed detectable levels for both protozoans at site S1 (23 and 8 oocysts per 100 L for *Cryptosporidium* and 15 and 16 cysts per 100 L for *Giardia*). The levels of *Cryptosporidium* decreased to below detection limits (<0.5 oocyts per 100L) at the outlet of the 17th St. Canal, whereas *Giardia* remained elevated (10 cysts per 100 L).

Bacterial assemblage data also showed fewer potential pathogens or related organisms in the Industrial Canal versus the 17th St. Canal and demonstrated a likely lower percentage of bacteria derived from contaminated (e.g. sludge, chemical etc.) study sites (Fig. 4a, b, c). Roughly 60% of the bacteria in samples collected from the 17th St. Canal showed top BLAST scores with microbes observed in uncontaminated aquatic environments; from 10 to 20% of the bacteria were typical of those found in sewage, with 13 to 18% associated with taxa that could be potentially pathogenic. The sample from the Industrial Canal, however, was characterized by a microbial community typical of uncontaminated aquatic environments (93%), with much smaller fractions associated with sewage sludge bacteria (1%) or potential pathogen groups (2%).

Concentrations of colony-forming units (CFUs) for putative *Vibrio* spp. averaged 1200 CFU per 100 ml (range 130 to 5600) in Oct. 2005, with highest total counts found in the canals and at stations near the outlet of the 17th St. Canal, and the lowest counts found at the stations farthest from shore (Fig 5). At this time, *V. cholerae* and *V. vulnificus* were present at nearly all sites and occasionally dominated the culturable *Vibrio* community, but *V. parahaemolyticus* was rare everywhere. *V. cholerae* was particularly dominant in the 17th St. Canal (79% of isolates), while *V. vulnificus* dominated the Industrial Canal (72% of isolates) and was most common on average, comprising 46% of isolates from all stations. The concentration of total putative *Vibrio* spp. was four-fold lower on average in Jan. 2006 with a mean of 300 CFU per 100 ml (range 32 to 680) and total CFUs on the *Vibrio*-selective media were again found to decline with distance from shore (Fig. 5). At this time, none of the three targeted species was common at any site, and *V. vulnificus*, in particular, was not detected in any sample.

The majority (92%) of the transect water samples from Oct. were positive for *Legionella* spp., but none of these was positive for *L. pneumophila* (Supplemental Table S-2). Lake samples collected in Nov., Dec., and Jan. were characterized by a smaller proportion of positives (50%) for *Legionella* spp., but one of the twenty samples evaluated was positive for *L. pneumophila* (site B2, 12-Dec. 2005). This positive sample was confirmed by sequence analysis of the recovered amplification product. By March 2006, the proportion of positive samples from the lake samples increased back to 100% with no positives for *L. pneumophila*, thereby suggesting that the presence of *Legionella* sp. may in part be affected by seasonal factors. The presence of *Legionella* was not correlated with levels of fecal indicator bacteria.

Chlorophyll-*a* concentrations of the lake transect sites ranged from 0.79 μg/L to 1.3 μg/L during the 8 Nov. 2005 sampling trip with no distinctive spatial pattern (Fig. 2). Samples collected from the canals were generally characterized by higher chlorophyll levels (3.7 μg/L). These data showed no evidence of significant algal blooms occurring at the time of the Nov. 2005 sampling, as concentrations were considerably lower than the 5 to 20 μg/L of chlorophyll found in LP in 1997 (*12*). Samples along the Louisiana coast near the Mississippi delta and in Chandeleur Sound ranged from 4.0 to 42 μg/L of chlorophyll-*a* 2 to 3 weeks after the hurricane (Fig. 2). These concentrations were similar to those found at earlier times (*13, 14, 15*), suggesting that algal blooms along the coast were not enhanced as a result of hurricane damage and flooding.

The shoreline sediments collected from the banks of the canals (sites R1 – R6), demonstrated the presence of fecal indicators in the range of 0.7 to 970 MPN/g by both the qPCR assay and the CS assay (Fig. 6 and Supplemental Table S-5). Samples of dried floodwater sediments collected from residential neighborhoods on 12 Nov. 2005 also demonstrated enterococci abundance at levels similar to the more contaminated canal shoreline sediments (270 to 980 MPN/g). Samples collected on 25 March 2006, approximately six months after flooding, also showed viable enterococci present in the shoreline sediments and in dried floodwater sediments from all three residential home sites, with the highest levels measured at 1,040 CFU/g sediment. The levels of indicator microbes appear to be related to the % organic matter of the sediments with all samples containing less than or equal to 3% organic matter characterized by indicator concentrations below 150 MPN/g whereas samples characterized by greater than 11%

organic content showed microbe levels greater than 350 MPN/g. All sediment samples collected during March 2006 were also positive for *Bifidobacterium*, with the exception of the one sample (R4) that was characterized by a relatively low level (15 CFU/g) of enterococci and low % organic content (Fig. 6). Microbial assemblage data for a residential sediment sample (sample Y3 collected on 25 March 2006) showed that roughly 80% of the bacteria were typical of those found in uncontaminated environments. Three percent were typical of those found in sewage sludge, with about 8% characterized by genera that may be associated with pathogenic microbes (Fig. 4d). Additional samples collected during June 2006 from both flooded (11 sites) and non-flooded (10 sites) residential neighborhoods (see Fig. 1 for locations), showed levels of enterococci in excess of 1,000 MPN/g (Fig. 6). However, the distribution of enterococci abundance in NO sediments was heterogeneous, and it did not necessarily correlate with flooding history. Some of the highest enterococci counts were found in both floodwater-exposed sediments (i.e. site Y7 at 6,200 MPN/g, site Y2 at 1,900 MPN/g) and in non-flooded sediments (i.e. site Z1 at 840 CFU/g, site Z6 at 7,600 CFU/g). Conversely, some sediments that were flooded at depth for an extended period of time, including a site in the Lower Ninth Ward area, demonstrated relatively low enterococci abundance (i.e. site Y4 at 60 MPN/g, site Y6 at 7 MPN/g, and site Y9 at 5 MPN/g).

Discussion

No evidence of a long-term algal bloom was observed in LP as a result of Hurricanes Katrina and Rita. Chlorophyll samples were collected from the lake starting in mid-October, after the dewatering of NO floodwaters was completed, so early blooms could have been missed. Even if an immediate bloom had occurred, evidence suggests that the lake would have recovered quickly as no significant impact was noted six weeks after the first hurricane.

The decline in concentrations of *Vibrio*, or positive samples for *Legionella*, from October to January is most likely attributable to normal seasonal cycles rather than recovery from a hurricane effect. Seasonal fluctuations in *Vibrio* spp. have been documented (*16*), with water temperature as a major driving variable (*17*). Average lake temperature in October was 25° C compared to 17° C in January, suggesting that induction into a viable but non-culturable state (*18,19*) could account for part of the observed decline in *Vibrio* colony-forming units. The warm temperature (*ca.* 31 °C) and low salinity (*ca.* 5-6 ppt) of floodwaters in downtown NO shortly after the storm (*4*) were conditions under which many *Vibrio* spp. would be expected to thrive, including the three targeted pathogens in this study, *V. cholerae* (*20*), *V. parahaemolyticus* (*21*) and *V. vulnificus* (*22, 23, 24*). *Legionella* and some *Vibrio* spp. have symbiotic relationships with other aquatic microbes. Their concentrations could therefore also vary in response to the general patterns of productivity among the plankton. Regardless of the cause, elevated concentrations of pathogenic *Vibrio* spp. in the lake waters flooding the city would have increased the risk of infections and thereby contributed to the spike in post-Katrina *Vibrio*-related infection and mortality (*5*).

The results of this study also indicate that fecal indicator bacteria in the offshore waters of LP, presumably introduced through the discharge of contaminated floodwaters from the city, had dissipated to low background levels within a few weeks after the dewatering process was complete. In contrast, levels of enterococci remained high in the near-shore waters of the lake and most canal waters, with pumping of inner city drainage resulting in an increase in indicator levels within the canals. Of note, the levels of fecal indicator bacteria (enterococci, *E. coli*, fecal coliform) were known to be high in NO interior-water discharges before the 2005 hurricane season, and the historic background levels of these fecal indicator bacteria in near-shore waters of LP have chronically exceeded recreational water quality standards for many decades (*4,25*). The source of contamination appears to be the chronic discharge of contaminated water from the interior portions of the city. The detection of *Cryptosporidium* and *Giardia* in these waters is a strong indication of the poor quality. Efforts are needed in the region to improve the sanitary infrastructure. These improvements should focus on reducing sewage contamination of groundwater seepage and stormwater drained from the NO region.

Fecal indicator bacteria in the sediments could not be attributed solely to the effects of Hurricanes Katrina and Rita, as counts of enterococci in both flooded and non-flooded areas were statistically the same. Elevated levels within sediments could have been due to sewage impacts or from environmental sources as there is a growing body of evidence that suggests persistence, and possibly regrowth, of fecal bacteria, particularly in subtropical and tropical environments in association with soils, marine sediments, and beach sand found near-shore (*26,27,28*). Because of this phenomenon additional evidence is generally needed in order to determine the possible cause of the elevated indicator levels within sediments. For example, a non-traditional indicator, *C. perfringens,* was used to document fecal impacts to agricultural soils in North Carolina in response to a series of hurricane events (*29*). In the current study, the detection of *B. adolescentis* along with elevated concentrations of enterococci in the majority of sediment samples supports the hypothesis that sewage contamination is the source of the fecal indicator bacteria in the shoreline sediments. Additional samples should be evaluated to determine if this co-occurrence is of statistical significance. The impacts from sewage (and possible presence of pathogens) is also supported by analysis of bacterial assemblage data which indicates that the sample sites characterized by higher levels of enterococci and *E. coli* (i.e. 17th St. Canal sites, flooded residential sediments, etc.) were also characterized by bacterial groups that have been commonly observed in sewage sludge, including the putative pathogens *Bacillus* spp. and *Mycobacterium* spp. (Figure 4d). Again, only one sediment sample was evaluated in this case, and further work is needed to determine whether this co-occurrence is of significance.

Conclusion

The floodwater pumped from the city into the lake was a small fraction (ca. 0.5%) of the total lake volume, and the large dilution effect appears to have limited the environmental impact on the lake and substantially reduced health risks associated with exposure to lake water to levels typically observed before the hurricane. Water quality within the city and in nearshore waters of LP continue to be impacted by discharges from interior portions of the city which appear to be contaminated with fecal microbes, a chronic condition that persisted in the area both before and after Hurricanes Katrina and Rita. Sediments also remain a cause for concern as they may serve as a potential source of on-going microbial exposure in near-shore waters and interior portions of NO. Given the history of this region with respect to extensive flooding of the city with sewage contaminated floodwaters induced by hurricanes, further investigation is needed to evaluate the microbial quality of floodwater sediments deposited in the NO area and to determine if there is an elevated risk of exposure to human pathogens through contact, ingestion, and inhalation of these sediments.

Materials and Methods

Enterococci and *E. coli* were measured in water samples using standard MF methods which included plating filters on mEI media (*30*) for enterococci and mTEC media (*31*) for *E. coli*. For enterococci, a commercially-available CS method (Enterolert™ by IDEXX, Westbrook, ME) was also utilized which including the distribution of the sample into a series of wells for enumeration. Samples analyzed for enterococci and *E. coli* by qPCR included concentrating water samples with a polycarbonate filter, extracting the DNA, followed by use of qPCR testing kits containing lypholyzed reagents from Cepheid (Sunnyvale, CA). Sediment samples were analyzed by eluting the microbes and analyzing the eluate by CS and qPCR as described above. Volatile organics were measured for the sediment samples gravimetrically before and after ignition.

Assays for alternative fecal indicators included measurements of FRNA coliphage by enrichment and culture on a host lawn of *E. coli* (32), *C. perfringens* by MF using mCP agar (33), fecal *Bacteroidales* using standard PCR and real-time PCR using the *Taq* nuclease assay (TNA) (34), and *Bifidobacterium adolescentis* by PCR (35). Some sediment samples were also assayed for *Bifidobacterium*. Bacterial diversity was evaluated through small subunit ribosomal RNA gene surveys using universal primer pairs that flank the V4-V8 region of the molecule and amplify SSU rRNA genes from all three domains of life (*Bacteria*, *Archaea* and *Eukarya*). This manuscript reports only the results from bacterial sequence data. General bacterial diversity assessments help determine the presence of sequence types related to known bacterial pathogens or may be used to infer the source of bacteria by noting the environments in which similar organisms are found (such as within sewage). The three pathogenic bacterial species in the genus *Vibrio* (i.e. *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae*) were assayed in samples from the lake transects by filter plating on CHROMAgar™ Vibrio and TCBS agar. Colonies were enumerated and a subset isolated for confirmatory identification based on their growth and color changes on selective media and by PCR testing with species-specific primers (36,37,38,39). *Legionella* species and *L. pneumophila* were assessed by PCR amplification (40,41) with amplification of *L. pneumophila* targeting the *mip* gene (42). *Cryptosporidium* and *Giardia* were analyzed by filtration (Filtamax™ by IDEXX, Westbrook, ME), elution, and purification using immuno-magnetic separation followed by epiflourescence microscopy (43). Additional information concerning the methods is available within the on-line supplemental information.

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Supplementary Information is linked to the online version of this paper at www.pnas.org/egi/content/full/XXXX. This supplementary information contains the materials and methods, supplemental tables S-1 to S-5 and supplemental figure S-1.

Acknowledgments This work was funded by NSF-NIEHS Oceans and Human Health Program (NSF OCE0432368, OCE0432479, OCE0430724 and NIEHS P50 ES12736, ES012740, ES012742), the NSF-SGER Program (OCE 0554402, OCE 0554674, OCE

0554850, OCE0600130), the NSF-REU Program, and by the Georgia Sea Grant College Program (NA04OAR170033). Samples from the Gulf of Mexico were provided through NOAA. The authors thank the Cepheid company, as well as R. Noble and D. Blackwood from UNC, for assistance with the Cepheid qPCR assays. The authors also thank M. Wright, A. Abdelzaher, J. Rocca, M. Dennett, and D. Moran for their assistance with logistics and sample processing. The IDEXX company provided Enterolert™ assay supplies free of charge. SERC Publication no. XX. CSTARS contribution #10.

Fig. 1: Floodwater Depths and Sampling Sites. Color shadings indicate depth of floodwater (in meters) in the City of NO as of 30 Aug. 2005. Droplet symbols indicate the location of sites where only water samples were collected including Lake Pontchartrain water sample sites and canal sites. Circles indicate the location of sites where sediment samples were collected. For sites adjacent to canals (R1 through R6), water samples were also collected. The cross shows the location of the 17th St. Canal Pump Station # 6, the main discharge point for the inner city canals into Lake Pontchartrain.

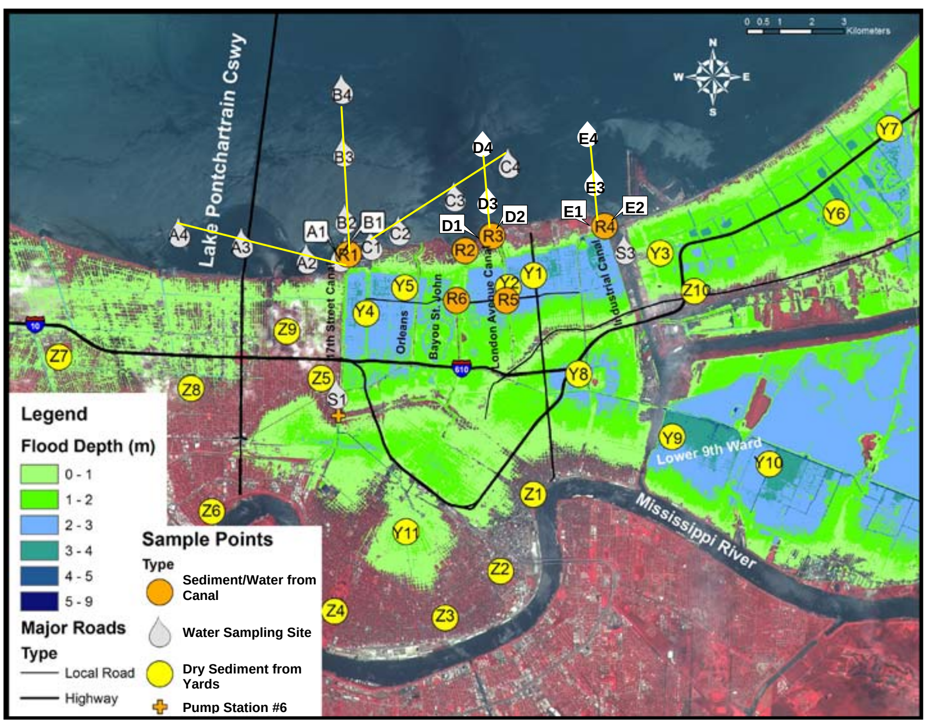

Fig. 2: Chlorophyll-*a* concentrations in Lake Pontchartrain, within the 17th St. and Industrial Canal of NO, in Chandeleur Sound – Mississippi River Delta area, and in the Gulf of Mexico following Hurricane Katrina. Samples were collected during two cruises (13 - 26 Sep. and 22 - 25 Sep. 2006) sponsored by NOAA. Data shown in the inset for Lake Pontchartrain were collected on 2 Nov. 2005.

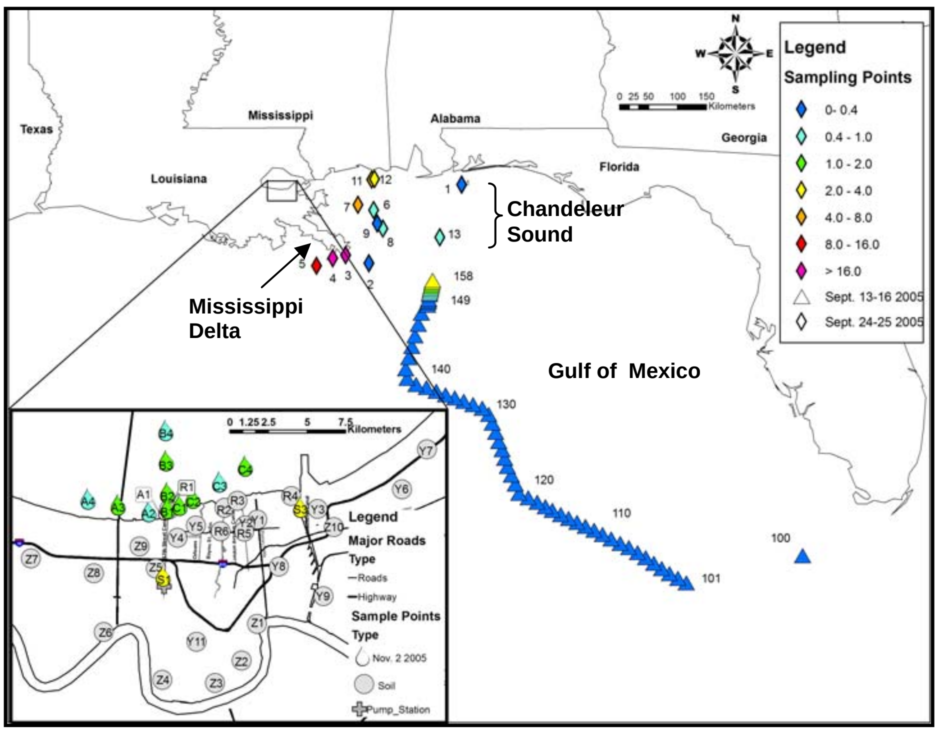

Figure 3: Fecal Indicators, enterococci and *E. coli*, in Lake Pontchartrain Transect B and Canal Sites including results from *Bacteroidales* analysis by PCR and *Bifidobacterium*. Results for the fecal indicators correspond to analysis using MF, CS, and qPCR. Error bars correspond to 95% confidence limits (students t-test). Data points shown with error bars correspond to the average for samples collected between 11 Oct. through 12 Nov. 2005. For lake transects, the samples were also averaged over transects A, B, and C. Site S1 was sampled both before and after pumping at the 17^{th} St. Canal Pump Station #6 For *Bacteroidales* and *Bifidobacterium* the number in the numerator corresponds to the number of positive samples and the number in the denominator corresponds to the total number of samples analyzed. "na" indicates not analyzed. Supporting data provided in supplemental tables S-2 and S-3.

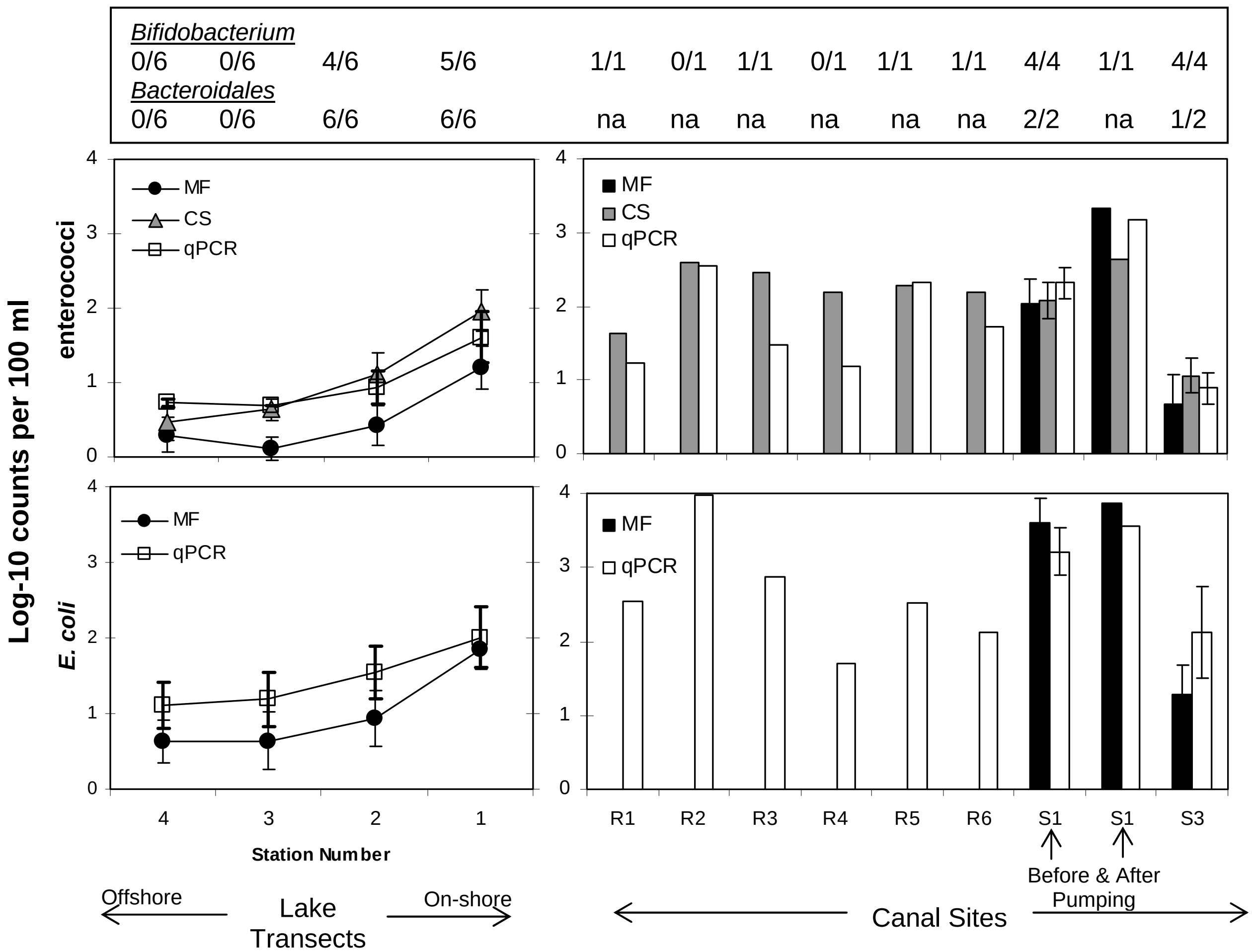
Bifidobacterium
0/6 0/6 4/6 5/6 1/1 0/1 1/1 0/1 1/1 1/1 4/4 1/1 4/4
Bacteroidales
0/6 0/6 6/6 6/6 na na na na na na 2/2 na 1/2
Log-10 counts per 100 ml
enterococci
E. coli
MF
CS
qPCR
Station Number
R1 R2 R3 R4 R5 R6 S1 S1 S3
Before & After Pumping
Offshore
Lake Transects
On-shore
Canal Sites

Figure 4: Comparative bacterial community analysis of samples collected from the 17th St. Canal on two separate occasions, from the Industrial Canal, and from a residential yard. Analyses are based on sequencing of random clones from libraries of PCR-amplified small-subunit rRNA gene fragments with sequences being binned into habitat types based on similarity to top scoring BLAST hits.

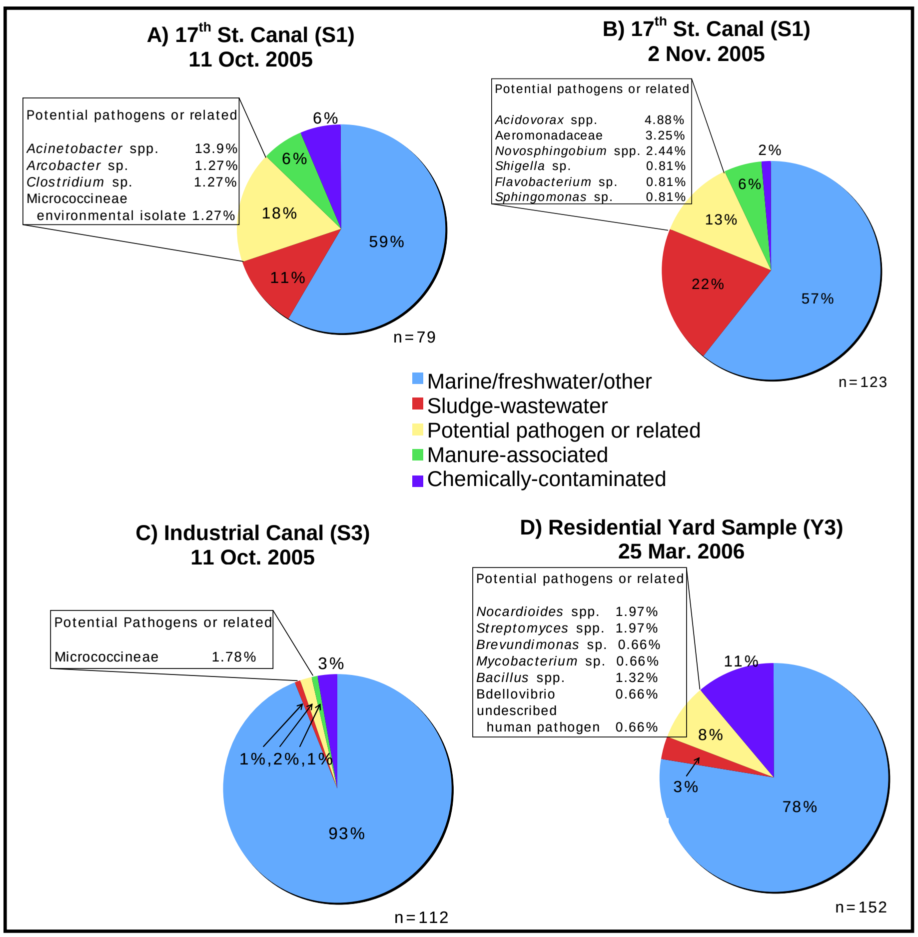

Figure 5: Abundance of *Vibrio* Species in Lake Pontchartrain Transects and Canal Sites During the 11 Oct. 2006 and 27 Jan. 2006 Sampling Dates. Total *Vibrio* spp. abundance is based on total colony-forming units on TCBS medium. Abundances of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* are estimated from the proportion of total colony-forming units testing positive for each of these species by biochemical and genetic tests. "Other" refers to those colonies not classified as one of the three targeted pathogens. Data shown are representative of the data collected. For lake transects, the samples were also averaged over 3 available transects. Supporting data provided in supplemental table S-4.

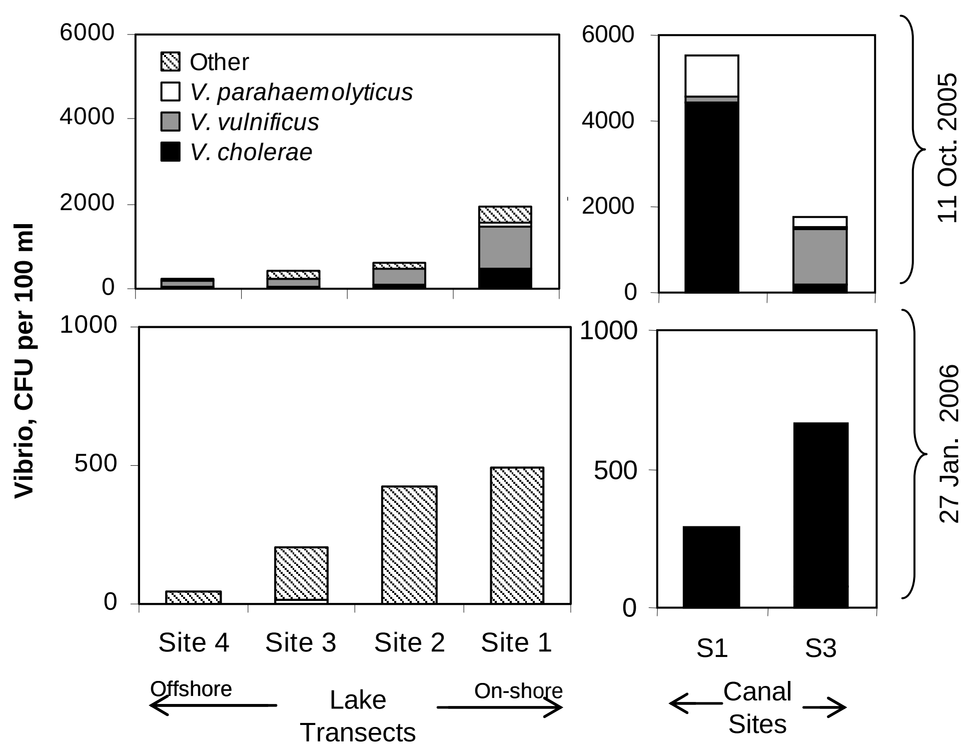

Figure 6: Abundance of Enterococci in Sediments of the NO area, including results from *Bifidobacterium* analysis for samples collected 25 Mar. 2006. (+: positive and -: not detected)**.** Enterococci results correspond to samples collected between 12 Nov. 2005 and 20 Jun. 2006 and analyzed using the CS method. Error bars correspond to 95% confidence limits (students t-test). Supporting data provided in supplemental table S-5.