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Little Venice Water Quality Monitoring Project: EPA Assistance Agreement X7-96410604-3 and FDEP contract SP674 & SP678

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Little Venice Water Quality Monitoring Project
EPA Assistance Agreement X7-96410604-3
and
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Final Report
July 2009

Submitted to
Environmental Protection Agency
and
Florida Department of Environmental Protection
by
Florida International University

Henry O. Briceño and Joseph N. Boyer
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Little Venice Water Quality Monitoring Project
Final Report

EPA Assistance Agreement X7-96410604-3
and FDEP Contracts SP674 & SP678

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EXECUTIVE SUMMARY

Water quality in the Little Venice area is the result of a dynamic interaction of complex environmental conditions with a man-modified landscape, where water masses from Florida Bay and ocean waters mix with runoff, ground waters, and seepage from onsite sewage disposal systems. Additionally, water quality may be influenced by flushing time and abundance of organic debris on the canal bottoms. The Little Venice neighborhood was selected in the Monroe County Sanitary Wastewater Master Plan as the first phase of wastewater improvements for the Marathon area because of its high development density, inadequate cesspool and septic systems, and known water quality problems in the canals.

The objective of the Little Venice Water Quality Monitoring Project was to detect changes in water quality as a function of remediation activities and included two phases. Phase 1 was executed prior to remediation, from May 2001 to December 2003. After the construction of the wastewater collection system was mostly completed, Phase 2 began in June 2005 and lasted until to May 2009. We use a Before–After Control-Impact Experimental Design with multiple sites to assess changes due to remediation. Observations and sampling were performed in three remedied canals (112th St., 100th St. and, 97th St. canals), in one control (reference) canal lacking remedial actions (91st St. canal) and a near shore site for comparison purposes (Fig. i).

Water samples were collected weekly for bacteriological analysis including enumeration of fecal coliforms (until November 2007) and Enterococci. Weekly field parameters measured at both the surface and bottom of the water column at each station included: salinity, temperature, and dissolved oxygen (DO). Weekly water samples from each station were analyzed for total nitrogen (TN), total phosphorus (TP), and chlorophyll a (CHLA). Additionally, monthly grab samples were analyzed for ammonium, nitrate, nitrite, soluble reactive phosphate, silicate, and total organic carbon. All water samples were analyzed by the SERC laboratory using standard methodology as outlined in our Quality Assurance Plan.
Non-parametric Mann-Whitney tests indicated statistically significant (p<0.05) declines in TN and increases in TP, surface and bottom DO, and CHLA in almost all sites. These changes were partially related to region wide variability as well as local condition and/or remediation actions. State of Florida Rule 62-302.530, for Class III marine waters, specifies that DO “shall never be less than 4.0 mg l⁻¹”. Prior to remediation, this threshold was exceeded in 57% and 67% of sampling events for surface and bottom water samples respectively. For Phase 2, the benchmark was exceeded 45% and 54% for surface and bottom DO, respectively. In spite of this improvement, low DO concentrations continue to be an issue of concern in Little Venice waters.

The Florida impaired water rule states that an estuary is impaired if the annual mean CHLA concentration is greater than 11 μg l⁻¹. Using this as a benchmark, annual mean CHLA concentrations for all canals and the offshore site were well below FL State standards during both Phase 1 (1.33 μg l⁻¹) and Phase 2 (2.14 μg l⁻¹); however, the overall increase during Phase 2 was statistically significant.

The Florida State standard for single counts of fecal coliforms in Class III-Marine waters is 800 CFU per 100 ml; the EPA recommended standard for *Enterococci* is 104 CFU per 100 ml.
During Phase 1, 0.4% of fecal coliform observations exceeded the FL State standard, and 6% of *Enterococci* counts exceeded the recommended EPA level. Fecal coliform analyses in Phase 2 indicated that 1% of observations exceeded the FL State standard. After 4 years into remediation (Phase 2), 4% of *Enterococci* counts exceeded the recommended EPA level, suggesting a slight improvement in water quality.

Bacterial count distribution along the year corresponded to both climatic conditions and site location. Higher counts occurred in the rainy season. In addition, the heads of the canals, having longer residence times, had significantly greater bacterial numbers than did the mouths. Figure ii is a plot of mean bacterial counts in Phase 1 versus the difference in mean values between Phase 1 and Phase 2. The positive sign of most differences and slope of the linear regression lines indicates that those stations in worse condition in Phase 1 experienced greater improvements after remediation. The magnitude of these slopes (0.77 and 0.65 for fecal coliforms and *Enterococci* respectively) and the high correlation coefficient of such regressions suggest that polluted sites may be improved by remediation actions, as those performed in Little Venice, by close to 78% for FC and 65% for EC. We feel this is an important result which has potential applications to future remediation projects in the Florida Keys and elsewhere.

Figure ii. Scatter plots of mean fecal coliforms and mean *Enterococci* counts in Phase 1 versus the difference mean Phase 1 – mean Phase 2. These figures indicate that larger improvements occurred in those stations having worse conditions in Phase 1. The regression slopes estimate this change as a function of the mean concentration in Phase 1. Translating that into % change, the “expected” changes would be 78% for FC and 65% for EC.
Remediation results for fecal coliforms and Enterococci may be masked by their re-growth in organic-rich (nutrient-rich) debris on the canal bottom or their re-supply by alternative sources as runoff, especially from storm action. Furthermore, regional trends in water quality may also affect local relationships and mask the potential improvements brought about by remediation. Hence, besides comparisons at individual sites between pre- and post-remediation, ratios of measured bacterial counts between remedied and control sites were used to test changes after remediation. This approach filters out those effects unrelated to remediation but occurring across the region which may have affected the results.

The outcome of these tests (Fig. iii) indicates that fecal coliforms ratios for the 112\textsuperscript{th} St canal (head and mouth) and the head of 100\textsuperscript{th} St canal were above 1.0 before remediation actions were implemented. This means that all of these sites were in worse conditions than their corresponding sites in the 91\textsuperscript{st} St control canal. Ratios for these stations in Phase 2 decreased considerably relative to the control canal (over 40\%) indicating a significant improvement. For the remaining stations, 100\textsuperscript{th} St canal mouth and 97\textsuperscript{th} St canal (head and mouth), ratios were below 1.0 in Phase 1, indicating better initial conditions than the control canal. After remediation, these sites remained in better conditions than the corresponding sites in the control canal, but the mouth of the 100\textsuperscript{th} St canal and the head of 97\textsuperscript{th} St canal have deteriorated as compared to the control canal (46\% and 19\% respectively), while the mouth of the 97\textsuperscript{th} St canal has improved by 50\%. For Enterococci counts (Fig. iii) all pre-remediation ratios for Phase 1 were above 1.0, meaning that all sites were initially in worse conditions than their corresponding sites in the 91\textsuperscript{st} St control canal. In Phase 2, ratios for all stations decreased considerably relative to the control canal (30\%-68\%), highlighting the improvement brought about by remediation.

In summary, this water quality monitoring program has rendered results which translate into encouraging signs of improvement in water quality in Little Venice as an outcome of remedial actions advocated by the Monroe County, the Environmental Protection Agency, the Florida Department of Environmental Protection and the community of Marathon.
Figure 7. Bar-graphs of fecal coliforms and Enterococci counts ratios between each remedied site (head or mouth) and the corresponding control site (head or mouth) in 91st St Canal, for Phase 1 and 2. Ratios above 1 indicate worse conditions than the control canal, and changes from Phase 1 to Phase 2 are expressed as percentages (improvements in black and deterioration in red). All stations for both indexes have improved except for fecal coliforms at the mouth of the 100th St canal and the head of the 97th St canals. These results highlight the improvement brought about by remediation.

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BACKGROUND

Since the early 1980’s several Florida counties began monitoring beaches and canals for Enterococci (EC) and fecal coliforms bacteria (FC), because elevated concentrations of these bacteria were believed to be strongly correlated with the presence of human pathogens. Given that onsite disposal systems (OSDS) and injection wells are known to be a source of microbial contamination of groundwater (Keswick, 1984), and because the ground waters and surface waters are very closely linked in the Keys, it is not surprising that fecal coliforms bacteria are common in canals waters (FDER, 1987).

The Little Venice neighborhood was selected in the Monroe County Sanitary Wastewater Master Plan as the first phase of wastewater improvements for the Marathon area because of the large concentration of cesspools and inadequate septic systems, small average size of lots, high development density, and known water quality problems in the canals in the area (Kruczynski 1999). Little Venice includes the ocean side area of Vaca Key from Vaca Cut (east) to 94th Street (west), Marathon, FL. The Little Venice Service Area includes ~540 Equivalent Development Units (Fig. 1).

Figure 1. Little Venice Subdivision area in Marathon Key; sampling stations are shown. ISCO sampling is only performed bimonthly at yellow coded sites.
Water quality in the 89th – 91st Street canals was thoroughly studied in 1984-1985 as part of the Florida Department of Environmental Regulation’s Monitoring Study (FDER, 1987). That study demonstrated significant nutrient enrichment of the canals, high chlorophyll-a content, and high coprostanol concentrations in sediments. Coprostanol is a break-down product of cholesterol and has been used as an indicator of fecal contamination.

During 2004 the Little Venice Service Area received a low-pressure, vacuum wastewater collection system to convey wastewater to a central treatment plant. The treatment plant produces effluents that meet or exceed the current advanced wastewater treatment (AWT) standards of 5:5:3:1 (Biological Oxygen Demand, Total Suspended Solids, Total Nitrogen, Total Phosphorous) and uses a Class V injection well for disposal of treated wastewater. Central collection and treatment of wastewater removes a substantial portion of nutrient loading into the canals by removing the sources of wastewater (septic tanks and cesspits).

The objective of the Little Venice Monitoring Project was to detect changes in water quality as a function of remediation activities. The initial experiment was conceptually developed as a Before–After Control-Impact Design with multiple sites (BACI; Eberhardt, 1976; Stewart-Oaten et al., 1986) and included two phases. Phase I, from year 2001 to year 2003, corresponded to the pre-remediation stage. Phase 2, which began in 2005 after the construction of the wastewater collection system and EDU connection, is the post-remediation phase. Four canals within the Little Venice Service Area were selected for study (Fig. 1). These canals are located adjacent to the 112th, 100th, 97th and 91st streets, and are lined with single-family residences that were constructed prior to 1970, which had inadequate sewage treatment systems with poorly functional septic systems or cesspits. The 91st Street canal was selected as a reference (control) canal because it is in close proximity to the remediated canals but was not subjected to remediation measures. Finally, a station located offshore of the 100th St canal was also sampled for additional comparisons.

Most of the sampling (82.6%), for both Phases, was performed within a usual time period, between 9:45 AM and 12:15 PM, during optimal conditions to detect human impact. Furthermore, hourly sampling indicated that median values for the parameters (TN, TP, temperature, DO, and salinity) deviated less than 6% from the full (24 hour) daily median (Boyer and Briceño 2006b), suggesting that no major differences with the daily median should be expected during the usual nutrient and bacterial sampling schedule. In summary, weekly
sampling under the actual daily schedule was shown to be sufficient to characterize daily variability in water quality in Little Venice.

**Regional scope**

Under a regional scope, water quality in the Little Venice area is the result of the dynamic interplay of an already complex environmental setting with that of a man made landscape, where neither ambient nor anthropogenic driving processes are constant. On the contrary, they are subjected to trends, seasonal changes and cycles of diverse periodicity and amplitude. The climate in South Florida is subtropical, with little temperature variation along the year but well defined wet (summer/fall) and dry (winter/spring) seasons (Lee et al., 2003). Storms are frequent during the wet season, eventually reaching extreme rain, winds and surge levels. Marine currents exert an important influence on the distribution, character and interactions of water masses (Fig. 2). The South Florida coastal region is bordered by strong, large-scale oceanic boundary currents (the Loop Current/Florida Current System) which link local coastal waters to Gulf of Mexico and Atlantic Ocean and even far upstream river sources (i.e. Mississippi River), by conveying coherent water masses contained within evolving eddy systems. Eddy formation, trapping of Loop Current waters on the shelf break and onshore transport are the proposed mechanisms by which Loop Current waters are transported onto the shelf (IMARS 2006). Furthermore, wind-driven southward coastal flows commonly transport low salinity water plumes coming from the Everglades to western Florida Bay and the Keys reef tract (Lee et al., 2001a, 2001b). In turn, flow direction through the Keys passages vary along the year, with southward flows predominating in winter and spring (dry season); north-northwest flows in the summer (wet season), and southwest flow towards the Tortugas in the fall (Nuttle et al., 2003).

This interaction between Atlantic, Gulf and continental waters affect biotic and abiotic processes in South Florida ecosystems, leading to even more complex responses, which in turn result in water quality diversity, both in time and space. Regional monitoring of the Florida Keys National Marine Sanctuary (FKNMS) allowed the grouping of water quality types into 8 clusters (Fig. 3), where the bulk of the stations fell into 6 large clusters (1, 3, 5, 6, 7, and 8) which described a gradient of water quality. The more relevant groups to the present study are clusters 3, 5 and 7, for which the overall nutrient gradient, from highest to lowest concentrations
is $7 > 5 > 3$, suggesting that this gradient is due to progressive mixing between a nutrient-poor marine end member and a nutrient-rich terrestrial-derived end member (Boyer & Briceño, 2006a).

Figure 2. Current circulation patterns in southwest Florida coasts (modified after Lee et al. 2003)

Figure 3. Results of cluster analysis showing station membership in distinct water quality groups (Boyer and Briceño, 2006a)
Local scope

At the local scale, the interaction occurs among water masses moving through Vaca Cut and along shore, including ocean waters, runoff, ground waters and seepage from cesspits. Water quality is affected by residence time in the canals, which in turn varies according to canal geometry (i.e. straight versus U-shaped), canal length, and seaward extension (i.e. 97th St. canal), bottom topography, accumulation of organic debris (Fig. 4) and tide and wind intensities, among other factors. These organic-rich bottom sediments, where bacteria thrive, are stirred back and forth during tides and may be resuspended in the water column (Fig. 4). Although the new input from cesspools and septic systems has been considerably reduced by the wastewater collection system, seepage from old installations and direct input from boats may still contribute to water quality degradation in the canals.

SAMPLING PROGRAM

The sampling program consisted of two Phases. Phase 1 was conducted for 2.5 years prior to the initiation of operation of the central sewage treatment system to establish pre-remediation conditions in the canals within the service area. Phase 2 began in June 2005, after initiation of the central sewage treatment system. Four canals within the Little Venice Service Area were selected for sampling (Figure 1). The first canal is a connected “U-shaped” canal system located at 112th Street, lined with single-family residences that were constructed prior to 1970. A high percentage of those residences had inadequate sewage treatment systems. This canal receives better tidal flushing than other canals within the Service Area because of the flow-through design and its relatively short length. The second canal is located adjacent to 100th Street and the third is located adjacent to 97th Street. Both 100th St. and 97th St. canals are dead-end canals that are lined with single-family houses and mobile homes. Many of these residences had inadequate sewage treatment systems. The 91st Street canal, located outside the Little Venice Service Area, was selected as a reference non-remedied canal. Finally, an additional reference site located offshore from the mouth of the 100th canal was also selected to monitor changes in the bay itself.
Weekly Canal Sampling

Nine sampling stations were chosen for this project: two per canal plus a nearshore site (Fig. 1). Stations were located at the mouth and head of each canal and the nearshore station (Sta. 2) which was located ~100 m offshore the 100th St. canal. Surface and bottom measurements of salinity (practical salinity units), temperature (°C), and dissolved oxygen (DO, mg l⁻¹) were performed at each station on a weekly basis. Duplicate water samples were collected in mid-channel at 20 cm below the surface. Water samples were also collected just below the surface for bacteriological analysis. Finally, for Phase 1, sampling commenced May 23, 2001 and ended Dec. 15, 2003. Phase 2 sampling began June 14, 2005 and ended in May 25, 2009.

Preliminary studies indicated that the greatest impact of potential terrestrial inputs occurred on the lowest, low tide (FDEP 2001) but daily tide drift did not allow regular sampling at that specific tide level. Hence, we deployed two ISCO auto-samplers at rotating sites to collect 12 samples per day over a two day period, with Hydrolab or YSI datasondes to measure and log temperature, salinity, DO, and pH on an hourly basis. The results of this experiment, which lasted until November 2007 (Boyer and Briceño 2007), indicate that:
• Morning hours rendered the highest probabilities of detecting potential man-derived influences (maximum TP and TN; minimum DO and salinity).

• Most of the sampling (82.6%), for both phases, was performed within a common period between 9:45 AM and 12:15 PM.

• Median values for the parameters (TN, TP, temperature, DO, and salinity) deviated less than 6% from the full (24 hours) daily median.

• No major differences with the daily median should be expected during the nutrient and bacterial sampling schedule.

The long-term sampling program performed during this Little Venice BACI experiment incorporates the temporal progression of tides rendering sampling events spanning the complete range of depths, and mixing proportions, during both phases. This, in turn, smoothed out tidal effects and did not affect final results for those variables controlled by tidal mixing (i.e. TN and TP). Enterococci exceedances preferentially occurred during the 9:30 to 12:00 period for both phases, and their distributions were not statistically different. Hence, we may consider that exceedances results have not been affected by a significant difference in sampling schedule. In summary, weekly sampling under the actual daily schedule was sufficient to characterize daily variability in water quality in Little Venice.

LABORATORY ANALYSIS

Nutrient Analysis

Water samples were analyzed for total nitrogen (TN), total phosphorus (TP), and chlorophyll a (CHLA, µg l⁻¹) by the SERC laboratory using standard methodology outlined in the Quality Assurance Plan. The ISCO water samples were analyzed only for TN and TP. Once a month, grab samples from each site were analyzed for the full suite of nutrients including ammonium (NH₄⁺), nitrate + nitrite (NOₓ⁻), nitrite (NO₂⁻), silicate (SiO₂), soluble reactive phosphate (SRP), and total organic carbon (TOC). Some parameters were not measured directly, but calculated by difference. Nitrate (NO₃⁻) was calculated as NOₓ⁻ - NO₂⁻, dissolved inorganic nitrogen (DIN) was calculated as NOₓ⁻ + NH₄⁺, and total organic nitrogen (TON) was defined as
All variables are reported in mg l\(^{-1}\) unless specified otherwise. The SERC Laboratory is a NELAP certified by the Florida Department of Health.

**Bacteriological Analysis**

During 2008 water samples were collected weekly as above and transported to SYNAGRO for enumeration of *Enterococci* (EPA 1600). All samples were kept at 4 °C and tested within 6 hours of sampling. The SYNAGRO lab is NELAP certified by the Florida Department of Health. Fecal coliform analyses were halted in November 2007, after realizing that results were strongly affected by re-growth in soil and canal bottom mud and were not an adequate and unbiased index for assessing changes due to remediation (Bonilla et al. 2006; Whitman et al. 2006; Fung et al. 2007; Briceño and Boyer 2008).

**DATA ANALYSIS**

The initial experiment was conceptually developed as a Before–After Control-Impact Design with multiple sites (BACI; Eberhardt, 1976; Stewart-Oaten et al., 1986). This design allows the application of traditional Before-After methods (Green, 1979; Smith, 2002) where the data are treated as independent samples and are compared using diverse statistics (absolute changes), and also comparisons with the control canal (relative changes).

**Comparison Methodologies**

We have adopted diverse methods to evaluate changes in water quality, both chemical and bacteriological, after remediation:

1. Comparing before and after mean concentrations
2. Comparing before and after number of exceedances
3. Comparing before and after concentration ratios between remedied and control stations

Methodologies 1 and 2 compare the sites with themselves to track absolute changes in the selected index but do not take into account the probability of potential secular or cyclic changes.
variability, unrelated to remediation, which may differentially affect the measured concentrations. Methodology 3 (ratio comparison) takes into account and theoretically eliminates the variability common to all stations but unrelated to remediation steps (i.e. trends, climate or bay water induced changes). Neither one of these methodologies is able to filter-out differentially induced variations such as eventual anthropogenic impacts (i.e. boat discharges, lawn irrigation, etc.).

**Detection limits**

Additional complications arise from the analytical method detection limits (MDL) of the techniques utilized in the determination of chemical and bacteriological indexes. Traditionally, censored concentrations below the detection limit (BDL) were replaced by either the MDL, \( \frac{1}{2} \) MDL or (MDL)\(^{1/2} \) (Nehls & Akland 1973; Harris et al. 2003) and even Federal agencies recommended the practice (EPA 1998; ACE 1988; Helsel 2005). Until now we have substituted BDLs by MDLs in our Little Venice data. BDL data must not be discarded because they are integral part of the dataset and bear important information on the system, but attempting substitutions, which are function of the laboratory precision, introduces an artificial signal unrelated to the data population and the phenomena under consideration (Helsel 2005). Taking these considerations into account, we have reassessed our previous data handling procedures incorporating software recently developed by Helsel (http://www.practicalstats.com/NADA) for treating censored data in Minitab® environment. These techniques estimate the mean and percentiles (Appendix 4) allowing comparisons without the introduction of extraneous signals due to laboratory precision or subjective decisions of the analyst.

**RESULTS**

**Bacteriological Analysis**

The head of the canals have greater bacterial numbers than the mouth (Fig. 5) as would be expected because of tidal mixing with offshore less polluted waters and the longer residence time at the canal head. Seasonality analysis (Fig. 6 and Appendix 2) indicates maximum values for head stations extended from June to September with the minimum concentration observed in
March. Canal mouth sites display the highest values in September and lowest values in February-to-April. Offshore data is more erratic along the year. Detected *Enterococci* counts (values above MDL; colony forming units per 100 ml, CFU) for the canals and reference stations for the complete period of record are shown in Appendix 1. In general, stations displayed a seasonal pattern with maxima centered about June-September and December-January, a persistent minimum in March-May, and a more subdued minimum in November (Fig. 6a; Appendix 2). These maxima seem to be in response to climatic conditions (rainy season in June-September). Conversely, the minima may be due to dryer conditions in March-May and October-November, which diminish runoff and seepage contributions to the canals. The seasonal pattern for the control and offshore stations is not as clear as in the remedied canal stations (Fig. 6b).

![Percent of Enterococci counts above Detection Limit of 20 CFU](image)

**Figure 5** Bar-graph of percent of detected *Enterococci* counts above the maximum DL=20 at each site, highlighting the occurrence of higher values at the head of canals.
Figure 6 Seasonality of Enterococci counts in Little Venice: (a) Overall Little Venice area displaying similar patterns for Phase 1 and 2. (b) Seasonality of Enterococci counts split by sample site position (head, mouth and offshore). Maximum values for head stations (blue bars) extend from June to September, and minimum concentration is observed in March-April. Canal mouths (red bars) display the highest values from June to October and minimum values in February-April. Offshore data (green bars) is more erratic without a clear pattern along the year.

The Florida State standard for single fecal coliform sample in Class III-Marine waters is 800 CFU 100 ml⁻¹ and the EPA recommended standard for Enterococci is 104 CFU 100 ml⁻¹. During Phase 1, 0.43% of fecal coliforms observations exceeded the FL State standard and 5.2% of Enterococci counts exceeded the recommended EPA level (Table 1). By November 2007, after 2.5 years into Phase 2, when FC determinations were suspended, 1% of fecal coliforms observations had exceeded the FL State standard. During Phase 1 EC exceedances were 5.73%, 6.27% and 0% for remedied, control and offshore stations respectively. During Phase 2 Enterococci counts exceedances declined to 3.95% at remedied stations and to 4.12% for the control canal. The offshore station slightly increased its exceedances to 0.55%.
Table 1 BACI comparison of FC and EC exceedances for each sampling site (FC= fecal colliforms; EC= Enterococci; Exc= exceedances)

To assess the magnitude of changes from Phase 1 to Phase 2 we plotted the mean fecal coliforms and mean Enterococci counts in Phase 1 versus the difference mean Phase 1 minus mean Phase 2 (Fig. 7). The positive sign of most differences and positive slope of the linear regression lines indicate that those stations in worse condition in Phase 1 experienced greater improvements after remediation. The magnitude of these slopes (0.78 and 0.65 for fecal coliforms and Enterococci respectively) and the high correlation coefficient of such regressions suggest that polluted sites may be improved by remediation actions, as those performed in Little Venice, by close to 78% for FC and 65% for EC, an important conclusion with potential applications to future remediation projects in the Florida Keys.

Figure 7. Scatter plots of mean fecal coliforms and mean Enterococci counts in Phase 1 versus the difference mean Phase 1 – mean Phase 2.
The general assumption that fecal coliforms and Enterococci are solely derived from fecal wastes has been demonstrated to be incorrect (Hardina and Fujioka 1991; Fujioka 1999; Byappanahalli 2000; Solo-Gabriele et al. 2000; Bonilla et al. 2006; Fung et al. 2007). These bacteria occur in natural environments and are commonly isolated from soil, sediments and plants (Devriese et al. 1987; Muller et al. 2001; Wheeler et al. 2002). Furthermore, residual bacteria survive for months in dried algae and readily grow upon re-hydration. Therefore, immediate remediation results for fecal coliforms and Enterococci may be masked by their re-growth in organic-rich (nutrient-rich) debris on the canal bottom (Fig. 4) or supplied by alternative sources as runoff, especially from storm action. In other words, the behavior of each station is affected by multiple factors which may mask the potential improvements bring about by remediation.

If such additional driving factors uniformly affect the overall area (i.e. rain, storms, winds, regional water circulation, etc) the Before–After Control-Impact Experimental Design allows us to use variations of the methodology to test the data set (Eberhardt, 1976; Smith, 2002). Ratios of measured parameters between control and remedied sites are used to test whether differences in before-and-after conditions of the treated canals are different from before-and-after conditions in the control canal. The overall assumption is that significant differences between treatment and control are due to remediation activity. With this goal in mind, we have calculated the quotients for bacterial counts between remedied and control stations, dividing the FC and EC counts at each head by the counts at the head of 91st St Canal for pre-remediation and post-remediation stages, and then plotted and tested these ratios with non-parametric statistics. Similar procedure was followed for canal mouth stations.

Figure 8 shows bacteria count ratio between each remedied site (head or mouth) and the corresponding control site (head or mouth) in 91st St Canal, for Phase 1 and 2. This approach filters out those effects unrelated to remediation uniformly occurring across the bay that may have affected and masked the results. Bacterial ratios for the 112th St canal (head and mouth) and the head of 100th St canal were >1.0 before remediation actions were implemented, meaning that all of these sites were in worse conditions than their corresponding sites in the 91st St Canal. Ratios for these stations in Phase 2 decreased considerably relative to the control canal (over 40%) indicating a significant improvement. For the remaining stations,100th St canal mouth and
97\textsuperscript{th} St Canal (head and mouth), bacterial ratios were below 1.0 in Phase 1, indicating better conditions than the control canal. After remediation the mouth of the 100\textsuperscript{th} St Canal and the head of 97\textsuperscript{th} St canal deteriorated as compared to the control canal (46\% and 19\% respectively), and the mouth of the 97\textsuperscript{th} St canal had improved by 50\%. These sites remained in better conditions than the corresponding sites in the control canal in Phase 2 (ratios below 1.0). For \textit{Enterococci} counts, all ratios for Phase 1 were above 1.0, meaning that all sites were in worse conditions than their corresponding sites in the 91\textsuperscript{st} St Canal during Phase 1. Ratios for all stations in Phase 2 decreased considerably relative to the control canal (30\%-68\%), highlighting the improvement brought about by remediation.

\textbf{Figure 8.} Bar-graphs of fecal coliforms and \textit{Enterococci} counts ratios between each remedied site (head or mouth) and the corresponding control site (head or mouth) in 91\textsuperscript{st} St Canal, for Phase 1 and 2. Ratios above 1 indicate worse conditions than the control canal, and changes from Phase 1 to Phase 2 are expressed as percentages (improvements in black and deterioration in red). All stations for both indexes have improved except for fecal colliforms at the mouth of the 100\textsuperscript{th} St canal and the head of the 97\textsuperscript{th} St canals. These results highlight the improvement brought about by remediation.

\textbf{Nutrient Analysis}

Results for nutrient analysis are presented as time-series in Appendix 3. In general, the most relevant observations on the distribution and level of nutrients in Little Venice are:

- Concentrations in the head of canals were higher than those at the canal mouth sites, suggesting higher terrestrial contribution (also supported by higher concentrations in the
untreated canal) and/or extended residence time (perhaps preferentially affecting the longest 97th St Canal).

- NO\textsubscript{3}\textsuperscript{-} was mostly driven by NO\textsubscript{3}\textsuperscript{-} concentrations (r\textsuperscript{2}=0.999). Both increase westward in the remedied canals area (Fig. 9), but were comparatively low in the control canal stations in both Phase 1 and 2.

![Figure 9. Box-plot of NO\textsubscript{3}\textsuperscript{-} concentration (ppm) in each phase and station.](image)

- NO\textsubscript{2}\textsuperscript{−} also seemed to increase westwards (Fig. 10) and along Phase 2, but the most conspicuous characteristic is the development of a different seasonality and high values in 2005-2007 (Phase 2) and the decline in 2008 (Appendix 3).

![Figure 10. Box-plot of NO\textsubscript{2}\textsuperscript{−} concentration (ppm) in each phase and station.](image)

- Higher NH\textsubscript{4}\textsuperscript{+} values were observed in the control canal stations and the head of the 112th St Canal (Fig. 11). Values in 2005 were significantly higher regionally, perhaps due to
hurricane impacts, translated into stirring of organic-rich sediments on canal bottom, leading to higher nitrification rates. Since 2005 all sites displayed a decreasing tendency in NH$_4^+$ concentrations. The un-remedied canal had significantly increased in NH$_4^+$ concentration in Phase 2.

![Figure 11](image1.png)

**Figure 11** Box-plot of NH$_4^+$ concentration (ppm) in each phase and station.

- TN also displayed an apparent westward increase within the remedied area (Fig. 12), and a statistically significant decline from Phase 1 to Phase 2 in all stations (Mann-Whitney test), despite the slightly increasing tendency since 2006. Station 7 (head of 97th St) and the un-remedied canal displayed the highest values. In general, all stations followed a similar pattern, suggesting a regional control on TN concentration which, in turn, developed a baseline on which site-specific trends are superimposed.

![Figure 12](image2.png)

**Figure 12.** Box-plot of TN concentration (ppm) in each phase and station.
- TON concentrations followed those of TN because most nitrogen is of organic origin. Hence after remediation values were significantly lower than those of Phase 1 for all stations (Mann-Whitney test), suggesting also a bay wide trend, perhaps not directly linked to remediation actions. The highest concentrations were those of the untreated control canal (Fig. 13).

![Figure 13](image)

**Figure 13** Box-plot of TON concentration (ppm) in each phase and station.

- In all stations, TP showed a well defined increasing trend up to 2006 and a decreasing trend towards 2008 and a return to higher levels in early 2009. These trends coincided with the regional TP trend (Boyer and Briceño 2009), suggesting a regional control on TP concentrations. Behavior of TP had been the opposite of that of TN until the end of 2006, when a parallel tendency began. There is an apparent westward increase in TP (Fig. 14).

![Figure 14](image)

**Figure 14.** Box-plot of TP concentration (ppm) in each phase and station.
• There was a significant contrast between lower SRP concentrations at the mouths and higher values at the heads of remedied canals (Fig. 15), something not well observed between the control canal stations. SRP increased significantly at the control canal mouth in early 2009.

![Figure 15. Box-plot of SRP concentration (ppm) in each phase and station.](image)

• Chlorophyll $a$ values were significantly higher in the post-remediation stage, with the head of the control canal dramatically displaying higher values than the rest of stations (Fig. 16). A regional CHLA increase was previously reported (Boyer and Briceño 2006b) and results from Little Venice seem to partially reflect such overall increase, which is more pronounced at canal head stations.

![Figure 16. Box-plot of CHLA concentration (µg l$^{-1}$) in each phase and station.](image)
• TOC values decreased slightly during Phase 2, with higher concentrations occurring in the control canal (Fig. 17). This decreasing trend coupled with the decline in TON, also observed in Florida Bay suggests that changes are regional and perhaps reflecting a connection with climate variability and not necessarily related to remediation activities.

![Figure 17. Box-plot of TOC concentration (ppm) in each phase and station.]

• Since 2006 (Briceño and Boyer 2008) silicate (SiO$_2$) showed two interesting tendencies (Fig. 18). First, there was a general increase to the west; and second, a decreasing trend during Phase 2. The first tendency suggests increased runoff contribution westwards. Second, the decline during Phase 2 is observed in both remedied and control canals, and may not be the exclusive result of remediation.

![Figure 18 Box-plot of SiO$_2$ concentration (ppm) in each phase and station]
• Surface and bottom salinity consistently increased during Phase 1 and up to 2005. Since then values have remained comparatively high, with a slight decrease in 2008 and 2009. This salinity trend is affecting all South Florida coastal waters, so saltier waters in the remedied canals can not be thought as a result of lower fresh water input after remediation, but the consequence of a regional drift. Head of canals are usually fresher than their mouths, and the head of 97th St canal (station 7) displays the lower salinities in Little Venice, suggesting a larger input from ground and runoff waters.

![Figure 19. Box-plot of water salinity in each phase and station.](image)

• DO in Little Venice waters are low, especially in bottom samples, and have decreased further in all stations until 2005, when a slight increasing trend began (Appendix 3). Despite this recent improving tendency, there are many values below the 4 mg l\(^{-1}\) level established for Class III marine waters by the State of Florida Rule 62-302.530.

![Figure 20. Box-plot of DO (mg l\(^{-1}\)) in each phase and station.](image)
The TN:TP ratio showed a significant decrease from Phase 1 to Phase 2, with the control canal displaying lower TN:TP ratios (closer to balanced conditions) than remedied stations. This shift in nutrient ratio, which favors biomass development in the water column, may be in part responsible for increases in CHLA and exacerbating DO levels, especially in the control canal.

In summary, TN and TON displayed statistically significant decreases (non-parametric Mann-Whitney tests) in all stations, contrasting with TP, CHLA, and salinity values showing statistically significant increases in all stations. These variables render statistical significant differences (p<0.05) between pre- and post-remediation, similar to the regional trend for the whole FKNMS. This behavior suggests that large scale conditions, which strongly influence regional water quality, have a great impact on local conditions and are not unique to Little Venice canals. The relevance of this fact is that regional processes may drive these nutrients concentration beyond changes induced by remediation activities.

There are no numerical nutrient standards for Florida marine waters. However, State of Florida Rule 62-02.300(13), F.A.C. states that “particular consideration shall be given to the protection from nutrient enrichment of those presently containing very low nutrient concentrations: less than 0.3 milligrams per liter total nitrogen or less than 0.04 milligrams per liter total phosphorus.” Prior to remediation, exceedances in the control canal ranged from 56% to 63% and for the remedied canals between 45% and 71%. After remediation all stations showed a decline in TN exceedances, with the control canal ranging from 31% to 35% and the remedied canals from 21% to 47% (Table 2). On the other hand, TP exceedances, which were rare during pre-remediation, have increased in most stations, but especially at the head of the control canal (Table 2).

CHLA concentrations showed an increasing concentration gradient from East to West with the highest values in the control canal (91st St Canal), and in the overall area for the post-remediation period. As for TP, CHLA exceedances (values >11 ppb) in Little Venice were rare in Phase 1, but have experienced a slight but statistically significant increase (p<0.0001) during Phase 2 (Table 2). It is important to point out that most of these exceedances are from the head of the control canal.
State of Florida Rule 62-302.530, for Class III marine waters, specifies that DO “shall never be less than 4.0” mg l\(^{-1}\). Prior to remediation, about 60% of the determinations of surface and bottom DO in canal waters exceeded this benchmark (Table 2). Percent exceedances in Phase 2 have declined (50%) but still suffer from low concentrations. Individually, all stations have improved their surface and bottom DO regarding frequency of exceedances, except for the mouth of the control canal. Low DO concentration remains as one of the most worrisome characteristics of Little Venice’s water, despite improvements brought about by remediation.

<table>
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<th>% Exceedances</th>
<th>TN</th>
<th>TP</th>
<th>CHLA</th>
<th>DO S</th>
<th>DO B</th>
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<td>Phase 1</td>
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<td>8%</td>
<td>0%</td>
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<td></td>
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<td>22%</td>
<td>4%</td>
<td>0%</td>
<td>44%</td>
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<td>Phase 1</td>
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<td>0%</td>
<td>0%</td>
<td>53%</td>
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<tr>
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<td>Phase 2</td>
<td>30%</td>
<td>4%</td>
<td>0%</td>
<td>35%</td>
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<td>2%</td>
<td>72%</td>
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<tr>
<td></td>
<td>Phase 2</td>
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<td>8%</td>
<td>45%</td>
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<td>0%</td>
<td>0%</td>
<td>55%</td>
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<tr>
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<td>21%</td>
<td>1%</td>
<td>1%</td>
<td>40%</td>
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<td>Phase 1</td>
<td>71%</td>
<td>0%</td>
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<td>0%</td>
<td>55%</td>
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<td>48%</td>
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<tr>
<td>Offshore control station</td>
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<td></td>
<td>Phase 2</td>
<td>14%</td>
<td>1%</td>
<td>0%</td>
<td>16%</td>
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</table>

Table 2. Exceedances in TN, TP, CHLA and DO (S=Surface; B=Bottom), in Little Venice for pre- and post-remediation stages.
CONCLUDING REMARKS

The Little Venice neighborhood was selected in the Monroe County Sanitary Wastewater Master Plan as the first phase of wastewater improvements for the Marathon area, and in 2004 received a low-pressure, vacuum wastewater collection system to convey wastewater to a central treatment plant, hoping to eliminate a substantial portion of bacteria and nutrient loading into the canals by removing the sources of wastewater (septic tanks and cesspits). After four years of remediation and continuous monitoring of water quality, there are encouraging signs of improvement in water quality in the remedied canals in Little Venice as compared to the un-remedied canal. Dissolved oxygen seems to be increasing, and bacterial counts (fecal coliforms and Enterococci) have declined significantly as compared to un-remedied canals, suggesting that in similar scenarios as that of Little Venice in the Florida Keys, polluted sites may be improved by remediation actions as those performed in Little Venice, achieving improvements close to 78% for fecal coliforms and 65% for Enterococci counts. Similarly, dissolved oxygen exceedances (below 4 mg L-1) may decline by 14% and 19% respectively. In spite of these improvements, low DO concentrations continue to be an issue of concern in Little Venice waters. Finally, these monitoring results have significant applications in decision making for future remediation actions, as they provide a framework of expected changes and their directions.
REFERENCES


FDEP, 2001. Spatial and Temporal Variability of Nutrient and Bacteria Samples in a Florida Keys Dead-end Canal. Florida Department of Environmental Protection. 14 p


APPENDIX 1

Enterococci counts time-series
(in all plots, red horizontal line is 104 CFU EPA recommended standard)

Figure 1.1. Detected Enterococci counts for Head and Mouth of 112th St. Canal.
Figure 1.2. Detected *Enterococci* counts for Head and Mouth of 100th St. Canal.

Figure 1.3 Detected *Enterococci* counts for Head and Mouth of 97th St. Canal.
Figure 1.4. Detected *Enterococci* counts for Head and Mouth of 91st St. Canal.

Figure 1.5. Detected *Enterococci* counts for Offshore station.
APPENDIX 2

Box-plot of *Enterococci* seasonality for all stations

![Box-plot of Enterococci seasonality for all stations](image)

**Figure 2.1** Seasonality of *Enterococci* counts (logarithmic scale) in the 112th St canal head.

![Box-plot of Enterococci seasonality for all stations](image)

**Figure 2.2** Seasonality of *Enterococci* counts (logarithmic scale) in the 112th St canal mouth.

![Box-plot of Enterococci seasonality for all stations](image)

**Figure 2.3** Seasonality of *Enterococci* counts (logarithmic scale) in the 100th St canal head.
Figure 2.4 Seasonality of *Enterococci* counts (logarithmic scale) in the 100th St canal mouth.

Figure 2.5 Seasonality of *Enterococci* counts (logarithmic scale) in the 97th St canal head.

Figure 2.6 Seasonality of *Enterococci* counts (logarithmic scale) in the 97th St canal mouth.
Figure 2.7 Seasonality of Enterococci counts (logarithmic scale) in the 91st St canal head.

Figure 2.8 Seasonality of Enterococci counts (logarithmic scale) in the 91st St canal mouth.

Figure 2.9 Seasonality of Enterococci counts (logarithmic scale) in the Offshore station.
APPENDIX 3

Time-series Diagrams for nutrients and Physical-chemical data
Head of 112th St Canal

DIN

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

TON

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

TP

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

SRP

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

CHLA

ug L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10
Mouth of 112th St Canal

DIN

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

TON

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

TP

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

SRP

mg L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

CHLA

ug L\(^{-1}\)

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10
Head of 100th St Canal

**DIN**

**TON**

**TP**

**SRP**

**CHLA**
Mouth of 100th St Canal

**DIN**

**TON**

**TP**

**SRP**

**CHLA**
Mouth of 100th St Canal

DO-B

mg L⁻¹

TEMP-S

°C

TEMP-B

°C
Head of 97th St Canal

**DO-B**

![Graph for DO-B showing data points for mg L⁻¹ over years 0-10.]

**TEMP-S**

![Graph for TEMP-S showing data points for °C over years 0-10.]

**TEMP-B**

![Graph for TEMP-B showing data points for °C over years 0-10.]

---

62
Mouth of 97th St Canal

TOC

mg L⁻¹

0
1
2
3
4

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

SI(OH)₄

mg L⁻¹

0.00
0.10
0.20
0.30
0.40
0.50

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

SAL-S

PSU

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

SAL-B

PSU

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10

DO-S

mg L⁻¹

0.00
2.00
4.00
6.00
8.00
10.00

Jan-01 Jan-02 Jan-03 Jan-04 Jan-05 Jan-06 Jan-07 Jan-08 Jan-09 Jan-10
Head of 91st St Canal

**DIN**

**TON**

**TP**

**SRP**

**CHLA**
Mouth of 91st St Canal

**DO-B**

- **mg L⁻¹**

**TEMP-S**

- **°C**

**TEMP-B**

- **°C**
Offshore Station

NOX

NO3

NO2

NH4

TN
## APPENDIX 4

Calculated percentiles for fecal coliforms and *Enterococci*

Calculations were performed using Robust Regression on Ordered Statistics methodology, as described by Helsel (2005) to cope with censored values (non-detects).

### Fecal Coliforms

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<th>90th</th>
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